UNIVERSITY OF CAPE COAST

CHEMICAL MODIFICATION OF THE BIOLOGICALLY ACTIVE COMPOUND, THYMOL

BY

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Thesis Submitted to the Department of Chemistry of the School of Physical Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Chemistry.

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ..... Date: 17-04-2018

Name: Justice Kwaku Addo

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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ABSTRACT

The study was carried to chemically modify a biologically active compound thymol, on the hydroxyl (-OH) functional group into its ester, ether and triazole derivatives. A generalised esterification and etherification reactions were employed to introduce an ester and ether functional groups respectively, and the azide-alkyne click reaction was employed to introduce the triazole moiety. Sixteen ester and nine ether derivatives of thymol were synthesised of which eight esters and five ethers are been reported for the first time. Ten novel 1, 2, 3-triazole derivatives of thymol were successfully synthesised. Two novel thymol-parthenin coupled products were successfully synthesised. All the prepared compounds were in excellent yields and of high purity. The compounds were characterised by one or more of the following spectral data; ^{1}H-NMR, ^{13}C-NMR, LC-QTOF/MS, GC-MS-EI/CI and IR. The larvicidal and adulticidal assay of the ester and ether derivatives against the *Anopheles gambiae s.s* revealed they possess moderate to excellent drug-like characteristics.The most potent larvicidal derivative, 2-Isopropyl-5-methylphenoxy-3-chloro methyl benzene (TM 2O) recorded an LC_{50} value of 1.90 mg/L after 12 hours of exposure time, about 8 folds higher in potency compared to the parent compound, thymol with an LC_{50} value of 15.01 mg/L after 72 hours of exposure time. The most potent adulticidal derivative is 2-Isopropyl-5-methylphenyl-2-methylpropanoate (TM 1C) with an LC_{50} value of 16.02 mg/L compared to thymol with an LC_{50} value of 27.60 mg/L after seven days of exposure time. These derivatives could serve as useful candidate insecticides against the larvae and adult female *Anopheles gambiae s.s* since they showed the highest bioactivity.

KEY WORDS

Derivative

Essential oils

Larvicides

Monoterpenoids

Natural products

Synthesis

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DEDICATION

To my lovely wife, Ivy Kesewaa Nkrumah and children: Christabel Asantewaa Baffour Addo and Vanessa Gyambea Baffour Addo.

TABLE OF CONTENTS

CHAPTER FIVE: SUMMARY,CONCLUSIONS AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ACRONYMS

ANOVA	Analysis of variance
GC	Gas Chromatography
CC	Column Chromatography
DMSO	Dimethyl Sulphoxide
Et_3N	Triethylamine
TLC	Thin-Layer Chromatography
IR	Infra-Red
CI	Chemical Impact Ionization
EI	Electron Impact Ionization
TM	Target Molecule
TMS	Tetramethyl Silane
KBr	Potassium Bromide
$CDCl_3$	Deuterated Chloroform
LC_{50}	Lethal Concentration at 50%
LC_{90}	Lethal Concentration at 90%
GC-MS	Gas Chromatography- Mass Spectrometry
LC-QTOF	Liquid Chromatography Quadrupole Time Of-Flight
MS	Mass Spectrometry
^{1}H-NMR	Proton Nuclear Magnetic Resonance
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
ppm	Parts Per Million
m/z	Mass-to-Charge Ratio
MHz	Mega Hertz
WHO	World Health Organisation

LIST OF ABBREVIATIONS

Ar	Aromatic
s	singlet
t	triplet
d	doublet
m	multiplet
TM 1A	2-Isopropyl-5-methylphenyl ethanoate
TM 1B	2-Isopropyl-5-methylphenyl propanoate
TM 1C	2-Isopropyl-5-methylphenyl 2-methylpropanoate
TM 1D	2-Isopropyl-5-methylphenyl butanoate
TM 1E	2-Isopropyl-5-methylphenyl-2-methyl butanoate
TM 1F	2-Isopropyl-5-methylphenyl pentanoate
TM 1G	2-Isopropyl-5-methylphenyl hexanoate
TM 1I	2-Isopropyl-5-methylphenyl 2-phenylethanoate
TM 1K	2-Isopropyl-5-methylphenyl benzoate
TM 1L	2-Isopropyl-5-methylphenyl-3-bromo-4-methylbenzoate
TM 1M	2-Isopropyl-5-methylphenyl 2-hydroxylbenzoate
TM 1N	2-Isopropyl-5-methylphenyl 2, 2-dichloroethanoate
TM 1P	2-Isopropyl-5-methylphenyl 4-ethylbenzoate
TM 1Q	2-Isopropyl-5-methylphenyl 3-chlorobenzoate
TM 1R	2-Isopropyl-5-methylphenyl 3-methoxybenzoate
TM 1U	Di-(2-Isopropyl-5-methylphenyl) hexanedioate
TM 2C	2-Isopropyl-5-methylphenoxy propane
TM 2D	2-Isopropyl-5-methylphenoxy methylethane
TM 2E	2-Isopropyl-5-methylphenoxy 1-methylpropane

TM 2F	2-Isopropyl-5-methylphenoxy butane
TM 2I	2-Isopropyl-5-methylphenoxy hexane
TM 2K	2-Isopropyl-5-methylphenoxy 2-chloroethane
TM 2N	2-Isopropyl-5-methylphenoxy methylbenzene
TM 2O	2-Isopropyl-5-methylphenoxy 3-chloromethylbenzene
TM 2P	2-Isopropyl-5-methylphenoxy 3-fluoromethylbenzene
TM 8A	1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-chloro-methylbenzene
TM 8B	1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-fluoro-methylbenzene
TM 8C	1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene
TM 8D	1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-chloro-methylbenzene
TM 8E	1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-fluoro-methylbenzene
TM 8F	1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene
TM 8G	1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro-methylbenzene
TM 8H	1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro-methylbenzene
TM 8I	1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-isopropyl-5-methyl-phenoxyethane
TM 8J	1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2,

	3-triazol-1-yl]-2- Isopropyl-5-methyl-phenoxyethane
TM 10A	7-(4-[(2-Isopropyl-5-methylphenoxy) methyl]-1H-1, 2, 3-triazol-1-yl)-octahydro-6-hydroxy-6α, 9α-dimethyl-3-methyleneazuleno [4, 5-β] furan-2, 9(9αH, 9βH)-dione
TM 10B	7-(4-[(4-chloro-2-Isopropyl-5-methylphenoxy) methyl]-1H-1, 2, 3-triazol-1-yl)-octahydro-6-hydroxy-6α, 9α-dimethyl-3-methyleneazuleno [4, 5-β] furan-2, 9(9αH, 9βH)-dione

CHAPTER ONE

INTRODUCTION

The reliance on medicinal plants as rich source of several biological active drugs is well researched but there is still an immense number of these bio-active natural products uncovered. Again, it is established that, some of these secondary products being produced by plants exhibit synergistic activity. This means that, when they are extracted and isolated from the plant source, they are likely to lose their biological activity entirely or there is a drastic reduction in their activity is reported. The activity of these natural products are related to the presence of certain functional group, hence there is the need to study the structure-activity relationship of existing compounds of natural source. This will allow for further modification of their structures to enhance their biological activity. Thus, this work seeks to explore the chemical modification of existing natural products, guided by their biological activity.

Background of study

Plants are sophisticated light-driven "green" factories able to synthesise an immense number of bio-active natural products (Jensen & Møller, 2010). These natural products are also referred to as secondary products or secondary metabolites since they are not directly essential for the basic processes of growth and development (Theis & Lerdau, 2003). The investigation of plant natural products has a long history that started about 200 years ago with the isolation of morphine by Friedrich Wilhelm Sertürmer. Since then the number of described secondary metabolites has risen to over 200,000 (Hartmann, 2007). They can be divided into two major classes, the first class formed by nitrogen-containing substances, such as alkaloids, amines, cyanogenic

glycosides, non-protein amino acids and glucosinolates, and the second class consisting of nitrogen-free substances which are represented by polyketides, polyacetylenes, saponins, phenolics and terpenes. Many of the secondary metabolites were found to serve plants as defenses against herbivores, pathogens and abiotic stresses (Huang *et al.,* 2010). In human society, plants play an irreplaceable role as food sources, not only for their nutritional value but also as spices and herbs which help preserve food or improve its taste. The plant compounds that add flavor to our food are mainly secondary products, such as capsaicin, an alkaloid, which is responsible for the hot taste of chili; or thymol, a terpene, which is one of the main flavoring components in herbs like oregano (Origanum sp.) or thyme (Thymus sp.). Oregano and thyme belong to the Lamiaceae plant family which include many other aromatic plants of great scientific and economic interest such as rosemary, sage, mint, and marjoram. The aroma associated with these plants arises from the essential oil found in peltate glandular trichomes on the aerial parts of the plant. These glandular trichomes consist of highly specialised secretory cells in which the components of the essential oil are synthesised and subsequently accumulate in a subcuticular storage cavity (Gershenzon, Maffei & Croteau, 1989; Turner, Gershenzon, Nielson, Froehlich & Croteau, 1999).The composition of the essential oils of oregano, thyme and marjoram is dominated by mono- and sesquiterpenes (Skoula & Harborne, 2002; Stahl-Biskup, 2002).These substances are responsible for the aroma and flavor of these herbs, and the extracted essential oils are used for the manufacturing of perfumes and cosmetics as well as for medicinal and pharmaceutical purposes as antimicrobial or antiseptic agents (Kintzios, 2002; StahlBiskup, 2002).

Mono- and sesquiterpenes are also thought to help defend the plant against herbivores and pathogens (Gershenzon & Dudareva, 2007).

Two monoterpenes of the Lamiaceae that have attracted much attention, thymol and carvacrol, are found in thyme and oregano but not in marjoram. These phenolic monoterpenes are especially known for their antiherbivore, antimicrobial, pharmaceutical and antioxidant activities (Isman, 2000; Hummelbrunner & Isman, 2001; Ultee, Bennik & Moezelaar 2002; Sedy & Koschier, 2003; Braga, Culici, Alfieri & Dal Sasso, 2008). They are even used to treat bee hives against the varroa mite without harming the bees (Floris, Satta, Cabras, Garau & Angioni, 2004). According to a prediction by Poulose and Croteau (1978a) the pathway for thymol formation proceeds from γ-terpinene via the aromatic compound, p-cymene, as an intermediate. However, despite extensive efforts to breed oregano or thyme varieties with a larger proportion of thymol and carvacrol for pharmaceutical use and the interest in these terpenes as plant defenses, no genes or enzymes responsible for thymol or carvacrol formation from γ-terpinene or p-cymene have been described.

Chemical pesticides cause serious problems like pesticide resistance, secondary pest outbreak, pest resurgence and toxic residues in the environment (Isman, 1999). Under greenhouse conditions, short life cycle and high reproductive potential of spider mites, combined with frequent pesticide applications, result in even more quick resistance to numerous miticides (Ambikadevi & Samarjit, 1997). Resistance and toxicity problems of the synthetic insecticides have resulted in the exigency of finding more effective and healthier alternatives. Hence, the alternative method for replacing the

using of synthetic insecticides is needed. Among existing methods, essential oils have been suggested as alternative sources for control. Essential oils derived from many plants are known to possess biological activity against prokaryotic (Deans & Ritchie, 1987) and eukaryotic organisms (Konstantopoulou, Vassilopoulou, Mavragani-Tsipidou, & Scouras, 1992). Also, many plants, including garlic (*Allium sativum L.*), rosemary (*Rosmarinus officinalis L.*), cinnamon (*Cinnamomum verum* J. Presl), and cedar (Cedrus spp.), have been used to control a variety of insects (Isman, 2004). Many of plant compounds are selective to pests because they do not have side effects on the environment and non-target organisms or their effect is slight (Isman, 2000).Thus, much attention has been focused on them as potential sources of commercial acaricide largely because certain plant essential oil preparations and their constituents meet the scales of minimum risk pesticides (USEPA 1996, 2009). Therewith, essential oils have a broad spectrum of insect and mite activity due to the presence of several modes of action including inhibition of molting, repellent and antifeedant activities and reduction in fecundity and growth (Saxena, 1989; Arnason *et al.*, 1993; Isman, 2000; Enan 2001; Akhtar & Isman, 2004).

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plant (Deans & Ritchie, 1987; Hammer, Carson & Riley, 1999; Sánchez, García & Heredia, 2010). The chemicals in essential oils are secondary metabolites, which play an important role in plant defense as they often possess antimicrobial properties (Hyldgaard, Mygind & Meyer, 2012). Essential oils have been used for centuries in medicine, perfumery, cosmetic,

and have been added to foods as part of spices or herbs. Their initial application was in medicine, but in the nineteenth century their use as aroma and flavor ingredients increased and became their major employment. Almost 3000 different essential oils are known, and 300 are used commercially in the flavor and fragrances market (Burt, 2004). Essential oils are considered to be secondary metabolites and important for plant defense as they often possess antimicrobial properties (Fraenkel, 1959; Tajkarimi, Ibrahim & Cliver, 2010). The antibacterial properties of secondary metabolites were first evaluated using essential oil vapors by De la Croix in 1881 (Burt, 2004). Since then, essential oils or their components have been shown to not only possess broad-range antibacterial properties (Deans & Ritchie, 1987; Oussalah, Caillet, Saucier & Lacroix, 2007),but also anti-parasitic (George, Smith, Shiel, Sparagano & Guy, 2009), insecticidal (Enan, 2001; Kim, Roh, Kim, Lee & Ahn, 2003), antiviral (Astani, Reichling & Schnitzler, 2011) , antifungal (Fitzgerald, Stratford & Narbad, 2003; Kalemba & Kunicka, 2003; Silva, Ferreira, Duarte, Mendonça & Domingues, 2011; Tserennadmid *et al.,* 2011), and antioxidant (Brenes & Roura, 2010) properties. Furthermore, they also function as growth enhancers for animals (Brenes & Roura, 2010; Ahmadifar, Falahatkar & Akrami, 2011). Although the food industry primarily uses essential oils as flavorings, they represent an interesting source of natural antimicrobials for food preservation.

Essential oils are also defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavour or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. Essential oils are found in

glandular hairs or secretory cavities of plant-cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots and/or fruits in different plants. The aromatic characteristics of essential oils provide various functions for the plants including (i) attracting or repelling insects, (ii) protecting themselves from heat or cold; and (iii) utilizing chemical constituents in the oil as defence materials. Many of the essential oils have other uses as food additives, flavourings, and components of cosmetics, soaps, perfumes, plastics, and as resins. Typically these oils are liquid at room temperature and get easily transformed from a liquid to a gaseous state at room or slightly higher temperature without undergoing decomposition. The amount of essential oil found in most plants is 1 to 2%, but can contain amounts ranging from 0.01 to 10%. For example, orange trees produce different composition of oils in their blossoms, citrus fruits, and/or leaves. In certain plants, one main essential oil constituent may predominate while in others it is a cocktail of various terpenes. In *Ocimum basilicum* (Basil), for example, methyl chavicol makes up 75% of the oil, β-asarone amounts to 70–80% in *Acorus calamus* rhizomes, linalool , in the range of 50– 60%, occurs in coriander seed and leaf oils procured from different locations at different time intervals and is by far the most predominant constituent followed by p-cymene, terpinene, camphor and limonene. Interestingly 2-decenol and decanal were the most predominant constituents in leaf oil (Lawrence & Reynolds, 2001). However, in other species there is no single component which predominates. Most essential oils comprise of monoterpenes compounds that contain 10 carbon atoms often arranged in a ring or in acyclic form, as well as sesquiterpenes which are hydrocarbons comprising of 15

carbon atoms. Higher terpenes may also be present as minor constituents. The most predominant groups are cyclic compounds with saturated or unsaturated hexacyclic or an aromatic system. Bicyclic (1, 8-cineole) and acyclic (linalool, citronellal) examples also make the components of essential oils. However, intraspecific variability in chemical composition does exist, which is relative to ecotypic variations and chemotypic races or populations.

Many plant essential oils show a broad spectrum of activity against pest insects and plant pathogenic fungi ranging from insecticidal, anti-feedant, repellent, oviposition, and deterrent, growth regulatory and anti-vector activities. These oils also have a long tradition of use in the protection of stored products (Koul, Walia & Dhaliwal, 2008). Recent investigations indicate that some chemical constituents of these oils interfere with the octopaminergic nervous system in insects. As this target site is not shared with mammals, most essential oil chemicals are relatively non-toxic to mammals and fish in toxicological tests, and meet the criteria for "reduced risk" pesticides. Some of these oils and their constituent chemicals are widely used as flavouring agents in foods and beverages and are even exempt from pesticide registration. This special regulatory status combined with the wide availability of essential oils from the flavour and fragrance industries, has made it possible to fast track commercialisation of essential oil-based pesticides. Though well received by consumers for use against home and garden pests, these "green pesticides" can also prove effective in agricultural situations, particularly for organic food production. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential-oil-based pesticides

owing to the complex mixtures of constituents that characterize many of these oils. Ultimately, it is in developing countries which are rich in endemic plant biodiversity that these pesticides may ultimately have their greatest impact in future integrated pest management (IPM) programmes due to their safety to non-target organisms and the environment. (Koul *et al.,* 2008).

The term terpenes are derived from turpentine (Sfaei-Ghomi, Meshkatalsadat, Shamai, Hasheminejad & Hassani, 2009) at. balsamum terebinthinae). Terpenes are a very large class of most abundant natural hydrocarbons and are commonly present in higher plants as constituents of essential oils. The fundamental building block of terpenes are the isoprene unit (2-methyl-1, 3-butadiene) linked in a head-to-tail fashion and is represented by the general structural formula $(C_5H_8)n$ where n is the number of linked isoprene units. The terpenes are classified into several classes depending on the number of isoprene units (2-methyl-1, 3-butadiene) in the structure. Out of this monoterpenes are class of terpenes that consist of two isoprene units joined head-to-tail and have the molecular formula $C_{10}H_{16}$. It is a hydrocarbons or as oxygenated moieties with aldehyde, alcohol, ketone, ester and ether functionalities.

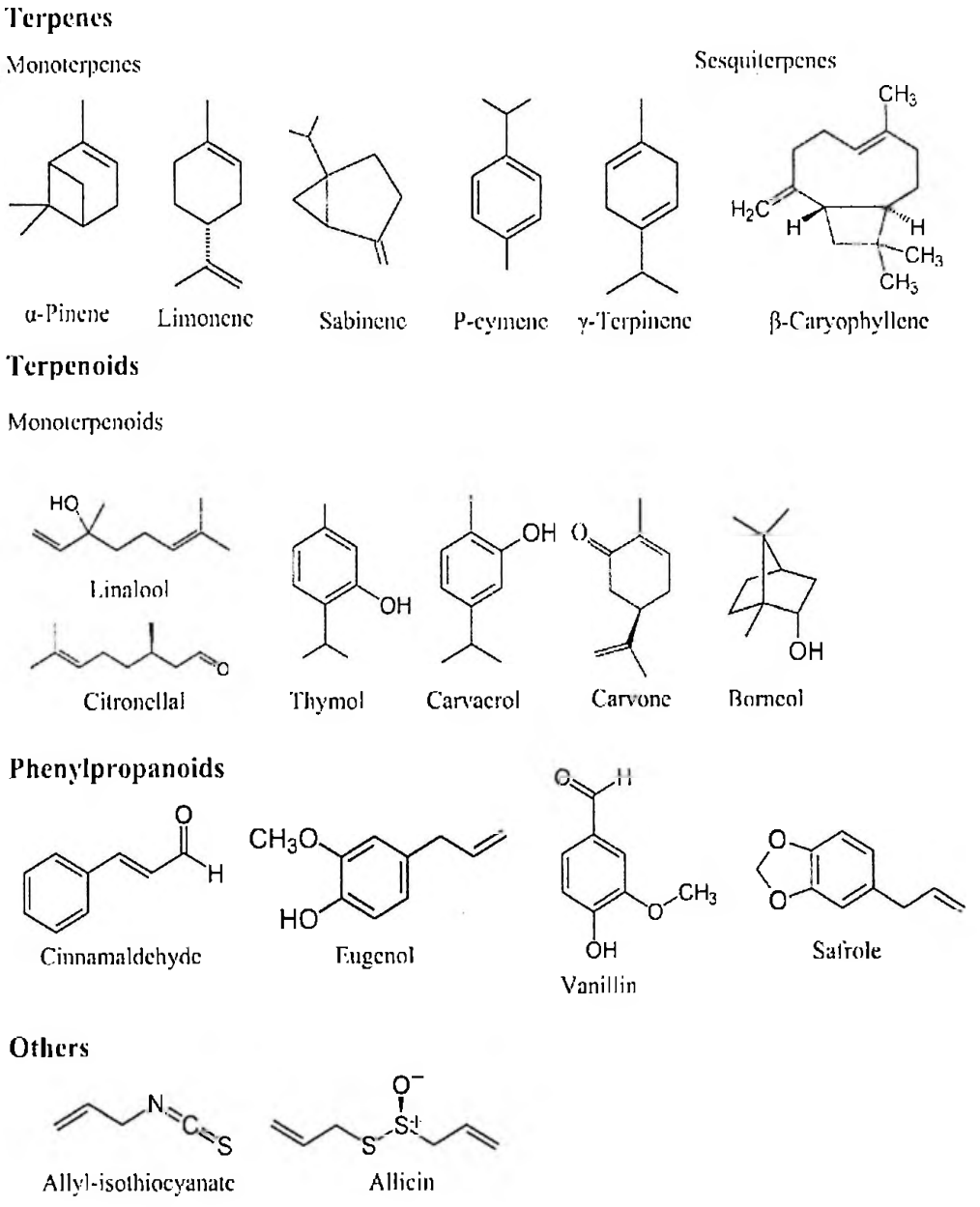


Figure 1: Chemical structures of selected essential oil constituents.

Terpenes are hydrocarbons produced from combination of several isoprene units (C_5H_8). Terpenes are synthesised in the cytoplasm of plant cells, and the synthesis proceeds via the mevalonic acid pathway starting from acetyl CoA. Terpenes have a hydrocarbon backbone which can be rearranged into cyclic structures by cyclases, thus forming monocyclic or bicyclic structures (Caballero, Trugo & Finglas, 2003). The main terpenes are monoterpenes ($C_{10}H_{16}$) and sesquiterpene ($C_{15}H_{24}$), but longer chains such as

diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{40}$), etc., also exist. Examples of terpenes include p-cymene, limonene, terpinene, sabinene, and pinene. Terpenes (also known as terpenoids or isoprenoids) form the largest group of natural products with more than 30,000 different structures (Buckingham, 1998) spread over the widest assortment of structural types with hundreds of different monoterpene, sesquiterpene, diterpene and triterpene carbon skeletons (Degenhardt, Köllner & Gershenzon, 2009). The majority of terpenes have been isolated from plants where their enormous structural variability leads to a great functional diversity. Terpenes play important roles in almost all basic plant processes, including growth, development, reproduction and defense (Gershenzon, 1999). For example, phytol, the side chain of the photosynthetic pigment chlorophyll, is the most abundant plant terpenoid (Davis & Croteau, 2000). Still, comparatively little is known about the actual role of most terpenes in nature despite this immense number and the importance of natural products in medicine, agriculture and industry (Gershenzon & Dudareva, 2007).

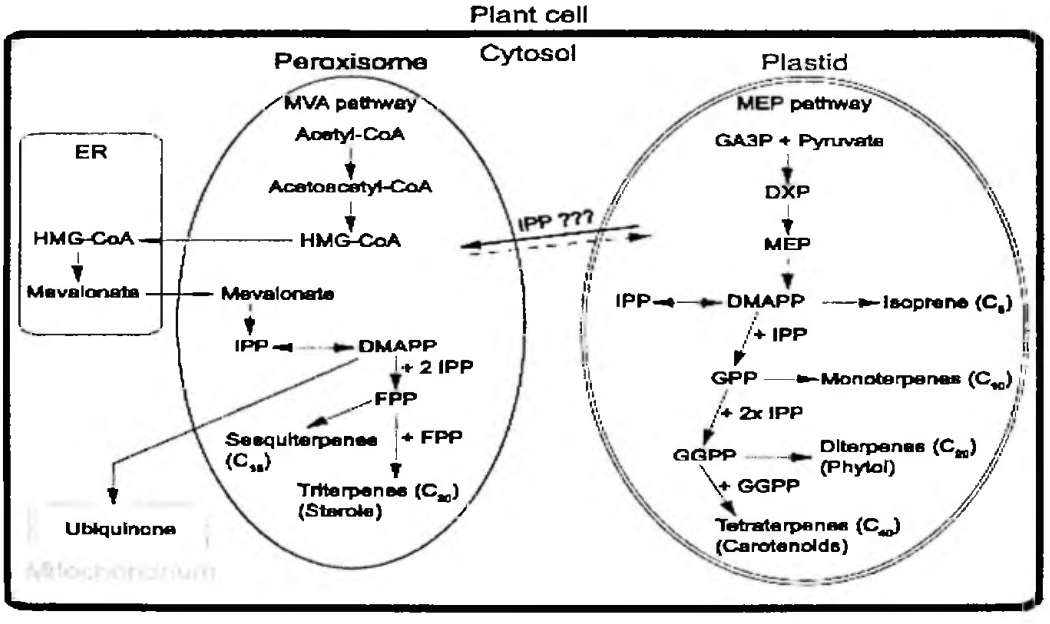


Figure 2: Compartmentation of plant terpene biosynthesis.

The Mevalonic acid (MVA) pathway is located in the cytosol in peroxisomes and in the endoplasmatic reticulum (ER). It starts with three units of AcetylCoA and the final product farnesyl pyrophosphate (FPP) is the precursor molecule for all sesquiterpenes.The Methyl-erythritol-phosphate (MEP) pathway is located in the plastids and the initial substrates are glyceraldehyde-3-phosphate (GA3P) and pyruvate. Geranyl diphosphate (GPP) is the precursor for all monoterpenes and geranyl geranyldiphsophate (GGPP) the precursor for diterpenes. Carotenoids are derived from two units of GGPP. DMAPP (dimethylallyl diphosphate) is the backbone to which different numbers of the isomer IPP (isopentenyl diphosphate) are added to form GPP, FPP or GGPP. Ubiquinone is formed in mitochondria. An exchange of IPP between different compartments is still under investigation. (Redrawn after (Sapir-Mir *et al.,* 2008; Sallaud *et al.,* 2009).

Much more is known about the biosynthesis and localization of terpenes within the plant cell.Terpenes are formed from the universal five-carbon building blocks, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), which are both synthesised by the plastidic methylerythritol pathway and the cytosolic mevalonate pathway (Gershenzon, 1999; Sapir-Mir *et al.,* 2008; Sallaud *et al.,* 2009) (Figure 2). DMAPP and IPP are fused by prenyltransferases to form geranyl diphosphate (GPP, C10), the usual precursor of the monoterpenes, and DMAPP and two units of IPP are fused to form farnesyl diphosphate (FPP, C15) the precursor of most sesquiterpenes. Next, the linear carbon skeletons of GPP and FPP are converted to the basic terpene skeletons by terpene synthases, a widespread class of enzymes responsible for the huge structural diversity of mono- and

sesquiterpenes since these enzymes often form multiple products (Tholl, 2006; Degenhardt *et al.*, 2009)

Terpenes do not represent a group of constituents with high inherent antimicrobial activity. For example, p-cymene, one of the major constituents in thyme, had no antimicrobial activity against several Gram-negative pathogens even at 85700 µg/mL concentration (Bagamboula, Uyttendaele & Debevere, 2004). In a large scale experiment, limonene, α-pinene, β-pinene, δ-3-carene,(+)-sabinene, and α-terpinene showed no or low antimicrobial activity against 25 different genera of bacteria that pose problems in animals, plants, and food products (Dorman & Deans, 2000). Koutsoudaki, Krsek and Rodger, (2005) compared the effect of α-pinene, β-pinene, p-cymene, β-myrcene, β-caryophyllene, limonene, and γ-terpinene against *Escherichia coli, Staphylococcus aureus*, and *Bacillus cereus,* and their antimicrobial activity were low or absent. P-cymene and γ-terpinene were ineffective as fungicides against *Saccharomyces cerevisiae* (Rao, Zhang, Muend & Rao, 2010). These in vitro tests indicate that terpenes are inefficient as antimicrobials when applied as single compounds.

The thymol and carvacrol precursor p-cymene is a monoterpene that has a benzene ring without any functional groups on its side chains. p-Cymene is not an efficient antimicrobial compound when used alone (Juven, Kanner, Schved & Weisslowicz, 1994; Mann, Cox & Markham, 2000; Aligiannis, Kalpoutzakis, Mitaku & Chinou, 2001; Bagamboula *et al.*, 2004), but it potentiate the activity of compounds like carvacrol (Ultee *et al.*, 2002; Rattanachaikunsopon & Phumkhachorn, 2010) and polymyxin B nonapeptide (Mann *et al.*, 2000). Several studies indicate that p-cymene is likely to act as a

substitutional impurity in the membrane, which partly perturbs the membrane of microorganisms. P-cymene has a high affinity for membranes and causes membrane expansion and affect the membrane potential of intact cells (Ultee *et al.*, 2002). Investigations on cell and vesicle systems confirm that p-cymene has no effect on the membrane permeability, but cause a decrease in the enthalpy and melting temperature of membranes (Cristani *et al.*, 2007), supporting the hypothesis that p-cymene acts as a substitutional impurity in the membrane. Even though the action of p-cymene on the cell membrane is well established, its effect on protein synthesis and cell motility has also been investigated .p-cymene had a negligible effect on the protein synthesis of *E. coli* cells (Burt *et al.*, 2007), while its effect on the membrane potential resulted in decreased cell motility, as a proton motive force is needed for flagellar movement (Gabel & Berg, 2003; Burt *et al.*, 2007).

Terpenoids are terpenes that undergo biochemical modifications via enzymes that add oxygen molecules and move or remove methyl groups (Caballero *et al.*, 2003). Terpenoids can be subdivided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides. Examples of terpenoids are: thymol, carvacrol, linalool, linalylacetate, citronellal, piperitone, menthol, and geraniol (Figure 1). The antimicrobial activity of most terpenoids is linked to their functional groups, and it has been shown that the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are important for antimicrobial activity. For example, the antimicrobial activity of the carvacrol derivatives carvacrol methylether and p-cymene were much lower than carvacrol (Dorman & Deans, 2000; Ultee *et al.*, 2002; Ben Arfa, Combes, Preziosi-Belloy, Gontard & Chalier, 2006). Exchanging the hydroxyl group of

Unfortunately, substantial yield losses occur due to insects and plant diseases caused by fungi, bacteria and viruses (Fletcher *et al.,* 2006). Fungi and bacteria also have unfavourable effects on quality, safety and preservation of food. Synthetic chemicals are widely used in the control of plant diseases. However, these chemicals may cause toxic residues in treated products (Barnard, Padgitt & Uri, 1997; Isman, 2000). Therefore, many researchers have sought natural antimicrobials from natural sources (Kim, Moon, & Hwang, 1999; Kubo, Lunde & Kubo, 1995), and some naturally occurring antimicrobial compounds have been found in medicinal plants, herbs and spice extracts (Hsieh, 2000; Larhsini, Oumoulid & Lazrek, 2001). The genus Inula, a variable perennial herb distributed in Asia, Europe and Africa, comprises ca. 100 species of the Compositae (Asteraceae) family, belonging to the tribe Inuleae (Editorial Committee of the Administration Bureau of Chinese Plant Medicine, 1979). Several species in this genus are used as traditional herbal medicines throughout the world. The roots of Inula hupehensis have been used to treat many diseases, including bronchitis, diabetes, and intestinal ulcers. The characteristic compounds of the genus Inula are sesquiterpenes and monoterpenes (Bokadia, MacLeod, Mehta, Mehta & Patel, 1986). In some Inula species, such as *I. britannica, I. salicina L., I. bifrons L., I. Conyza* DC. and *I. spiraeifolia L.*, thymol derivatives, rather than sesquiterpenoids, are the major root constituents (Bohlmann, Mahanta, Jakupovic, Rastogi & Natu, 1978; Bohlmann & Zdero, 1977). Recently, much attention has been paid to thymol derivatives, due to their diverse biological activities. Thymol derivatives, isolated from many species of Inula, have shown antibacterial activities (Stojakowska, Kedzia & Kisiel, 2005; Yoshida, Mori & He, 1995).

The usefulness of thymol derivatives as insecticides and transdermal drug delivery enhancers has also been reported (Grodnitzky & Coats, 2002; Wagner, Suter & Merfort, 2004). However, there have been few reports on the inhibitory activity of thymol derivatives against plant pathogenic fungi (Tawata, Taira, Kobamoto, Ishihara & Toyama, 1996). The mode of action of thymol, a phenolic monoterpenoid and one of the major constituents of thyme oil, has received much attention from researchers. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different position on the phenolic ring (Figure 1).

The antimicrobial action of phenolic compounds, such as thymol and carvacrol, are expected to cause structural and functional damages to the cytoplasmic membrane (Sikkema, De Bont & Poolman, 1995). The primary mode of antibacterial action of thymol is not fully known, but is believed to involve outer and inner membrane disruption, and interaction with membrane proteins and intracellular targets. For the above reasons, there is the need to find new derivatives of thymol through chemical modification of the existing structure. The incooperation of the triazole nucleus as a linkage to other functional groups on the thymol moiety is an area worth researching.

The triazole nucleus is one of the most important and well known heterocycles which is a common and integral feature of a variety of natural products and medicinal agents. Triazole nucleus is present as a core structural component in an array of drug categories such as antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anaesthetic, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, anti-Parkinson's, antidiabetic,

antiobesity and immunomodulatory agents, etc. The broad and potent activity of triazole and their derivatives has established them as pharmacologically significant scaffolds. The basic heterocyclic rings present in the various medicinal agents are 1, 2, 3-triazole and 1, 2, 4-triazole. A large volume of research has been carried out on triazole and their derivatives, which has proved the pharmacological importance of this heterocyclic nucleus (Kharb, Sharma & Yar, 2011). For the above reasons, the in cooperation of the triazole moiety on the biological template monoterpenoid thymol is much desirable.

Statement of Purpose

Several human pathogens can acquire resistance against the available antimicrobial compounds or would need prolonged time of therapy, which may cause toxicity. A Surveillance program carried out by Habon *et al.,* (2001) in the United State, Canada, Europe, Latin America and Asia Pacific region have shown that resistance to several antimicrobial drugs continues to emerge.

Again, research by Mwangi, Berkley, Lowe, Peshu, Marsh & Newton, 2002; Iwalokun, Gbenle, Smith, Ogunledun, Akinsinde & Omonigbehin, 2001; Kuaban, Bercion, Jifon, Cunin & Ngu Blackett, 2000) have indicated high prevalence of antimicrobial resistance bacteria in many Africa regions including Nigeria, Cameroun and Kenya. This has compromised the outcomes of many infections that were, until recently treatable and remain the most common diseases in Africa.

The loss of quality and safety of food largely results from microorganisms. The use of antimicrobials and antiseptics is a common alternative to control bacteria in food (Tauxe, 1990). However, the widespread

use of antibiotics in human medicine and agriculture has caused serious problem of bacterial resistance (Beovic, 2006). Currently, many prescribers use antibiotics that are no longer effective due to increased prevalence of resistance, eventually requiring multiple chemotherapeutic courses to effect a cure. Conversely, expensive agents that are employed in life-threatening situations may be substituted for cheaper agents, if local susceptibilities are known.

The environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and public in recent years. It has been estimated that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually. The reasons for this are twofold: (1) the high toxicity and non-biodegradable properties of pesticides and (2) the residues in soil, water resources and crops that affect public health. (Koul *et al.,* 2008)

Justification

Considering the socio-economic impact of people suffering from pathogenic diseases as well as the loss of quality and safety of food through agricultural practices and the fact that, drug resistivity of microorganisms is on the increase, there is the need to find other alternative antimicrobial agents through syntheses by modifying functional groups of existing antimicrobial agents that will show better activity than the parent compound.

The need to search for new, highly selective and biodegradable pesticides to solve the problem of long term toxicity to mammals and the development of environmentally friendly pesticides can be achieved through chemical modification of existing natural products of known biological activity. Natural

products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts on human health and the environment. The move toward green chemistry processes and the continuing need for developing new crop protection tools with novel modes of action makes discovery and commercialisation of natural products and their derivatives as green pesticides an attractive and profitable pursuit that is commanding attention (Koul *et al.*, 2008).

Major thrust by the whole of the pharmaceutical industry is focused towards design and development of new innovative/indigenous plant based drugs through investigation of leads from traditional system of medicine (Nagle, Pawar, Sonawane, Nikum, Patil & More, 2013). Recent years, ethno-botanical and traditional uses of naturally occuring compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. It is best classical approach in the search of new molecules for management of various diseases. Thorough screening of literature available on thymol researchers can explore the therapeutic potential that are not known (Nagle *et al.*, 2013).

Natural products with biological activity can be further modified to enhance their efficacy and potential as pesticides and insecticides without any adverse toxicity to mammals, crops, water resources and the environment at large.This can be achieved by studying the structure activity relationship of these compounds to identify ways of improving on their biological activity. This would enable synthetic chemist to introduce functional groups on an existing natural product during modification process, thereby enhancing the potency and efficacy of the newly derivatives of the parent biologically active

natural products. These semisynthetic products would be safe and eco-friendly as the parent natural products .This will eventually help to overcome the above disadvantages.

Objectives

General Objective

To synthesise a number of derivatives of thymol, through chemical modification of the hydroxyl functional group on thymol. Some chemical reactions to be employed include esterification reactions, etherification reactions, Michael Addition reaction, Azide-Alkyne "Click" Reaction, Duff reaction, and chlorination reaction. Again, to screen for the biological activity of the synthesised molecules against the Anopheles mosquito (larvae and adult mosquitoes). The study therefore seeks to achieve the following specific objectives:

Specific Objectives

1. a. To synthesise ester derivatives of thymol
   b. Screen for larvicidal and adulticidal potency of the derivatives on *Anopheles gambiae s.s*
   c. Characterise all ester derivatives using GC-MS (EI and CI) and FT-IR.
2. a. To synthesise ether derivatives of thymol
   b. Screen for larvicidal and adulticidal potency of the derivatives on *Anopheles gambiae s.s*
   c. Characterise all ether derivatives using GC-MS (EI and CI) and FT-IR.

3. a. To synthesise derivatives of thymol with the 1, 2, 3-Triazole moiety.

   b. To characterise all the thymol derivatives with the 1, 2, 3-triazole moiety using LC-QTOF (ESI), GC-MS (EI & CI), FT-IR, ^{1}H-NMR, and ^{13}C-NMR.

4. To synthesise other thymol derivatives of interest as intermediates to other products.

5. a. Coupling of thymol and its derivatives to parthenin with 1, 2, 3-triazole moiety.

   b. To characterise all the thymol-parthenin coupled derivatives with 1, 2, 3-triazole moiety using LC-QTOF (ESI), FT-IR, ^{1}H-NMR, and ^{13}C-NMR.

Summary

The various class of natural products are potential starting materials to be modified into semi-synthetic compounds with an enhanced biological activity. This research will concentrate on one of such class of natural products, the terpenoids. Thymol, which is a monoterpene and Parthenin, a sesquiterpene will be modified chemically into their derivatives. These derivatives will be characterised by a number of spectral analyses and also screen for their biological activity.

CHAPTER TWO

LITERATURE REVIEW

Introduction

Thymol, a monoterpene is a mild local irritant. It resembles phenol in its systemic actions, but less toxic, partly because it is less soluble. The less toxicity of thymol serves as an appropriate template for the development of other compounds. Thymol can be derivatised by the incooperation of the triazole nucleus which will make it more soluble in water and alcohol. Thymol can also serve as an alkylating agent on Parthenin through the use of the triazole nucleus as a linker which will lead to the discovery of many biologically active compounds.

Thymol

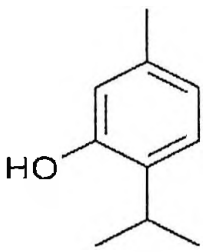
Thymol (also known as 2-isopropyl-5-methylphenol, IPMP) is a natural monoterpene phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with Carvacrol, found in oil of thyme, and extracted from *Thymus vulgaris* (common thyme) and various other kinds of plants as a white crystalline substance of a pleasant aromatic odor and strong antiseptic properties. Thymol also provides the distinctive, strong flavor of the culinary herb thyme, also produced from *T. vulgaris.* Thymol is a naturally occurring phenolic monoterpenoid. It was discovered by Neumann in 1719. It was purified in 1853 by M. Lalleman.

Thymol is an aroma compound present in the essential oil of *Nigella sativa L.* seeds. It is produced by these plant species as a chemical defence mechanism against phytopathogens (Vazquez, Fente, Franco, Vazquez & Cepeda, 2001). Therefore, this compound has attracted much attention in food industry, as it has been used in foods such as cheese as natural preservative to

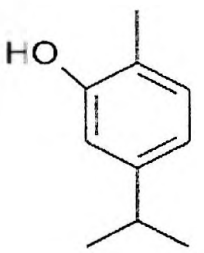
prevent fungal growth. (Juven *et al.*, 1994; Vazquez *et al.*, 2001; Venturini, Blanco & Oria, 2002). Thymol has also been used in medicine because of its pharmacological importance as antiseptic, antispasmodic, tonic and carminative (Didry, Dubreuil & Pinkas, 1994).

Isomers of Thymol

There are two isomers of thymol (iso-propyl-metacresol) and carvacrol (iso-propyl-orthocresol) that exist in nature.



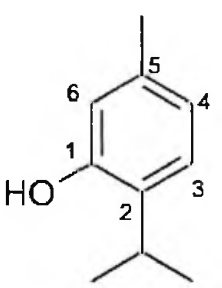
(Thymol)



(Carvacrol)

Nomenclature

Its common name is thyme camphor; thymic acid and trade name as thymocide; Topps. Nomenclature of thymol have been stated from the hydroxyl group and their IUPAC name is 5- Methyl-2-(1-Methylethyl) phenol or 2-isopropyl -5- methyl-1-hydroxy benzene.



Monograph of Thymol

Molecular Formula:- $C_{10}H_{14}O$, Molecular Weight (gm): 150.21, Composition: C = 79.95 %, H = 9.39 %, O = 10.65 %, Melting point: 51.5 ^{O}C, Boiling point: 233 ^{O}C, Density d425: 0.9699, Refractive index D25 : 1.5204,

Solubility: 1 g dissolves in 1000 mL water, 1 mL alcohol and 0.7 mL chloroform, 1.5 mL ether, 1.7 mL olive oil at 25 ^{O}C, Toxicity: Oral LD50 in rats, 980 mg/Kg, IR spectrum (cm^{-1}):- 3484, 1629, 1592, 1148, 1080, 940, 850, 805, PMR spectrum ($CDCl_3$, ppm) : 1.24 (d, 6H, 2–CH_3 gem.), 2.26(s, 3H, Ar.-CH_3), 3.20 (septate/m, 1H, >CH-), 4.78 (bs, 1H, -OH), 6.55, 6.75, 7.18 (s,d,d, 3H, Ar.-H), Mass spectrum m/e: 135 (100%), 150, 91, 117, 107, 105, 121.

Natural occurrence

Thymol is a naturally occurring monoterpenoid. It occurs in different plants in different concentration as follows:

Thyme (*Thymus vulgaris L*)

Thymol exists in oil of thyme. Thyme contains thymol in 30 – 40%. The air-dried leaves of four species viz Thymus species: *Thymus persicus*, *Thymus eriocalyx*, *Thymus daenensis* subsp. daenensis and *Thymus serpyllum L*. These plants found in Lorestan area in the western part of Iran contain thymol in 4.23 %, 66.34%, 10.38, 7.39% respectively concentrations (Sfaei-Ghomi *et al.*, 2009).

Carum copticum (Ajwan)

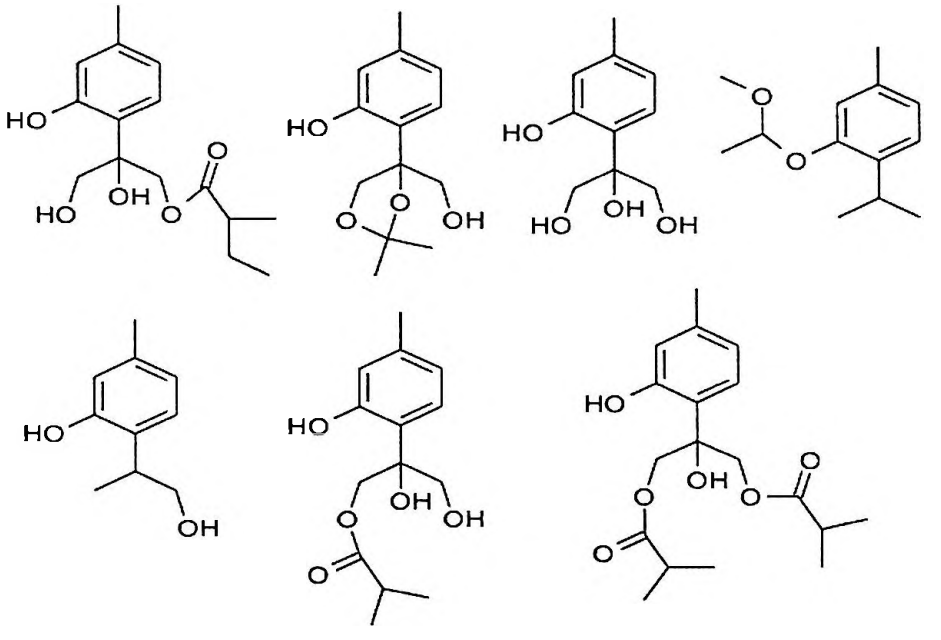
Carum copticum (Ajwan) contain thymol (35-60%) and some carvacrol. Thymol easily crystalizes from the oil on cooling and commonly known as Ajwain ka phool or Satajwain. The remainder of oil is called thymine on account of its similarity with the corresponding portion of *Thyme vulgaris* (Pathak *et al.*, 2010; Krishna, 1966).

Baccharisgrise bachii

The major constituents (concentrations higher than 3.5 %) were thymol (18.3%), thymol methyl ether (16.7%), thymyl acetate (10.9%), alpha pinene (7.2%), alpha humulene (7.2%) and globulol (3.7%) (Hadad *et al.,* 2007).

Centipeda minima

Two new monoterpenoids, 8, 10-dihydroxy-9(2) methylbutyryloxythymol and 10-hydroxy-8, 9-dioxyisopropylidene-thymol, together with five known thymol derivatives: 8,9,10 trihydroxythymol, thymol-β-glucopyranoside, 9-hydroxythymol, 8, 10- dihydroxy-9-isobutyryloxythymol, and 8-hydroxy-9, 10-diisobutyryloxythymol, were isolated from *Centipeda minima* (Liang *et al.,* 2007).

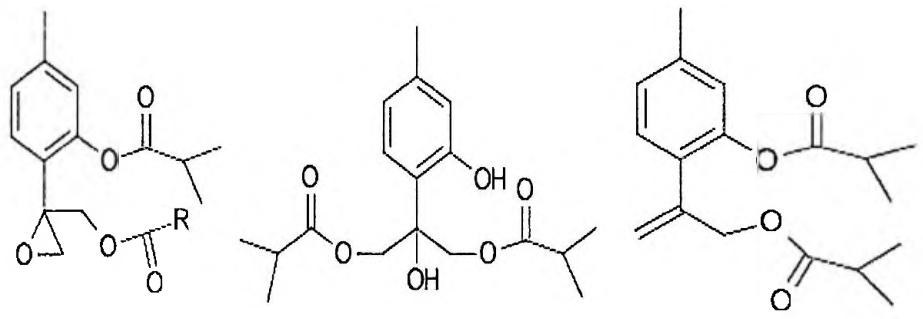


Inula cuspidata

Thymol, thymyl isobytarate, thymyl isovalerate and other constituents have been isolated from steam volatile extract of *Inula cuspidata* (Mathela, Tiwari, Padalia & Chanotiya, 2008).

Arnica montana

It is a perennial growing in the middle, southern, eastern parts of Europe. Five known thymol derivatives were isolated from roots of *Arnica montana* transformed with Agrobacterium rhizogenes LBA9402 (Weremczuk-Jeżyna, Kisiel & Wysokińska, 2006).



Biological Activity

Thymol is part of a naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with other biocides such as carvacrol. In addition, naturally-occurring biocidal agents such as thymol can reduce bacterial resistance to common drugs such as penicillin (Palaniappan & Holley, 2010). Numerous studies have demonstrated the antimicrobial effects of thymol, ranging from inducing antibiotic susceptibility in drug-resistant pathogens to powerful antioxidant properties (Ündeğer, Başaran, Degen & Başaran, 2009). Research demonstrates that naturally occurring biocides such as thymol and carvacrol reduce bacterial resistance to antibiotics through a synergistic effect, (Palaniappan & Holley, 2010) and thymol has been shown to be an effective fungicide,(Ahmad, Khan, Khan, Yousuf & Manzoor, 2010) particularly against fluconazole-resistant strains. This is especially relevant given that opportunistic Candida (fungus) infections can cause severe systemic infections in immunocompromised patients and current treatments are highly toxic, often result in drug-resistant

Candida strains, and have low efficacy. Compounds in the essential oils of one type of oregano have demonstrated antimutagenic effects, and in particular carvacrol (isomeric with thymol) and thymol were demonstrated to have a strong antimutagenic effect (Mezzouga *et al.,* 2007). In addition, there is evidence that thymol has antitumor properties (Anderson, 2006). Though the exact mechanism is unknown, there is evidence to suggest that thymol possesses at least some of its biocidal properties via membrane disruption (Trombetta *et al.,* 2005).

Studies have shown that thymol interacts with cell membranes. The interaction affects membrane permeability, and this has been documented by loss of membrane potential, cellular uptake of ethidium bromide, and leakage of potassium ions, ATP, and carboxyfluorescein (Helander *et al.*, 1998; Lambert, Skandamis, Coote & Nychas, 2001; Walsh, Maillard, Russell, Catrenich, Charbonneau & Bartolo, 2003; Xu, Zhou, Ji, Pei & Xu, 2008). Although the protective properties of lipopolysaccharide (LPS) against thymol had been confirmed using random transposon-insertion mutants, treatment of *E. coli* cells with thymol caused release of LPS and disruption of the outer membrane (Helander *et al.*, 1998; Shapira & Mimran, 2007). The outer membrane disruption could not be prevented by addition of magnesium, suggesting that thymol did not disrupt the membrane by chelating cations (Helander *et al.*, 1998). Thymol integrates at the polar head-group region of a lipid bilayer causing alterations to the cell membrane, which at low concentrations induce adaptational changes in the membrane lipid profile in order to compensate for thymol's fluidifying effects and to maintain the membrane function and structure (Turina, Nolan, Zygadlo & Perillo, 2006; Di

Pasqua, Betts, Hoskins, Edwards, Ercolini & Mauriello, 2007). In addition to interacting with membrane phospholipids, evidence has accumulated that documents thymol's interaction with membrane proteins and intracellular targets, which hinder cell recovery after temporary exposure. The ability of thymol to interact with proteins was examined using the protein bovine serum albumin (BSA) and the organic compound deferoxamine, which is also rich in amine groups but otherwise known for its Fe^{3+}chelating properties. These compounds react similarly to that of amine groups in bacterial membrane proteins (Juven *et al.*, 1994).

Based on the antimicrobial activity of thymol in the absence and presence of the thymol-inhibiting deferoxamine or BSA (Juven *et al.,* 1994), it was hypothesized that thymol forms a complex with membrane-bound or periplasmic proteins by means of hydrogen bonds and hydrophobic interactions. Interaction with membrane proteins was further supported by Di Pasqua, Mamone, Ferranti, Ercolini and Mauriello (2010) who exposed *Salmonella enterica* to sub-lethal concentrations of thymol, and observed accumulation of misfolded outer membrane proteins and upregulation of genes involved in synthesis of outer membrane proteins. Contrarily, down-regulation of outer membrane proteins was shown in Erwinia spp. (Horváth, Kovács, Kocsis, & Kustos, 2009). Upon exposure to thymol, *S. enterica* upregulated production of the chaperon proteins Heat Shock Protein 60 (GroEL), and Heat Shock Protein 70 (DnaK), which are key proteins in the protection against thermal stress and misfolding of proteins (Di Pasqua *et al.*, 2010; Hartl, Bracher & Hayer-Hartl, 2011). Thymol also impaired the citrate metabolic

pathway and affected many enzymes directly or indirectly involved in the synthesis of ATP (Di Pasqua *et al.*, 2010).

Thymol's intracellular action indicates that it affects important energy-generating processes, which lower a cells' ability to recover after exposure to thymol. The mode of action of thymol against yeast and fungi has been sparsely investigated, but studies point to interactions with the cell envelope and intracellular targets. Thymol disrupted vesicles and cell membranes, and impaired ergosterol biosynthesis in Candida strains, which consequently affected cell membrane integrity because ergosterol regulates membrane fluidity and asymmetry similarly to cholesterol in animal cells (Ghannoum & Rice, 1999; Cristani *et al.,* 2007; Ahmad *et al.*, 2011). Interestingly, thymol induced cell lysis and only altered the cell structure of proliferating *S. cerevisiae* cells, indicating the effect of thymol depends on cell proliferation (Bennis, Chami, Chami, Bouchikhi & Remmal, 2004). Contrary to this, Rao *et al.* (2010) proposed that thymol activates specific signalling pathways in yeast, rather than causing non-specific lesion of membranes. This proposal was based on the observation that thymol caused cytosolic Ca^{2+} bursts and transcription responses similar to Ca^{2+} stress and nutrient starvation (Rao *et al.*, 2010). Irrespective of such biological and pharmacological activities, its use is limited, because of its poor aqueous solubility (1 g in 1 L) (The Merck index2006), sublimation, photo reactivity and poor heat sensitivity (Ghosheh, Houdi & Crooks, 1999).

Uses

Thymol has been used in alcohol solutions and in dusting powders for the treatment of tinea or ringworm infections, and was used in the United States to

treat hookworm infections. It is also used as a preservative in halothane, an anaesthetic, and as an antiseptic in mouthwash. When used to reduce plaque and gingivitis, thymol has been found to be more effective when used in combination with chlorhexidine than when used purely by itself (Filoche, Soma & Sissons, 2005).Thymol is also the active antiseptic ingredient in some toothpastes, such as Euthymol.

There is evidence supporting the belief that thymol, when applied two to three time's daily, can eliminate certain kinds of fungal infections that affect fingernails and toenails in humans. Regular application to the affected nail over periods of about three months has been shown to eliminate the affliction by effectively preventing further progress by simply cutting the nail as one normally would, all infected material is eventually eliminated. The antifungal nature of thymol is caused by thymol's ability to alter in the hyphal morphology and cause hyphal aggregates, resulting in reduced hyphal diameters and lyses of hyphal wall (Numpaque, Oviedo, Gil, García & Durango, 2011). Additionally, thymol is lipophilic, enabling it to interact with the cell membrane of fungus cells, altering cell membrane permeability permitting the loss of macromolecules (Šegvić Klarić, Kosalec, Mastelić, Piecková & Pepeljnak, 2007).

Recent medical research on rats concludes that "Thyme extract had relaxing effects on organs possessing β2-receptors (uterus and trachea) (Wienkötter, Begrow, Kinzinger, Schierstedt & Verspohl, 2007). In a 1994 report released by five major cigarette companies, thymol was listed as one of 599 additives to cigarettes.

Thymol has been used to successfully control varroa mites and prevent fermentation and the growth of mold in bee colonies, methods developed by beekeeper R.O.B. Manley. Thymol is also used as a rapidly degrading, non-persisting pesticide (Hu & Coats, 2008).

Derivatives of thymol and carvacrol with increased antimicrobial activities have been developed (Mathela, Singh & Gupta, 2010).The preparation of methacrylic and p-styrenesulfonic acid esters of thymol could lead to less toxic macromolecular biocides, which can be attached to a polymeric backbone (Moszner, Salz & Rheinberger, 1994). A minor use of thymol is in book and paper conservation: Paper with mold damage can be sealed in bags with thymol crystals to kill fungal spores. However, this practice is not currently recommended due to apparent accelerated degradation suffered by these objects.

Thymol—named after the herb itself—is the primary volatile oil constituent of thyme, and its health-supporting effects are well documented. In studies on aging in rats, thymol has been found to protect and significantly increase the percentage of healthy fats found in cell membranes and other cell structures. In particular, the amount of DHA (docosahexaenoic acid, an omega-3 fatty acid) in brain, kidney, and heart cell membranes was increased after dietary supplementation with thyme. In other studies looking more closely at changes in the brains cells themselves, researchers found that the maximum benefits of thyme occurred when the food was introduced very early in the lifecycle of the rats, but was less effective in offsetting the problems in brain cell aging when introduced late in the aging process.

Thyme also contains a variety of flavonoids, including apigenin, naringenin, luteolin, and thymonin. These flavonoids increase thyme's antioxidant capacity, and combined with its status as a very good source of manganese, give thyme a high standing on the list of anti-oxidant foods.

Synthesis of Thymol

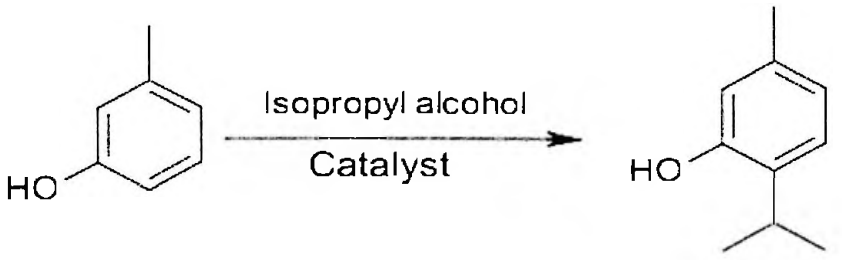
Various synthetic methods have been documented for thymol. It can be synthesised from a variety of starting materials and a few of them are listed as follows:

Preparation from m-cresol

M-cresol is the most commonly used starting material for the synthesis of thymol.

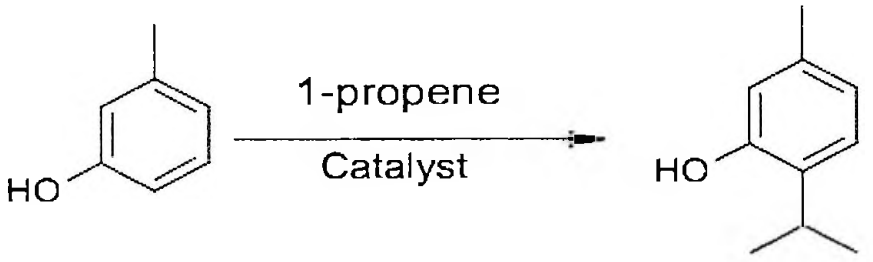
Reaction of m-cresol with Alcohol

M-cresol reacts with isopropene in the presence of acidic catalyst (Yamanaka, 1976), Magnesium Aluminium hydrotalcites (MgAl-HTS) (Grabowska, Mišta, Trawczyński, Wrzyszcz & Zawadzki, 2001), Zinc Aluminate spinel (Zn Al_2O_4 spinel) (Amandi, Hyde, Ross, Lotz & Poliakoff, 2005), γ – Al_2O_3 and supercritical CO_2, $scCO_2$ (Grabowska & Wrzyszcz, 2001) to give thymol. Alkylation of m-cresol with n- and iso propanol in the presence of a catalyst that contains oxides of Fe, Si, Cr and K (Ali & Gaikar, 2011). A continuous method for the preparation of thymol was developed, using a carbonized sulfonic acid (CSA) resin as the catalyst, under the influence of microwave (Benjamin, Nogbou, Ado, Azzaro-Pantel & Davin, 2007).



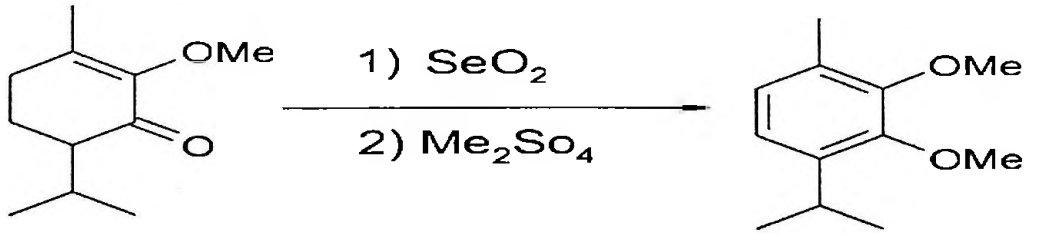
Reaction of m-cresol with Propene

Synthesis of thymol is also carried out by the Friedal Craft alkylation of m- cresol with propene in the presence of $Fe_2 (SO_4)_3/\gamma\text{-}Al_2O_3$ (Wimmer & Buysch, 1991). Zeolites of Li, K, Mg, Cu, Zn, Ca, Ti, Zr, Sn, Cr, Fe, Mn, Co, Ni as a catalyst (Phillips, 1920).



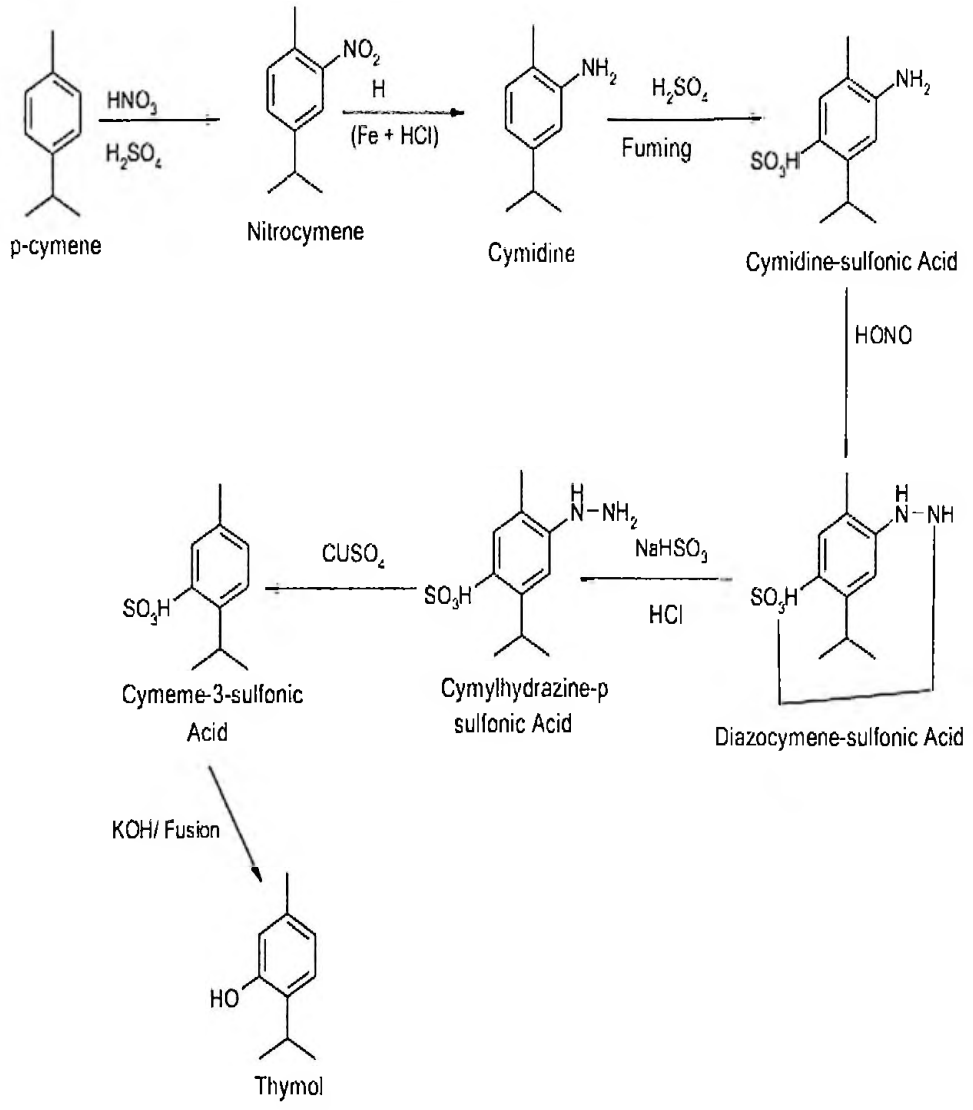
Preparation of 2-methoxymethyl Thymyl Ether

The methyl ether was dehydrogenated with Selenium dioxide and then by methylation with dimethylsulphate to get 2, 3 dimethoxy –p-cumene (Mathela *et al.*, 2010).



Preparation from p- Cymene

Max Phillips has reported synthesis of thymol from p-cymene in seven steps. The p-cymene has been found suitable starting material for this method due to better yield at each step of reaction (Wahidullah & Paknikar, 1988).



Reactions of Thymol

The thymol have a particularly well studied chemistry with certain exceptions, most efforts have been expended on their preparation. However, some reactions have received attention, and these are summarised as follows:

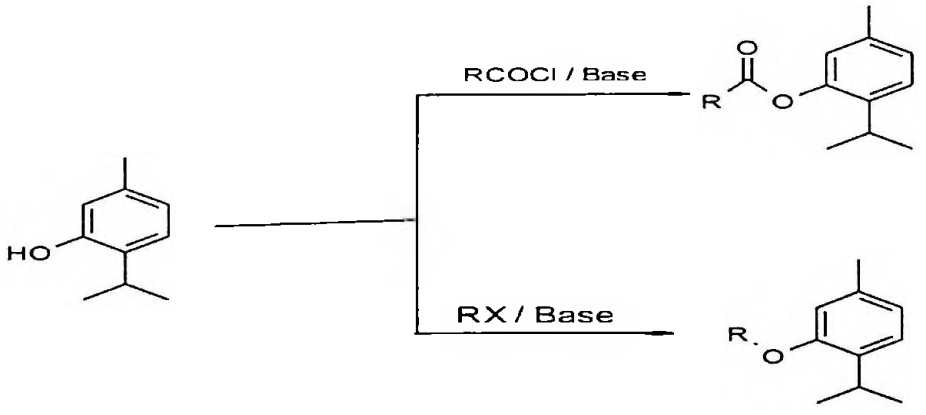
O-alkylation and O-acylation

Various types of esters of thymol have been synthesised using triethyl amine and DCM at $0^{O}C$ (Kank, Rho, Hwang & OH, 2003). The esters viz hydroxyl/ alkoxybenzoates, and 3, 4, 5-trimethoxycinnamate containing thymol moiety exhibits high-level inhibitory activity against melanin synthesis in cultured melanocytes (Kumbhar & Dewang, 2001). Ether and ester

derivatives of thymol were synthesised having antifungal potency (Kumbhar & Dewang, 2001; Satzinger & Herramann, 1976). Some of natural esters like thymyl isobutarate and thymyl isovalerate were synthesised in the presence of triethyl amine (Mathela *et al.,* 2008). Ester of 4-hydroxy of novel ether of thymol was reported by G. Satzinger (More, Pawar, Dewang, Patil & Mahulikar, 2004).

Thymyl ether and ester can also be synthesised by using polymer support reaction by activated Amberlite IRA-400 (Goankar & Kirtany, 1991; Varma & Narayanan, 1985). Thymyl ether and ester were synthesised from thymol with different alkyl and acyl halide in the microwave fly ash as a support (Goankar & Kirtany, 1991; Varma & Narayanan, 1985).

6-Isopropyl-3-methylphenoxyacetic acid (Thymoxyacetic acid) can be synthesised by reaction of sodium salt of thymol with chloroacetic acid (Singh, Shukla, Dwivedi & Khanna, 1989).

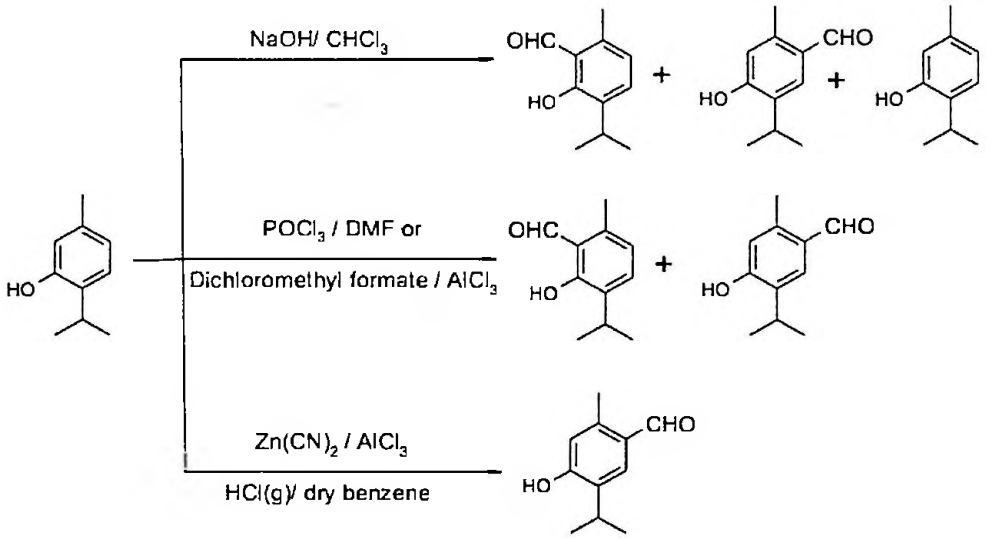


Formylation

Different types of formylation method have been reported. Formylation of thymol by the Reimer- Tiemann reaction gives the ortho and para isomer with some unreacted Thymol (Bell & Henry, 1928). It also undergoes Reimer-Tiemann reaction in the presence of carbon tetrachloride gives 4-thymotic acid

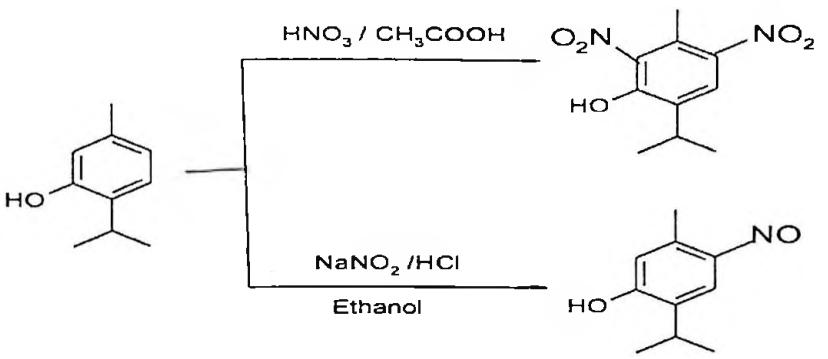
(Adams & Levine, 1923). Similar reaction of thymol with $POCl_3$ in DMF (formylation), dichloromethyl formate in DCM in the presence of $AlCl_3$ as a catalyst also gives the ortho and para formyl thymol with different yield.

Gatermann formylation was also carried out which involves the reaction of thymol with zinc cyanide, $AlCl_3$, in dry benzene by the continuous flow of dry HCl gas giving only 4-formyl thymol in quantitative yield. HCN which is very poisonous is formed; therefore this method was less adopted (Osorio, Arango, Robledo, Munoz, Jaramillo & Velez, 2007).



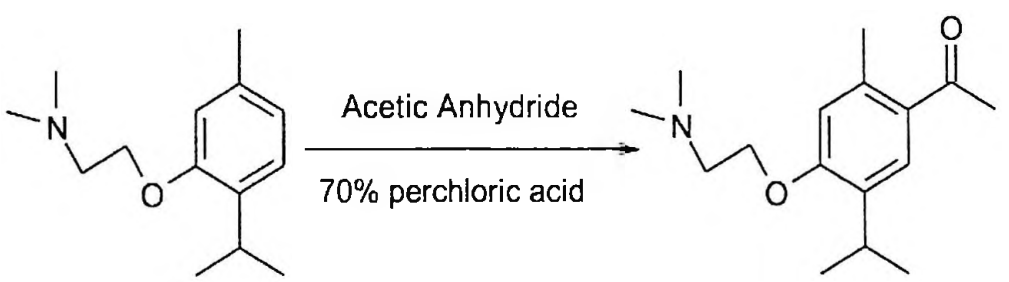
Nitration

Nitration of thymol by the use of HNO_3 and acetic acid gives 6-isopropyl-3-methyl-2, 4-dinitro-phenol (Robledo *et al.*, 2005). Sara Robledo and co-workers synthesised the 4-nitroso thymol by reaction of sodium nitrite on thymol in the presence of concentrated HCl to give 6-isopropyl-3-methyl-4-nitroso-phenol in good yield (Vashi & Shah, 1996). It was further used for the synthesis of 6-isopropyl-3-methyl-4-amino-phenol by H_2S (g) /Aqueous NH_3 (Monza, Belli & Novara, 1982).



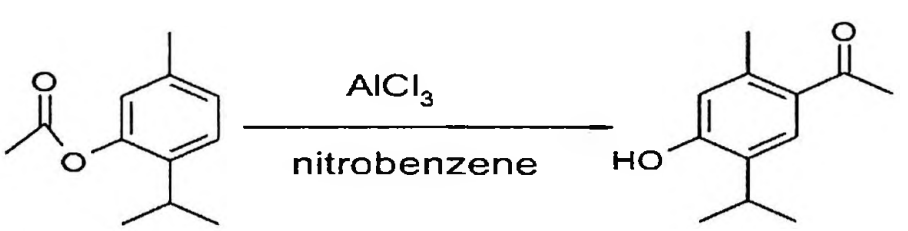
Friedal- Craft Acylation

2-(2-isopropyl-5-methylphenoxy)-N, N-dimethylethanamine undergoes Friedal Craft acylation by acetic anhydride and perchloric acid as catalyst to give 1-(4-(2 (dimethylamino) ethoxy)-5-isopropyl-2-methylphenyl) ethanone (Furka & Szell, 1960).

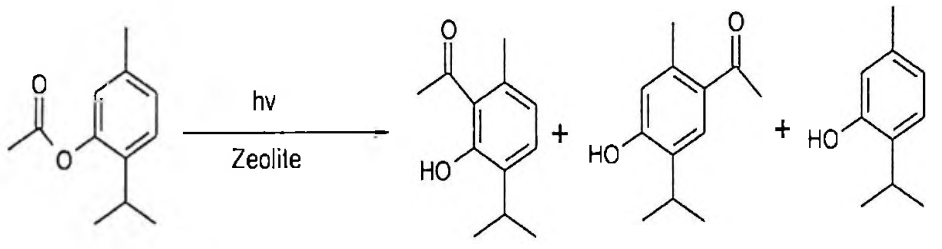


Fries- Rearrangement

Thymol undergoes Fries rearrangement e.g. 2-isopropyl-5-methylphenyl acetate, which on Fries rearrangement in the presence of anhydrous $AlCl_3$ and nitrobenzene at room temperature afforded 1-(4-hydroxy-5-isopropyl-2-methylphenyl) ethanone in good yield (Suau, Torres & Valpuesta, 1995).

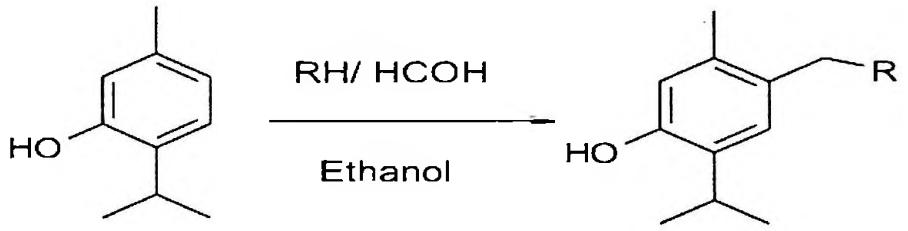


The compound 2-isopropyl-5-methylphenyl acetate undergoes photo fries with zeolite as a catalyst to give a mixture of 1-[4-hydroxy-5-isopropyl-2-methylphenyl] ethanone, 1-(4-hydroxy-5-isopropyl-2-methylphenyl) ethanone and thymol (Shen, Huang, Liao & Wang, 2005).



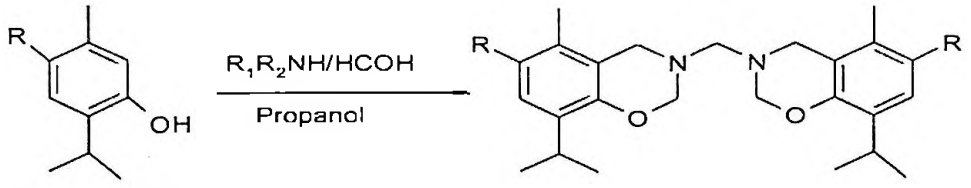
Mannich Reactions

Thymol has been reported to undergo Mannich reactions with formaldehyde solution and morphaline or pyrrolidine forming 4-Morpholinomethyl-2-isopropyl-5-methylphenol and 4-pyrrolidine-2-isopropyl-5-methylphenol respectively (Dwivedi, Shukla, Bhandari, Setty, Kamboj & Khanna, 1991).



1, 3- Oxazine Reaction

Reaction of thymol or 4-formyl thymol with paraformaldehye and primary amine gave the corresponding substituted 1,3 benamines whereas similar reaction involving primary amine and thymol/ 4-formylthymol gave the corresponding substituted bis-(1, 3) –benzoxazines (Kamat & Paknikar, 1981)



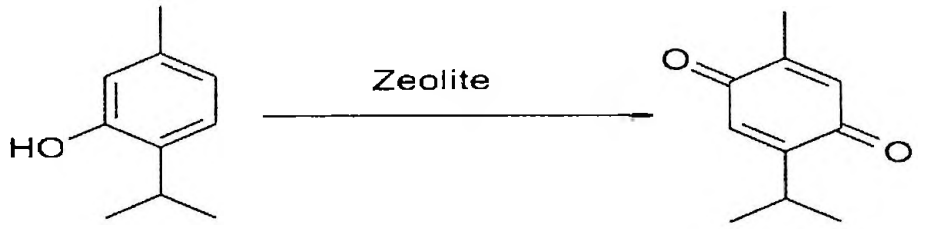
Where R: H, COH

Duff Reaction

Thymol on reaction with hexamine and acetic acid gives o-thymoldehyde (Madadi & Rahimi, 2010).

Oxidation

Alev Gune have synthesised the thymoquinone by oxidation of thymol with zeolite (Koshti *et al.,* 2008).



Diazotization

Thymol easily undergo diazotization reaction with various aromatic amines to give (E)-4-(2- aryldiazenyl)-2-isopropyl-5-methylphenol (Nath, Sethi, Srivastava, Jain & Srivastava, 1997).

Pharmacology of Thymol and its Derivatives

Thymol exhibits a number of pharmacological activities which are listed as follows:

Anti-lithiatic Properties

T. ammi is among a list of 14 indigenous medicinal plants that were reported to have been used for abortion as well as been investigated for the oestrogenic content of some herbs. These were traditionally used to increase milk yield in dairy cattle. The *T. ammi* has also been traditionally used as a

galactogogue in humans (Dwivedi, S.K & Dubey N.K., 1993; Srivastava K.C., 1988).

Anti-hyperlipidaemic Activity

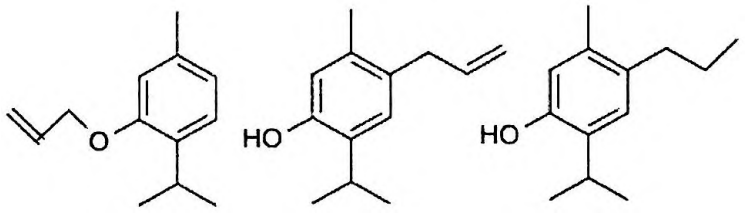
Trachyspermum ammi is reported to have platelet aggregation inhibitory action, (Aftab, Atta-Ur-Rahman & Usmanghani, 1995) antifungal potency (Arrigoni, 1977) and blood pressure lowering action (Azuma, Ozasa, Ueda & Takagi, 1986). Antihyperlipidaemic effect of *T.ammi* seed have been observed in albino rabbits (Dwivedi & Dubey, 1993).

Anti-inflammatory Effects

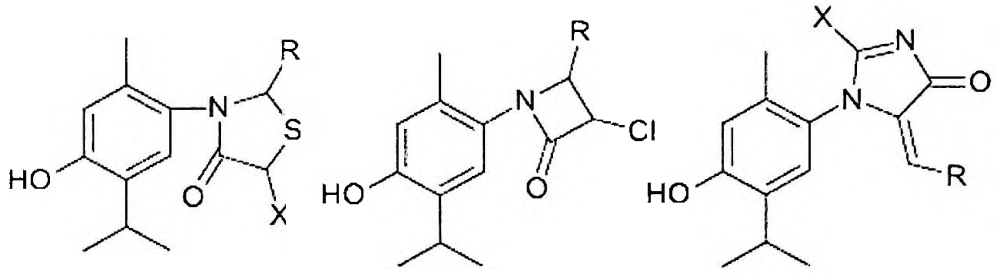
Extract of *Carum copticum. Linn* containing thymol shows anti-inflammatory activity by affecting kinnin, prostaglandin, bradykinin and lysozyme syntheses (Dwivedi & Dubey, 1993) and by inhibition of leukocyte chemotaxis (Braga, Dal Sasso, Culici, Bianchi, Bordoni & Marabini, 2006). It has also helpful effects in controlling the inflammatory processes present in many infections, inhibiting fMLP-induced elastase release in a concentration dependent manner (Lupo *et al.,* 2000).

Antibacterial Activity

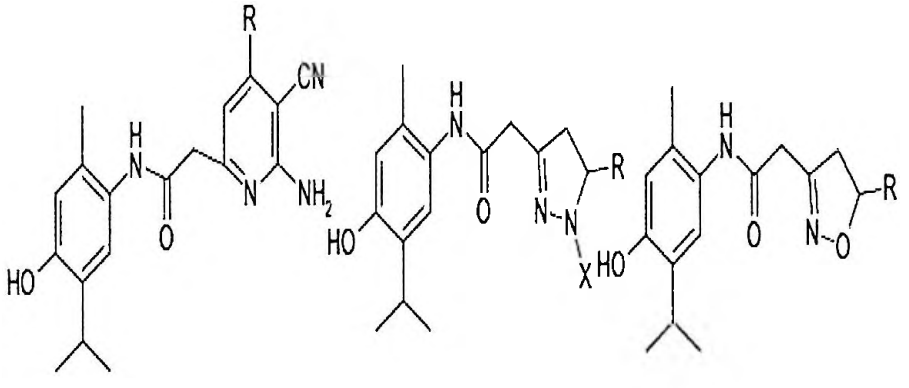
A. T. Lupo and co-workers have shown that modified thymol derivatives below show good antibacterial activity against *S. aureus* 6538, *E.coli* 11229 (Vashi, Mehta & Shah, 1995).



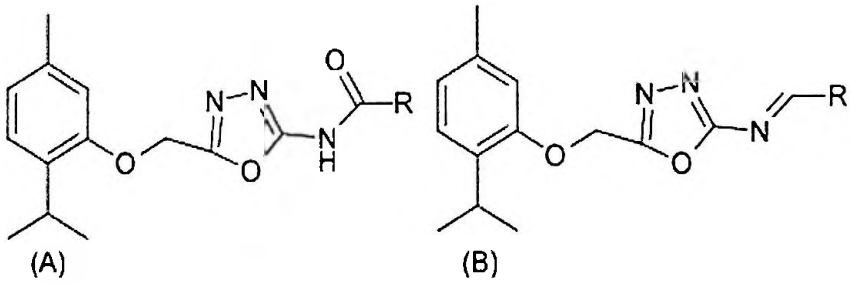
Also, 4-thiazolidinones, 2-azetidinones, and 4-imidazolinone derivatives derived from 4-nitroso thymol show moderate to good antimicrobial and anti tuberculostatic activity (Desai & Shah, 2003).



Cyanopyridine, isoxazole, pyrazoline derivatives were synthesised and screened for antimicrobial activity (Roda & Vansdadia, 1988).



Roda, Vansdadia & Parekh have reported the syntheses and antimicrobial activity of 1,3,4 oxadiazole derivatives of thymol (Khanuja, 2004).



(A) (B)

Antibiotic Resistant Antibacterial Activity

"Thymol" kills the bacteria resistant to even prevalent third generation antibiotics and multi drug resistant (mdr) microbial pathogens and thus useful as a plant based fourth generation herbal antibiotic formulation

(Gallucci, Casero, Oliva, Zygadlo & Demo, 2006). Qiu *et al.* show that thymol has promising activity against the antibiolic resistant *S. aurous* (Qiu *et al.*, 2010; Tassou, Koutsoumanis & Nychas, 2000).

Antifungal Activity

The Ajwain Ethanol Extract (AEE), was assessed for antibacterial and antifungal activity against selected pathogenic bacteria and fungi (Juglal, Govinden & Odhav, 2002). The antifungal activity of the AEE was studied by agar well assay against various fungi *A.flavus, A. ochraceus, A. niger, A. orzyae, Fusarium moniliforme, Penicillium* sp. (Guo *et al.*, 2009). Thymol was found to have *in vitro* antifungal activity against 24 fluconazole (FLC)-resistant and 12 FLC-susceptible clinical isolates of Candida albicans, standard strain ATCC 10231 and one experimentally induced FLC-resistant (Rasooli, Fakoor, Allameh, Rezaee & Owlia, 2009). The inhibitory effects of the *Thymus kotschyanus* and *Zataria multiflora Boiss* against *Aspergillus parasiticus* were tested. It is well known that a phenolic-OH group is very reactive and can easily form hydrogen bonds with the active sites of enzymes (Pelczar, Chan, & Krieg, 1988).

Nematicidal Activity

Thymol and carvacrol were very effective against Pin Wilt Nematode (PWN) by interfering with the neuromodulator octopamine (Guo *et al.*, 2009) or GABA-gated chloride channels of insect pests which are used as a potent nematicidal agent (Kostyukovsky, Rafaeli, Gileadi, Demchenko & Shaaya, 2002; Singh, Maurya, Catalan & De Lampasona, 2004; Kong, Lee, Moon, Lee & Ahn, 2006; Choi, Shin & Park, 2007; Murthy, Borse, Khanum & Srinivas, 2009).

Anthelmintic Activity

Thymol might exert its anthelmintic activity by interference with the energy metabolism of parasites through potentiation of ATPase activity and thus loss of energy reserves (Priestley, Williamson, Wafford & Sattelle, 2003). The first scientific evidence of anthelmintic activity of *T. ammi* in mixed natural helminth infestations in animals, although preliminary studies of its effect against specific helminths, e.g. *Ascaris lumbricoides* in humans and *Haemonchus contortus* in sheep, have been reported (Park, Kim, Lee & Shin, 2007; Tamurab & Iwamoto, 2004). The plant has also been reported to possess cholinergic activity, which might also be a contributory factor to its anthelmintic activity, with added effect from the known facilitatory effect of cholinergic agents on the peristaltic movements of the gut, thus helping in expulsion of intestinal parasites (Jabbar, Iqbal & Khan, 2006; Patel & Srinivasan, 2001).

Digestive Stimulant Actions

Thymol reduced food transit time and enhanced the activity of digestive enzymes (Sethi & Singh, 1989).

Abortifacient Activity

T. ammi that contains thymol effective as an abortifacient. In cases where pregnancy was continued in spite of herbal drug administration, foetuses showed various skeletal defects and several other visceral defects; they expressed concern at the remarkable potential of the putative abortifacient herbal drugs to affect foetuses adversely (Gilani, Jabeen, Ghayur, Janbaz & Akhtar, 2005).

Hypotensive Activity

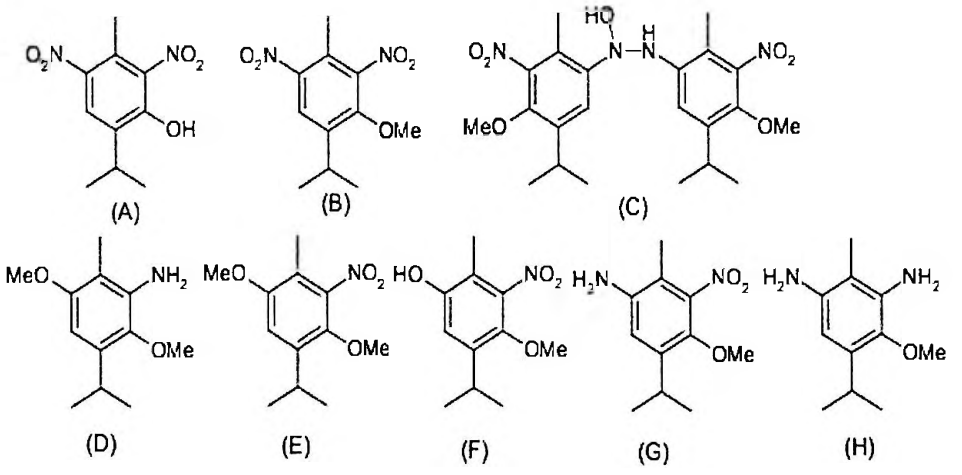
The *T. ammi* extract administered *Intra* veinas was found to have hypotensive effect (Velazhahan *et al.*, 2010).

Detoxification of Aflatoxins by *Trachyspermum ammi*

Thymol were evaluated for their ability to detoxify aflatoxin G1 (AFG1) by enzyme-linked immunosorbent assay (ELISA) (Osorio *et al.*, 2007).

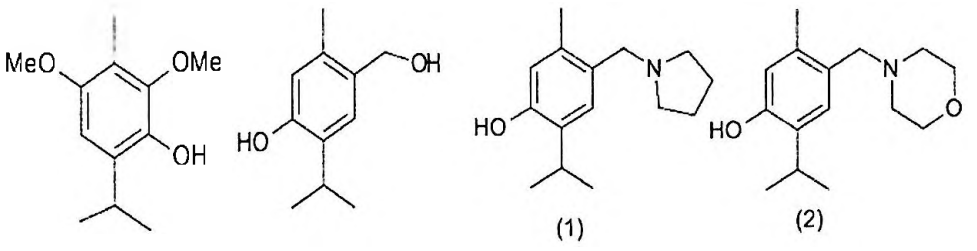
Antileishmanial and Cytitoxic Effect

Edison Osorio *et al* suggest that thymol and its synthetic derivatives A-H may be the leading compounds of anti-leishmanial and it also shows less toxicity against U-9937 (Osorio *et al.*, 2007; Rojano *et al.*, 2008; Robledo *et al.*, 2005).



Antioxidant

Benjamin Rojano and coorworker demonstrated that isoespintanol is a potential anti-oxidant because of the high stability of its radicals (Rojano *et al.*, 2008; Nagle, Pawar, Sonawane, Bhosale & More, 2011).

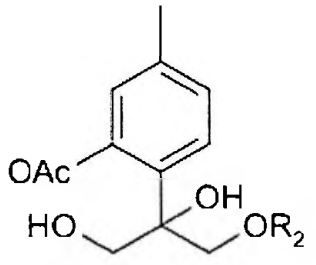


(1) (2)

4-[hydroxymethyl]-5-isopropyl-2-methyl phenol shows remarkably better antioxidant properties by DPPH method. Shen, Huang, Liao, & Wang report the synthesis and antioxidant activity of Compounds (1) & (2) above (Kamat & Paknikar, 1981). Pramod Nagle and coworkers report the synthesis and antioxidant activity of 2-pyridone of Thymol (Ok *et al.*, 2001).

Anticancer

Thymol derivative below show promising antitumor activity (He, Mo, Hadisusilo, Qureshi & Elson, 1997).



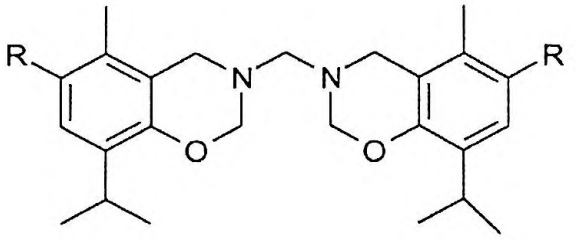
The study estimated that thymol required structurally diverse isoprenoids to inhibit the increase in a population of murine B16 (F10) melanoma cells (Magyar, 2003).

Antihypertensive

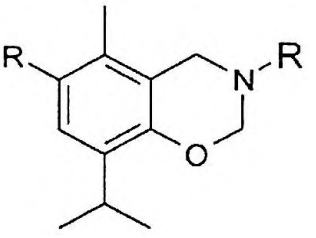
Jonos Magyar from Debrecen University report that thymol show significant antihypertensive effect on human ventricular cells (Singh *et al.*, 1989).

Spermicides

1, 3-benzoxazines derivative of thymol display potential spermicidal activity by A. K. Dawadi (Ok *et al.*, 2001). Two derivatives of thymol gossypol [1] and hemigossypol [2] shown below have been used as potential male antifertility agent in all over the world (Kank *et al.*, 2003).



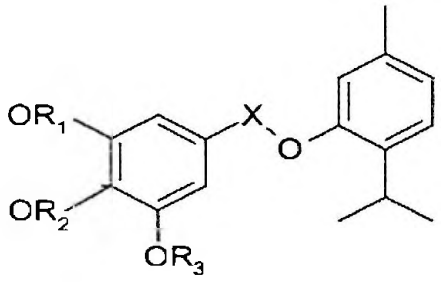
[1]



[2]

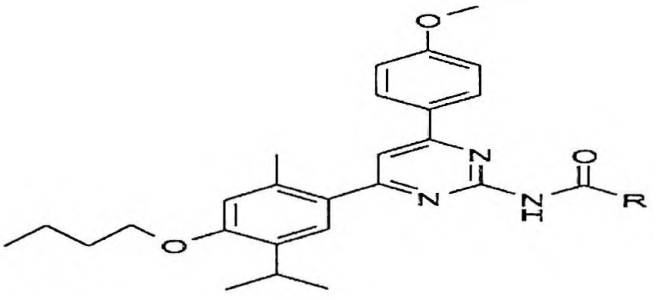
Depigmenting Activity

Alkoxy benzoates or alkoxy cinnamates of thymol show good to moderate depigmenting activity with low toxicity (Nagle *et al.*, 2011).



Adenosine A1 Receptor Antangonist

Nagle and coworkers report the 2, 4, 6 trisubstituted pyrimidine containing the thymol nucleus as a Selective Adenosine A1 Receptor Antagonists docking study (Nagle, Pawar, Sonawane, Bhosale & More, 2012; Leonardi, Riva, De Toma, Boi, Pennini & Sironi, 1994).



α_1 Adrenoreceptor Antagonist and Uroselectivity

Leonardi A. *et al* report that thymol and its derivatives have good affinity for α1, α2, 5HT1A, 5HT2 and D2 receptors (Leonardi *et al.*, 1994).

Biotransformation and Antifungal Activity.

Stem-end rot (*Botryodiplodia theobromae*) and anthracnose (*Colletotrichum acutatum*) are two serious diseases that contribute significantly to harvest and postharvest loss of tamarillo, avocado, mango, papaya, and citrus in Colombia (Afanador-Kafurì, Minz, Maymon & Freeman, 2003; Martínez, Hío, Osorio & Torres, 2009). The use of synthetic chemicals as fungicides is the primary method of control of postharvest fungal decay caused by both diseases. However, the rapid development of tolerance to commercial fungicides by *C. acutatum* and *B. theobromae* has led to an increase in the quantities of these compounds that have to be used. Consequently, the presence of fungicide residues on the fruits decreases their quality and can prevent their export to some foreign markets (Tripathi & Dubey, 2004). Furthermore, the use of synthetic chemicals to control pre- and postharvest deterioration of food commodities is restricted, due to their possible carcinogenicity, teratogenicity, acute toxicity, environmental pollution and side effects on human beings (Tripathi & Shukla, 2007). Therefore, the fruit industry urgently demands alternative pre- and postharvest treatments that are free of synthetic fungicides and acceptable to consumers.

Given these facts, the use of essential oils and some of their constituents can be a very attractive method for pre- and postharvest disease control of fruits, due to their relative safety and wide acceptance by consumers (Ormancey, Sisalli & Coutiere, 2001). Carvacrol (5-isopropyl-2-methylphenol) and thymol (2-isopropyl-5-methylphenol) are the main components of the essential oils of some Laminaceae members like oregano, thyme, and savory. They are produced by these plant species as a chemical defense mechanism against phytopathogenic microorganisms (Vázquez *et al.,* 2001). Accordingly, the potent antimicrobial and fungitoxic properties of Carvacrol and Thymol against various plant pathogens have been previously documented (Sokovic, Tzakou, Pitarokili & Couladis, 2002; Falcone, Speranza, Del Nobile, Corbo & Sinigaglia, 2005). It has been found that these agents cause alterations in the hyphal morphology and hyphal aggregates, resulting in reduced hyphal diameters and lyses of hyphal wall, as these interact with the cell membrane of the pathogen (Soylu, Yigitbas, Soylu & Kurt, 2007). In addition, chemical modification of these phenolic compounds to various ether and ester derivatives has been reported to result in change in biological activity (Mathela *et al.,* 2010).

Despite their antimicrobial characteristics, Chamberlain & Dagley (1968) found a *Pseudomonas* strain able to degrade thymol completely and carvacrol partially. The authors proposed a metabolic pathway for thymol that involves *meta*-ring opening of a trihydric phenol, 3-hydroxythymo-1, 4-quinol to 3, 7-di-methyl-2, 4, 6-trioxo-octanoate. Hydrolysis of the latter, catalyzed by β-ketolase, yields acetate, 2-ketobutyrate and isobutyrate. Thus, biotransformation experiments provide information on the detoxification

mechanism used by phytopathogenic microorganisms and give an indication of the structural modifications that may be necessary if substrates of this type are to be further developed as selective fungal control agents (Daoubi, Hernández-Galán, Benharref & Collado, *2005).*

Essential Oils

Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavour or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. Essential oils are found in glandular hairs or secretory cavities of plant-cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots and/or fruits in different plants. The aromatic characteristics of essential oils provide various functions for the plants including (i) attracting or repelling insects, (ii) protecting themselves from heat or cold; and (iii) utilizing chemical constituents in the oil as defence materials. Many of the essential oils have other uses as food additives, flavourings, and components of cosmetics, soaps, perfumes, plastics, and as resins. Typically these oils are liquid at room temperature and get easily transformed from a liquid to a gaseous state at room or slightly higher temperature without undergoing decomposition. The amount of essential oil found in most plants is 1 to 2%, but can contain amounts ranging from 0.01 to 10%. For example, orange tree produces different composition of oils in their blossoms, citrus fruits, and/or leaves. In certain plants, one main essential oil constituent may predominate while in others it is a cocktail of various terpenes. In *Ocimum basilicum* (Basil), for example, methyl chavicol makes up 75% of the oil, β-asarone amounts to 70–80% in *Acorus calamus* rhizomes,

linalool , in the range of 50– 60%, occurs in coriander seed and leaf oils procured from different locations at different time intervals and is by far the most predominant constituent followed by p-cymene, terpinene, camphor and limonene. Interestingly 2-decenol and decanal were the most predominant constituents in leaf oil (Lawrence & Reynolds, 2001). However, in other species there is no single component which predominates. Most essential oils comprise of monoterpenes compounds that contain 10 carbon atoms often arranged in a ring or in acyclic form, as well as sesquiterpenes which are hydrocarbons comprising of 15 carbon atoms. Higher terpenes may also be present as minor constituents. The most predominant groups are cyclic compounds with saturated or unsaturated hexacyclic or an aromatic system. Bicyclic (1, 8-cineole) and acyclic (linalool, citronellal) examples also make the components of essential oils. However, intraspecific variability in chemical composition does exist, which is relative to ecotypic variations and chemotypic races or populations.

Essential Oils as Green Pesticides

Naturally green concept suggests the avoidance of use of any pesticide via public education and awareness-raising program, developed to inform public about the potential risk of pesticide use and alternatives that are available. In fact, such programs support the policy of "prudent avoidance".

Essential oils are usually obtained via steam distillation of aromatic plants, specifically those used as fragrances and flavourings in the perfume and food industries, respectively, and more recently for aromatherapy and as herbal medicines. Plant essential oils are produced commercially from several botanical sources, many of which are members of the mint family

(Lamiaceae). The oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes. Examples include 1,8-cineole, the major constituent of oils from rosemary and eucalyptus; eugenol from clove oil; thymol from garden thyme; menthol from various species of mint; asarones from calamus; and carvacrol and linalool from many plant species. A number of source plants have been traditionally used for protection of stored commodities, especially in the Mediterranean region and in Southern Asia, but interest in the oils was renewed with emerging demonstration of their fumigant and contact insecticidal activities to a wide range of pests in the 1990s (Isman, 2000). The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (Kostyukovsky *et al.*, 2002) by some oils and with GABA-gated chloride channels by others (Priestley *et al.*, 2003). The purified terpenoid constituents of essential oils are moderately toxic to mammals (Table 1), but, with few exceptions, the oils themselves or products based on oils are mostly nontoxic to mammals, birds, and fish (Stroh, Wan, Isman & Moul, 1998), therefore, justifying their placement under "green pesticides". Owing to their volatility, essential oils have limited persistence under field conditions; therefore, although natural enemies are susceptible via direct contact, predators and parasitoids reinvading a treated crop one or more days after treatment are unlikely to be poisoned by residue contact as often occurs with conventional insecticides (Koul *et al.*, 2008).

In fact, effects on natural enemies have yet to be evaluated under field conditions. Recent evidence for an octopaminergic mode-of-action for certain

monoterpenoids (Bischof & Enan 2004; Kostyukovsky *et al.*, 2002), combined with their relative chemical simplicity may yet find these natural products useful as lead structures for the discovery of new neurotoxic insecticides with good mammalian selectivity. There are several examples of essential oils like that of rose (*Rosa damascene*), patchouli (*Pogostemon patchouli*), sandalwood (*Santalum album*), lavender (*Lavendula officinalis*), geranium (*Pelargonium graveolens*), etc. that are well known in perfumery and fragrance industry. Other essential oils such as lemon grass (*Cimbopogon winteriana*), *Eulcalyptus globulus*, rosemary (*Rosemarinus officinalis*), vetiver (*Vetiveria zizanoides*), clove (*Eugenia caryophyllus*) and thyme (*Thymus vulgaris*) are known for their pest control properties. While peppermint (*Mentha piperita*) repels ants, flies, lice and moths; pennyroyal (*Mentha pulegium*) wards off fleas, ants, lice, mosquitoes, ticks and moths. Spearmint (*Mentha spicata*) and Basil (*Ocimum basilicum*) are also effective in warding off flies. Similarly, essential oil bearing plants like *Artemesia vulgaris*, *Melaleuca leucadendron*, *Pelargonium roseum*, *Lavandula angustifolia*, *Mentha piperita*, and *Juniperus virginiana* are also effective against various insects and fungal pathogens (Kordali, Cakir, Mavi, Kilic & Yildirim, 2005).

Table 1: Mammalian Toxicity of Some Essential Oil Compounds

Compound	Animal tested	Route	LD_{50} (mg/kg)
2-Acetonaphthone	Mice	Oral	599
Apiol	Dogs	Intravenous	500
Anisaldehyde	Rats	Oral	1510
trans-Anethole	Rats	Oral	2090
(+) Carvone	Rats	Oral	1640
1,8-Cineole	Rats	Oral	2480
Cinnamaldehyde	Guinea pigs	Oral	1160
	Rats	Oral	2220
Citral	Rats	Oral	4960
Dillapiol	Rats	Oral	1000–1500
Eugenol	Rats	Oral	2680
3-Isothujone	Mice	Subcutaneous	442.2
d-Limonene	Rats	Oral	4600
Linalool	Rats	Oral	> 1000
Maltol	Rats	Oral	2330
Menthol	Rats	Oral	3180
2-Methoxyphenol	Rats	Oral	725
Methyl chavicol	Rats	Oral	1820
Methyl eugenol	Rats	Oral	1179
Myrcene	Rats	Oral	5000
Pulegone	Mice	Intraperitoneal	150
γ-terpinene	Rats	Oral	1680
Terpinen-4-ol	Rats	Oral	4300
Thujone	Mice	Subcutaneous	87.5
Thymol	Mice	Oral	1800
	Rats	Oral	980

Source: Dev and Koul (1997); FAO (1999); Koul (2005)

Studies conducted on the effects of volatile oil constituents of Mentha species are highly effective against *Callosobruchus maculatus* and *Tribolium castanum,* the common stored grain pests (Tripathi, Veena, Aggarwal & Sushil, 2000). Essential oils derived from eucalyptus and lemongrass have also been found effective as animal repellents, antifeedants, insecticides, miticides

and antimicrobial products; thus finding use as disinfectants, sanitizers, bacteriostats, microbiocides, fungicides and some have made impact in protecting household belongings. Essential oil from *Cinnamomum zeylanicum*, *Cymbopogon citratus*, *Lavandula angustifolia* syn. *L. officinalis*, *Tanacetum vulgare*, *Rabdosia melissoides*, *Acorus calamus*, *Eugenia caryophyllata*, *Ocimum spp.*, *Gaultheria procumbens*, *Cuminum cymium*, *Bunium persicum*, *Trachyspermum ammi*, *Foeniculum vulgare*, *Abelmoschus moschatus*, *Cedrus spp.* and Piper species are also known for their varied pest control properties. Citronella (*Cymbopogon nardus*) essential oil has been used for over fifty years both as an insect repellent and an animal repellent. Combining few drops each of citronella, lemon (*Citrus limon*), rose (*Rosa damascena*), lavender and basil essential oils with one litre of distilled water is effective to ward off indoor insect pests. The larvicidal activity of citronella oil has been mainly attributed to its major monoterpenic constituent citronellal (Zaridah, Nor Azah, Abu Said & Mohd Faridz, 2003). Vetiver (*Vetiveria zizanioides*) essential oil obtained by steam distillation of aromatic roots contains a large number of oxygenated sesquiterpenes. This oil is known to protect clothes and other valuable materials from insect attack when placed in closets, drawers, and chests. Catnip (*Nepeta cateria*) essential oil is highly effective for repelling mosquitoes, bees and other flying insects. The most active constituent in catnip has been identified as nepetalactone. It repels mosquitoes ten times more than DEET. It is particularly effective against *Aedes aegypti* mosquito, a vector for yellow fever virus. Oil of *Trachyspermum sp.* is also larvicidal against *A. aegypti* and southern house mosquito, *Culex quinquefasciatus* say (LC_{50} = 93.19–150.0 ppm) (Vrushali, 2001).

Essential Oils in Food Preservation

Food-borne diseases are a growing public health problem worldwide. It is estimated that each year in the United States, 31 species of pathogens cause 9.4 million cases of food-borne illnesses (Scallan *et al.*, 2011). Successful control of food-borne pathogens requires the use of multiple preservation techniques in the manufacturing and storage of food products. A recent consumer trend toward preference for products with lower salt and sugar content presents an increased need for efficient food preservatives, as lowering the salt and sugar content would otherwise compromise the product's shelf-life (Zink, 1997). A wide range of preservatives are used to extend the shelf-life of a product by inhibiting microbial growth. However, an increasingly negative consumer perception of synthetic food additives has spurred an interest in finding natural alternatives to the traditional solutions (Zink, 1997). Although originally added to change or improve taste, the antimicrobial activity of essential oils makes them an attractive choice for substituting synthetic preservatives.

Perspectives and Limitations in Application of Essential Oils in Food

A range of essential oil components have been accepted by the European Commission for their intended use as flavorings in food products. The registered flavorings are, e.g., linalool, thymol, eugenol, carvone, cinnamaldehyde, vanillin, carvacrol, citral, and limonene, all of which are considered to present no risk to the health of the consumer. The United States Food and Drug Administration (FDA) also classifies these substances as generally recognized as safe (GRAS). The crude essential oils classified as GRAS by FDA include amongst others clove, oregano, thyme, nutmeg, basil,

mustard, and cinnamon. There are regulatory limitations on the accepted daily intake of essential oils or essential oil components, so before they can be used in food products, a daily intake survey should be available for evaluation by FDA. Despite the demonstrated potential of essential oils and their constituents *in vitro*, their use as preservatives in food has been limited because high concentrations are needed to achieve sufficient antimicrobial activity. In many food products, the hydrophobic essential oil constituents are impaired by interactions with food matrix components, such as fat (Rattanachaikunsopon & Phumkhachorn, 2010; Cava-Roda, Taboada-Rodríguez, Valverde-Franco & Marín-Iniesta, 2010), starch (Gutierrez, Barry-Ryan, & Bourke, 2008), and proteins (Cerrutti & Alzamora, 1996; Kyung, 2011). Furthermore, the antimicrobial potency of essential oil constituents also depends on p^H (Juven *et al.*, 1994), temperature (Rattanachaikunsopon & Phumkhachorn, 2010), and the level of microbial contamination (Espina, Somolinos, Lorán, Conchello, García & Pagán, 2011). Extrapolation of results from *in vitro* tests to food products is thus difficult at best, and a lower performance of the antimicrobial compound must be expected. For example, Cilantro oil had significant antibacterial activity at 0.018% *in vitro*, but when applied to a ham model, even 6% cilantro oil had no antimicrobial activity (Gill, Delaquis, Russo & Holley, 2002). Before being added to food products, it is therefore useful to investigate how essential oils or their constituents interact with food components in vitro. Food matrix interactions with the essential oils or their constituents can be investigated by measuring the growth of microorganisms in culture medium containing a range of concentrations of fat, protein, or starch as well as the antimicrobial

compound of interest. Such experiments have been performed using a so-called food model media (Gutierrez, Barry-Ryan & Bourke, 2009) and can be used to provide quick answers to which kind of food products the compound in question can be used in. The intense aroma of essential oils, even low concentrations, can cause negative organoleptic effects exceeding the threshold acceptable to consumers (Lv, Liang, Yuan & Li, 2011). Having to increase the concentration of essential oils to compensate for their interactions with food matrix components is therefore highly unfortunate and limits their application to spicy foods where the acceptable sensory threshold is relatively high. Different strategies can be used to circumvent this problem. One option is to use essential oils in active packaging rather than as an ingredient in the product itself. Essential oils can be encapsulated in polymers of edible and biodegradable coatings or sachets that provide a slow release to the food surface or to the headspace of packages of, e.g., fruit, meat, and fish (Pelissari, Grossmann, Yamashita & Pineda, 2009; Sánchez-González, Vargas, González-Martínez, Chiralt & Cháfer, 2011). Sachets that release volatile essential oils into the headspace environment are simply placed within an enclosed food package (Ahvenainen, 2003). The advantage of incorporating volatile components of essential oils in films or edible coatings is that the diffusion rate of the agents away from the food product can be reduced thereby maintaining the active compounds in the headspace or on the product surface for extended periods of time (Phillips & Laird, 2011; Sánchez-González *et al.*, 2011). A way to minimize organoleptic effects of essential oils added to the matrix of a food product is to encapsulate essential oils into nanoemulsions. This approach increases the stability of volatile components,

protecting them from interacting with the food matrix, and increases the antimicrobial activity due to increased passive cellular uptake (Donsì, Annunziata, Sessa & Ferrari, 2011). Lowering the concentration of essential oils without compromising their antimicrobial activity can also be obtained by applying them in combination with other antimicrobial compounds that provide a synergistic effect (Nguefack *et al.*, 2012). Synergies are known to occur for essential oil combinations, and it is therefore a field with countless opportunities to find potent antimicrobial blends, which may be the key to implementing essential oils in food preservation without simultaneous organoleptic effects.

Synergies between Essential Oil Components

The interaction between antimicrobials in a combination can have three different outcomes, synergistic, additive, or antagonistic. Synergy occurs when a blend of two antimicrobial compounds has an antimicrobial activity that is greater than the sum of the individual components. An additive effect is obtained when the combination of antimicrobials has a combined effect equal to the sum of the individual compounds. Antagonism occurs when a blend of antimicrobial compounds has a combined effect less than when applied separately (Davidson & Parish, 1989; Burt, 2004). The combined effect of a blend is analysed by using measurements of the MIC to calculate the fractional inhibition concentration index (FIC Index) according to the formulas defined by (Davidson & Parish, 1989): FICA=MICA+B/MICA, FICB=MICB+A/MICB,FIC Index=FICA+FICB. The MICA+B value is the MIC of compound A in the presence of compound B, and vice versa for MICB+A. Calculating the FIC value for either substance A or B then requires

determination of the MIC for the individual components. Theoretically, a FIC Index near 1 indicates additive interactions, while below 1 implicates synergy, and above 1 antagonism (Davidson & Parish, 1989). However, this definition has been replaced by a more general one where the FIC Index results are interpreted as synergistic if FIC Index <0.5, additive if 0.5<FIC Index <4, or antagonistic if FIC Index >4 (Odds, 2003).

The antimicrobial activity of a given essential oil may depend on only one or two of the major constituents that make up the oil. However, increasing amounts of evidence indicate that the inherent activity of essential oils may not rely exclusively on the ratio in which the main active constituents are present, but also interactions between these and minor constituents in the oils. Various synergistic antimicrobial activities have been reported for constituents or fractions of essential oils when tested in binary or ternary combinations (Delaquis, Stanich, Girard & Mazza, 2002; Pei, Zhou, Ji & Xu, 2009; García-García, López-Malo, & Palou, 2011; Nguefack *et al.*, 2012). For example, García-García *et al.* (2011) found the most synergistic binary combination against *L. innocua* to be carvacrol and thymol, and the most active ternary combination to be carvacrol, thymol, and eugenol. Reports on greater antimicrobial activity of crude essential oils compared to blends of their major individual components suggests that trace components in the crude essential oils are critical to the activity and may have a synergistic effect (Marino, Bersani & Comi, 2001; Delaquis *et al.*, 2002; Burt, 2004; Koutsoudaki *et al.*, 2005).

In contrast to this, trace components may also cause antagonistic interactions, which were seen by comparing the antimicrobial effect of pure

carvacrol to oregano oil where carvacrol is a major constituent. Pure carvacrol was 1500 times more effective than the crude essential oil (Rao *et al.*, 2010). Among individual essential oil constituents, synergy has been observed for carvacrol and p-cymene on *B. cereus* (Ultee *et al.*, 2002; Rattanachaikunsopon & Phumkhachorn, 2010). It appears that p-cymene swells bacterial cell membranes, probably enabling easier entrance of carvacrol into the cell membrane where it exerts its action (Ultee *et al.*, 2002). Furthermore, Bassolé *et al.* (2010) showed that if linalool or menthol was combined with eugenol it showed the highest synergy, suggesting that a monoterpenoid phenol combined with a monoterpenoid alcohol is an effective combination.

Little is currently known about what governs synergy and antagonism among essential oil constituents. Four theoretical mechanisms of antimicrobial interactions produce synergy: (i) sequential inhibition several steps in a particular biochemical pathway, (ii) inhibition of enzymes that degrade of excrete antimicrobials, (iii) interaction of several antimicrobials with the cell wall, or (iv) interaction with the cell wall or membrane that leads to increased uptake of other antimicrobials (Davidson & Parish, 1989; Eliopoulos, Moellering & Pillai, 1996). Another possibility for synergistic effects could be that antimicrobials have different mode of actions, thereby attacking two different sites on or in the cell, which indirectly depend on each other. Even less is known about the cause antagonism, it is hypothesized to occur when: (i) combining bacteriostatic and bactericidal antimicrobials, (ii) antimicrobials have the same site of action, (iii) antimicrobials interact with each other (Davidson & Parish, 1989), Larson (1985) in Roller (2003). The hypothesised synergistic or antagonistic interactions are based on 15year old results, and

with the emergence of new techniques this field is likely to see some significant advances in our understanding of how antimicrobial compounds affect each other when acting in concert. In practice, the knowledge needed to exploit synergistic combinations of essential oils in food products is (i) the site and mode of action of each essential oil constituent, and (ii) the mechanisms resulting in synergy or antagonism between several compounds, and (iii) how each compound interacts with food matrix components in a way that affects is antimicrobial properties. When the mechanistic details for synergistic interactions are better understood, it will be easier to exploit synergies using intelligent combinations of constituents to combat food spoilage microorganisms.

Larvicidal and Adulticidal Activity of Monoterpenes

Govindarajan, Jebanesan & Pushpanathan, (2008) reported the ovicidal and Larvicidal efficacy of methanolic leaf extracts of *Cassia fistula* against *Anopheles stephensi* and *Culex quinquefasciatus*. Similarly efficacy of crude extract of *Cassia fistula* was evaluated against *Culex tritaeniorhynchus* and *Anopheles subpictus*. Results shown excellent larvicidal potential against both mosquitoes (Govindarajan & Sivakumar, 2011). The methanolic extracts of *Cassia fistula* showed 89% mortality against adult mosquitoes at 70 ppm dose rate after 48 hrs (Mehmood, Lateef, Omer, Anjum, Rashid & Shehzad, 2014). In another study, Govindarajan (2009) reported the bioefficacy of *Cassia fistula* leaf extracts with different solvents like benzene, acetone and methanol against dengue vector *Aedes aegypti*. The larvicidal activity of methanolic leaf extracts of *Cassia fistula* showed highest efficacy in dengue vector. Kumar, Warikoo and Wahab, (2010) reported larvicidal potential of ethanolic extracts

of dried fruits of three species of peppercorns against different instars of dengue fever mosquito, *Aedes aegypti*. The ethanolic extracts of three species of peppercorns were long pepper, black pepper and white pepper. Ethanolic extracts of all the three pepper species were 11-25 times more toxic against 3rd instar larvae as compared to the early 4th instar larvae. It showed the extracts of piper nigrum have compounds which are potentially active against insects. Nath, Bhuyan and Goswami, (2006) reported the Larvicidal activities of methanolic extracts of 19 different indigenous plants against 3rd instar larvae of *Aedes albopictus* and *Culex quinquefasciatus*. Among these tested plants, *Piper nigrum* showed 2nd highest Larvicidal mortality against 3rd instar larvae of *Aedes albopictus* and *Culex quinquefasciatus*. Several other studies had also reported that plant extracts are good alternatives to insecticides (Govindarajan, 2009; Rajkumar, Jebanesan & Nagarajan, 2011).

Sesquiterpenes

Sesquiterpeniodal structural framework in natural products has opened new vistas for medicinal and biological chemistry. SLs provide a template for structural modifications as these are generally functional group rich chemical entities. Moreover, these biologically significant motifs are reported to be non-toxic, less susceptible to multidrug resistance (MDR) and highly bio-available owing to their capability to penetrate the biological membranes. In recent times, the anti-cancer property of various sesquiterpenes has been of much interest and extensive studies have been carried out to characterize the anti-cancer activity, the molecular mechanisms, and the potential chemo preventive and chemo-therapeutic application of sesquiterpenoid lactones (Gershenzon & Dudareva, 2007). Cytotoxicity and many other biological activities of

sesquiterpenoid lactones, is known to be mediated by the presence of potentially alkylant structural elements capable of reacting covalently with biological nucleophiles, thereby inhibiting a variety of cellular functions (Schmidt, 1999) which direct the cells into apoptosis (Dirsch, Stuppner & Vollmar, 2001). This is also true to the skeleton with a α, β-unsaturated function whereby the assimilation of heteroatom (S, N & O) has been reported to augment or modulate the biological activities of newly generated chemical entities (Amslinger, 2010). Literature is full of examples underscoring natural products with α, β-unsaturated carbonyl moiety exhibiting cancer chemo-preventive and chemo-protective activities (Figure 3). The hallmarks of the biological profile of α, β-unsaturated carbonyl compounds are their ability to act as Michael acceptors (Talalay, De Long & Prochaska, 1988). Although traditionally shunned in modern drug discovery (McGovern, Caselli, Grigorieff & Shoichet, 2002), trapping of nucleophiles like thiols by covalent coupling represents an important mechanism of biological activity. This has led to the discovery of many biologically relevant pathways to understand the mechanism of action of the particular drug candidate. Among the sesquiterpenoid lactones, it is reported that they affect the function of many enzyme systems and transcription factors so that their cytotoxicity is probably a consequence of interference with various target structures within the cell. Despite the plethora of experimental studies found in literature on the cytotoxicity of particular sesquiterpenoid lactones against many cell lines, little is known on the effects of different alkylant structure elements and of other structural factors on cytotoxicity in terms of structure-activity relationships (SAR). This, however, would be an important step in the

direction of rational lead optimization. It can be assumed that any attempt to find SAR for sesquiterpene lactones, cytotoxicity must be taken into account with the capability of the molecules to engage in Michael-type addition to biological nucleophiles such as glutathione (GSH). This capability will for a large part depend on the presence of alkylant structure elements. Using helenalin and parthenolide as models, it has been well established that DNA binding of NF-κB is prevented by alkylation of cystiene in the p65/NF-κB subunit, which is considered to be the general mechanism for SL bearing *α, β*-unsaturated carbonyl structures (Heilmann, Wasescha & Schmidt, 2001).

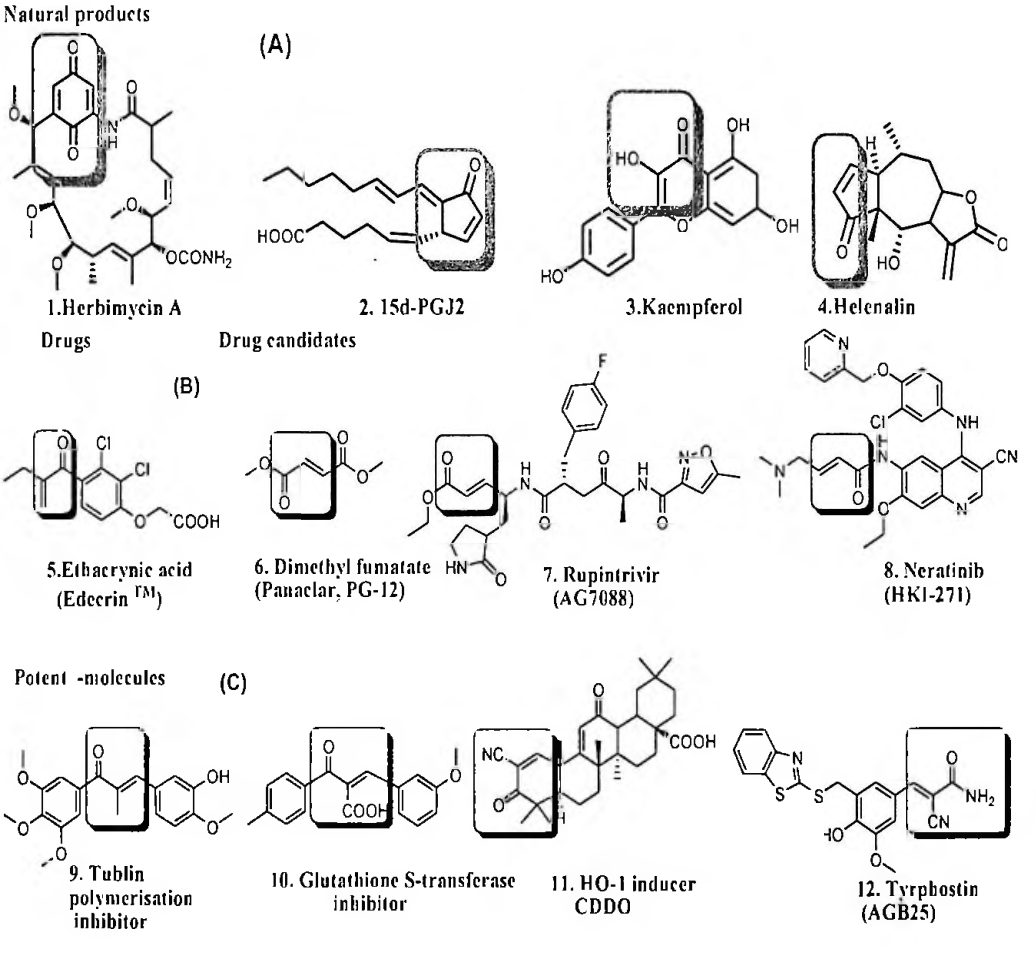


Figure 3: Bio-active compounds (A=Natural products), (B=Drugs), (C=Potent molecules) containing *α-β* unsaturated carbonyl groups.

Owing to the biological importance of sesquiterpenoid lactones and in order to find out the role of different structural alkylating elements towards

cytotoxicity, Parthenin Figure 4, a major constituent of the aggressive and obnoxious herb, *Parthenium hysterophorus L.* (*Compositae*) would be coupled with thymol and its derivatives as alkylating elements. The compound is a sesquiterpenoid having a pseudoguanolide structure. It contains α-methylene-γ-butyrolactone moiety (ring C) along with other functionalities and five chiral centers. The compound is interesting for its structural pattern as well as for its bioactivity.

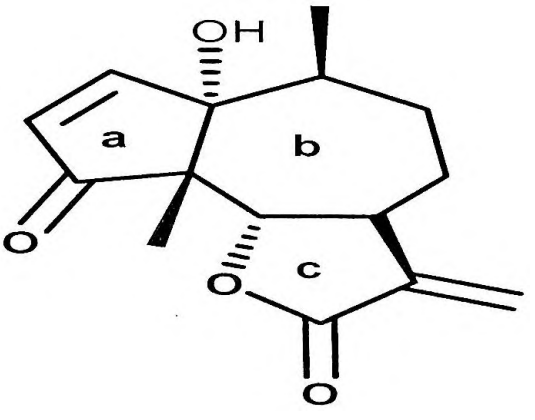


Figure 4: Structure of Parthenin.

This sesquiterpenoid lactone has attracted the attention of chemists as well as biologists due to its interesting structure and reported activities like anti-cancer, antibacterial, antiamoebic, anti-inflammatory, lipid peroxidation inhibition, and trypanocidal activity (Talakal, Dwivedi & Sharma, 1995; Ramos, Rivero, Victoria, Visozo, Piloto & Garcıa, 2001; Kim, Oh & Kim, 2005; Modzelewska, Sur, Kumar & Khan, 2005; Fraga, 2006). There are various reports available on the modification work of parthenin owing to presence of multiple reactive sites. Much emphasis has been given to α, β-unsaturated ketone moiety and α-methylene group. There are two views regarding its SAR. According to one view endocyclic double bond is important for its activity while according to other reports exo methylene is important for biological activity. Thus, a detailed investigation of the importance of α, β--unsaturated group in ring A and α-methylene group in ring C is much desirable.

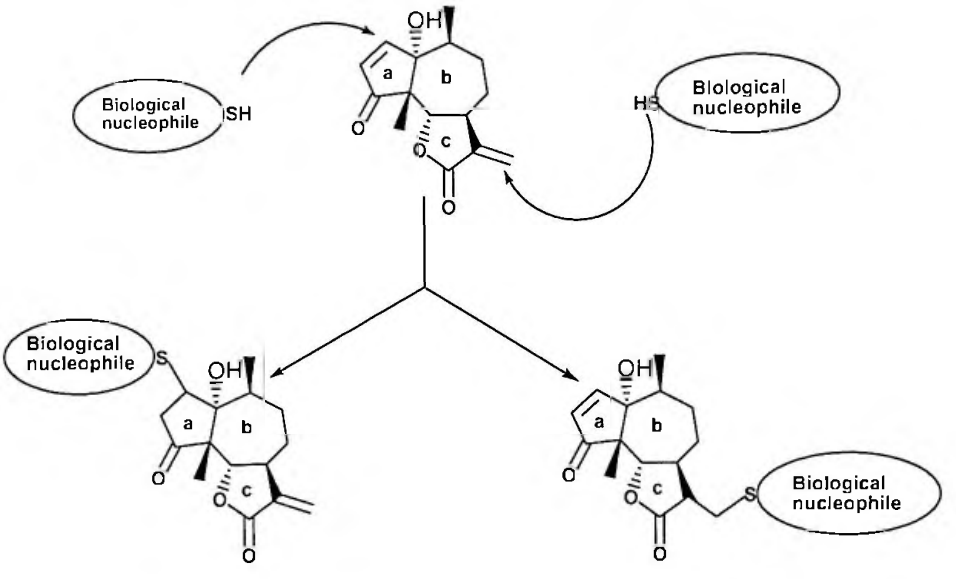
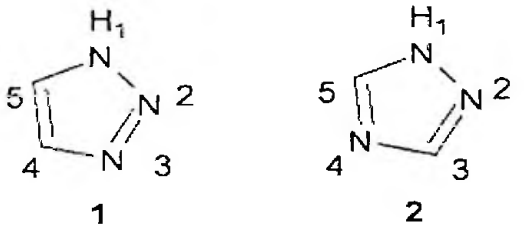


Figure 5: Attack of biological nucleophiles on Parthenin.

Triazole Derivatives

Triazole is a white to pale yellow crystalline solid with a weak, characteristic odour, it is soluble in water and alcohol, melts at 120°C and boils at 260°C. It occurs as a pair of isomeric chemical compounds 1, 2, 3-triazole, **1**, and 1, 2, 4-triazole, **2** with molecular formula $C_2H_3N_3$, and a molecular weight of 69.06 (Kharb *et al.,* 2011). The two isomers are:



Triazole heterocyclic compounds have been paid special attention due to their potential applications as medicinal agents, agrochemicals, supramolecular ligands, biomimetic catalysts, etc (Bai, Zhou & Mi, 2007; Chang, Wang, Zhang, Zhou, Geng & Ji, 2011). Triazole ring is an important five-membered heterocycle with three nitrogen atoms, possesses aromaticity and is an electron

rich system (Asif, 2015). This unique structure endows triazole derivatives to readily bind with a variety of enzymes and receptors in biological system and display a broad spectrum of biological activities (Mi, Wu & Zhou, 2008; Mi, Zhou & Bai, 2007; Wang & Zhou, 2011). Triazole compounds have showed great potential and been paid special attention. Furthermore, triazole ring can be used as an attractive linker to combine different pharmacophore fragments to produce innovative bifunctional drug molecules, providing a convenient and efficient pathway to develop various bioactive and functional molecules (Ouellette, Jones & Zubieta, 2011; Liu, Zhu, Li, Zhang, Leng & Zhang, 2011; Rodriguez-Fernandez, Manzano, Benito, Hermosa, Monte & Criado, 2005). The triazole ring is also an important isostere of imidazole, oxazole, pyrazole, thiazole, amide moiety in designing various types of new drug molecules. Various triazole-based derivatives have been extensively prepared and investigated for their biological activities, which is one of the most active areas in the researches and developments of new drugs. Triazole derivatives, with pharmacological activity, less adverse effects, low toxicity, high bioavailability, good pharmacokinetics property, fewer multi-drug resistances and drug-targeting, diversity of drug administration, broad spectrum, better curative effect, have been frequently becoming clinical drugs or candidates for the treatment of various types of diseases. All these showed wide potential of triazole-based compounds as medicinal agents (Zhou, Zhang, Yan, Wan, Gan & Shi, 2010; Zhou, Zhang, Gan, Zhang & Geng, 2009; Zhou *et al.,* 2009). The researches and developments of the whole range of triazole compounds as medicinal drugs from the reported as: antifungal, anticancer, antibacterial, antitubercular, antiviral, anti-inflammatory, analgesic, anticonvulsant,

antiparasitic, antidiabetic, anti obesitic, antihistaminic, anti-neuropathic, antihypertensive and so on (Prajapati, Goswami & Patal, 2013; Waghamale & Piste, 2013; Hunashal & Satyanarayana, 2012; Jordão, *et al.,* 2011; Singh, Kaur, Kumar & Kumar, 2010; Bay *et al.,* 2010; Guo, Wei, Jia, Zhao & Quan, 2009; Demirbas, Karaoglu,; Mathew, Keshavayya, Vaidya & Giles, 2007; Demirbas & Sancak, 2004).

Parthenin

Much emphasis has been given to the active methylene group on lactone ring towards anticancer activity of parthenin.

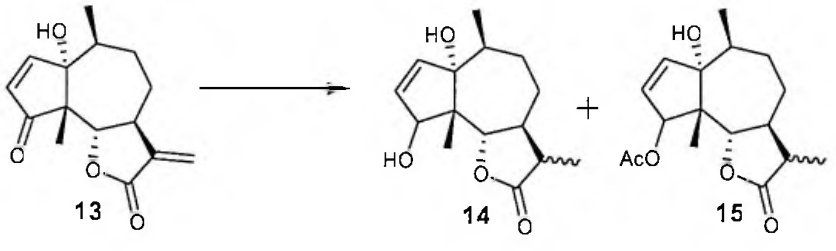
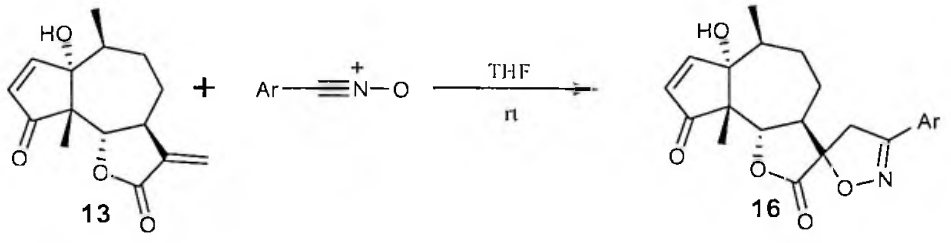


Figure 6: Reduction of parthenin; Loss of bifunctionality.

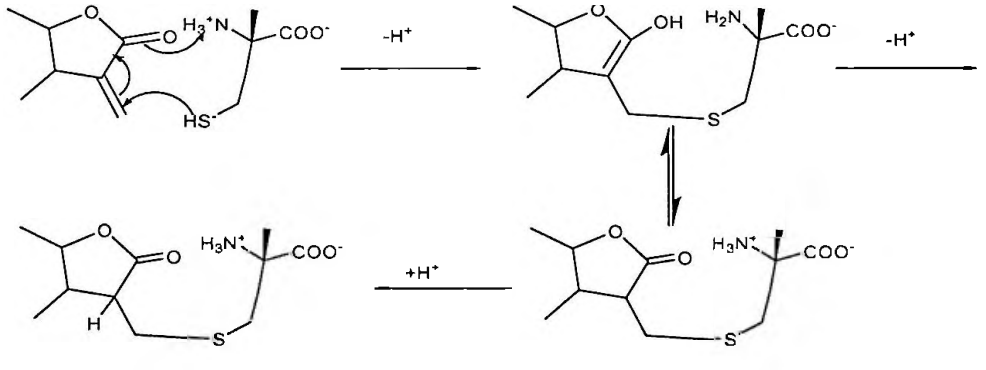
According to Shah *et al* (2009) reduction of the keto group and the exocyclic double bond has led to complete loss of bioactivity, which indicates that cytotoxicity is due to the bifuctionality i.e., α,β-unsaturated carbonyl group and exo-methylene group. Loss of bifuctionality leads to loss of activity.

According to Reddy *et al* (2011) conservation of α, β-unsaturated ketonic moiety of parthenin is crucial for retaining the anti-cancer activity of the ligand whereas the modification of the α-methylene-γ-butyrolactone would facilitate better protein modulation thus enabling improved activity and bioavailability through fine tuning of hydrophilic lipophilic balance.



Scheme 1: Synthesis of spiro heterocycles on ring C of parthenin.

Even though several groups world over, have been working on the structural modification of parthenin, either out of curiosity or with a view to developing secondary leads but none of these reports reveal a focused and rational approach to the modification of parthenin in order to develop a SHAL (small molecule high affinity ligand) with better anticancer activity. Recently, there are literature reports which consider that the major activity of Sesquiterpeniods has been linked mainly to the α-methylene-γ-lactone functionality, which is prone to react with suitable nucleophiles e.g., sulfhydryl groups of cysteine, in a Michael addition fashion. These reactions are nonspecific, leading to the inhibition of a large number of enzymes or factors involved in key biological processes (Polo *et al.,* 2007, Rozalski *et al.,* 2007; Nakagawa *et al.*, 2005; Lee, Wu & Hall, 1977; Kupchan, Eakin & Thomas, 1971).



Scheme 2: Attack of thiol group of cysteine on α, β-unsaturated carbonyl group.

Reaction of the α-methylene lactone moiety with cysteine proceeds by a concerted mechanism in which the ammonium group activates the α, β-unsaturated carbonyl for simultaneous addition of the thiol group. This mechanism explains the dramatic increase in affinity of the methyl lactone towards free cysteine. While few reports claim the importance of both cyclopentenone and α-methylene-γ-butyrolactone ring. Thus even though several modifications have been brought around this molecule, so far no concrete structure-activity relationship (SAR) has been drawn with respect to its anticancer activity and mode of action of this molecule *visa*-a-*viz* the target protein.

In the light of promising therapeutically prospective of this natural product as reported as anticancer molecule, a systematic approach is conceived by us for deciphering the SAR of this natural product, thereby transforming the primary lead molecules into better secondary leads as improved protein modulators. To establish the role of exo/endocyclic double bonds towards the anticancer activity, a strategy to selectively react one of these double bonds has been devised. Out of few chemical transformational possibilities available to achieve the above goal, the present approach involves the selective addition of nucleophile $TMSN_3$ to β position of α,β-unsaturated cyclopentenone in order to generate a focussed library of 1,2,3 triazoles. By screening the anticancer activity of these novel 1, 2, 3-triazole derivatives we can easily establish the pharmacological importance of α-methylene-γ-butyrolactone ring over the cyclopentenone ring, thereby establishing the SAR of the molecule unequivocally.

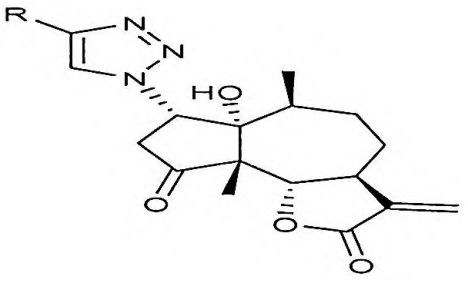
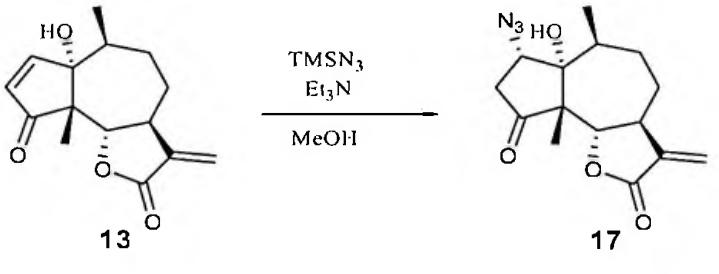


Figure 7: Diagrammatic representation of synthesis of triazoles on ring A of parthenin.

Modification of the Ring A

In order to comprehend the role of double bond in the ring A of parthenin for anticancer activity, we envisaged to study the effect of Michael addition to *α, β*-unsaturated carbonyl group of ring A. Accordingly, a solution of parthenin in methanol was treated with a basic solution of nucleophile $TMSN_3$ at 0°C temperature (Scheme 3). For the optimization of the reaction conditions, the Michael addition was carried out at varying pH using different organic and inorganic bases (Table 2).

It was observed that a p^H of 8-8.5 would be optimal for reaction. Among the various bases used, the most favourable was triethylamine in terms of yield, reaction time and minimal side product formation. After the formation of the product and processing, it was purified through silica gel (100-200 mesh size) column chromatography.



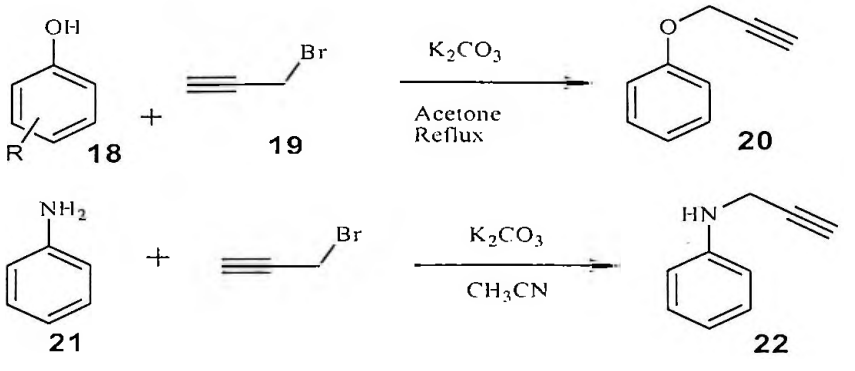
Scheme 3: Synthesis of azide group at β position of *α, β*-unsaturated cyclopentanone ring.

Table 2: Standardisation of Reaction Conditions for Michael Addition

S. No.	Base used	pH	Time (h)	Yield%
		7.5	6	35
1	NaOAc	8	6	40
		8.5	6	40
		7.5	6	45
2	DIPEA	8	6	50
		8.5	6	53
		7.5	3.5	75
3	Et_3N	**8**	„	**80**
		8.5	„	**88**
		9.5	„	65*
4		7.5	6	40
	C_5H_5N	8	„	40
		8.5	„	40
	Pyrolidine	7.5	6	35
5		8	„	40
		8.5	„	40

The structure of the product **17** was confirmed by ^{1}H, ^{13}C and mass spectroscopic data in which disappearance of peaks corresponding to the double bond was observed. Further the presence of azido group was confirmed by the IR spectrophotometer wherein the representative absorption peak for azido group was observed at 2100.89 cm^{-1}. Stereo chemistry at C-2 position was confirmed through ^{1}H by observation of the position and splitting pattern of the signal for proton (δ 3.3, t, J = 9.6 Hz) which is the characteristic J value of alpha orientation (Bhat & Nagasampagi, 1988). While for beta orientation characteristic (Choudhary, Yousuf, Nawaz & Ahmed, 2004) J value is 15.4 Hz. Also another reason for alpha attack is the steric hindrance posed by the methyl group and the possible hydrogen bonding between the hydroxyl group and nitrogen of azide group which makes the alpha orientation more stable.

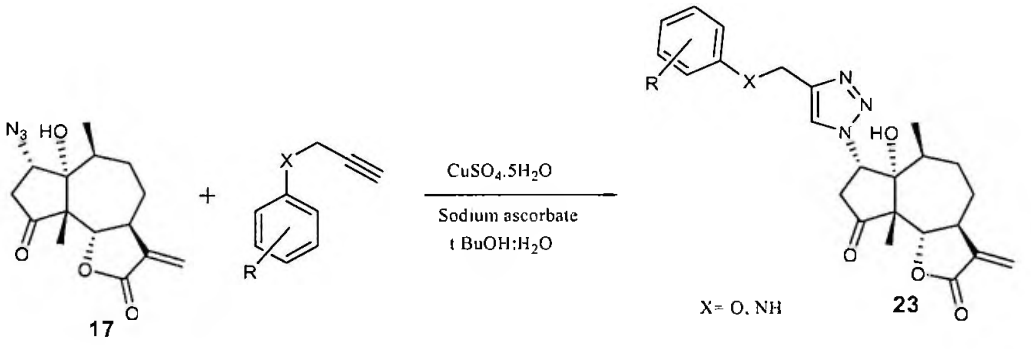
The intermediate **17** was further carried for 3+2 cycloaddition reactions with various terminal alkynes. Terminal alkynes were generated from phenols and anilines according to the literature procedures (Reddy *et al.*, 2008) given in Scheme 4.



Scheme 4: Synthesis of terminal alkynes intermediates.

Synthesis of Triazole Derivatives of Parthenin

Various terminal alkynes including O-propargylated phenols and anilines were reacted with azido parthenin **17** under click chemistry conditions to obtain the cycloaddition products *i.e.*, 1, 2, 3-triazole derivatives 23.



Scheme 5: Synthesis of 1, 2, 3-triazoles on ring A of parthenin.

The formation of 1,2,3-triazole ring was confirmed by the presence of triazolyl proton at δ 7.7 ppm in ^{1}H NMR spectra and the absence of absorption peak at 2103.89 cm^{-1} corresponding to the azido group in IR spectra. A focused library of 20 compounds of 1, 2, 3-triazolyl derivatives of parthenin **17** has been synthesised by varying the hetero-atoms (O/N; bridged between

aromatic ring and propargyl group) and the nature and position of the substitutions over aromatic ring in order to obtain a clear structure-activity relationship (SAR).

Table 3: 1, 2, 3 - Triazolyl Derivatives of Parthenin

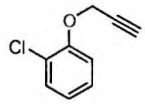
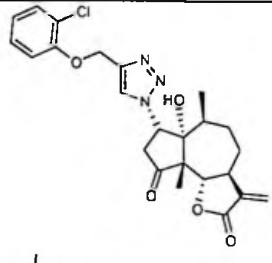
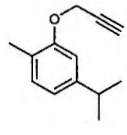
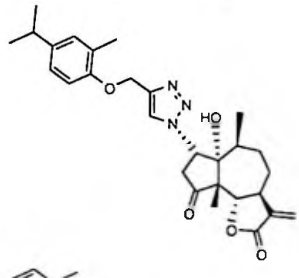
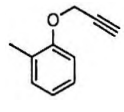
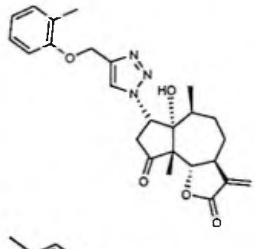
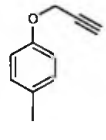
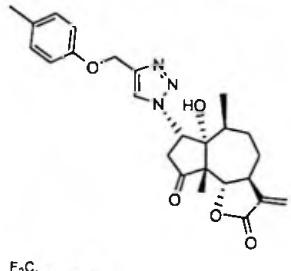
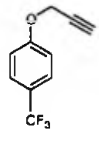
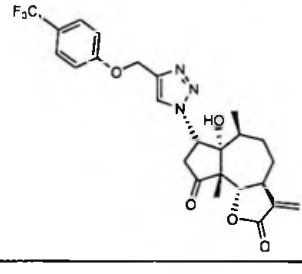
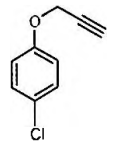
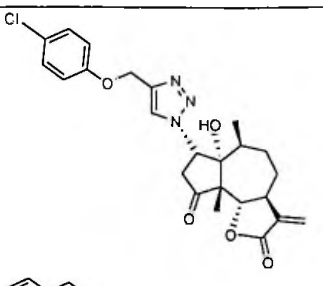
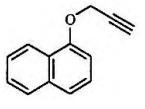
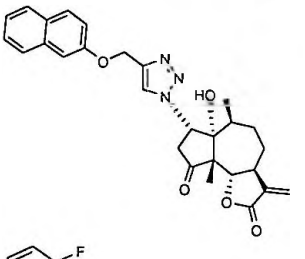
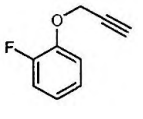
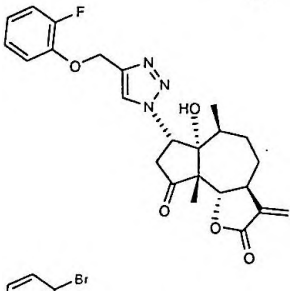
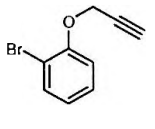
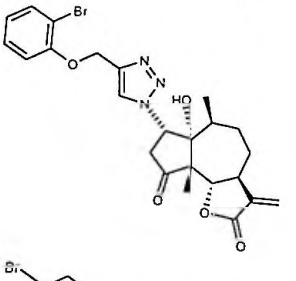
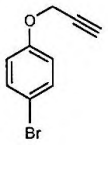
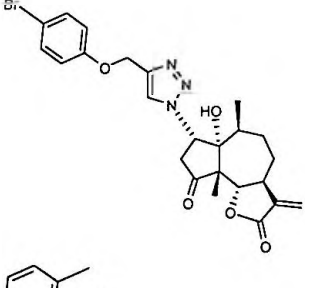
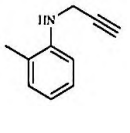
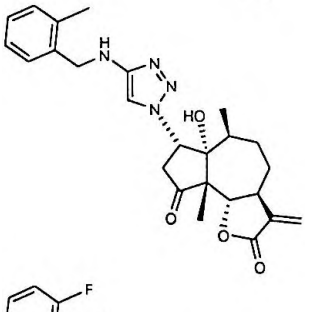
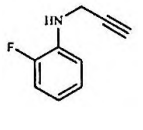
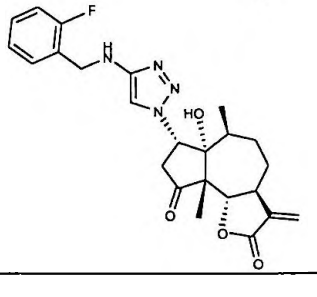
Entry	Reactant	Product[a]	(Yield%)[b], Time, h
24			3(95)
25			3(92)
26			3(95)
27			3(90)
28			2(95)

Table 3 continued

29			3(95)
30			3(96)
31			2(95)
32			2(90)
33			2(97)
34			3(90)
35			3(95)

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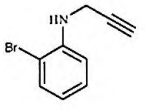
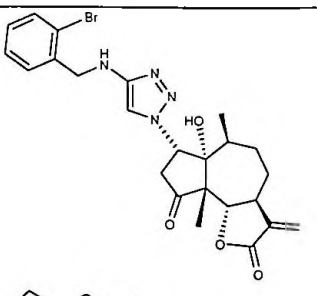
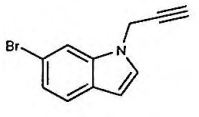
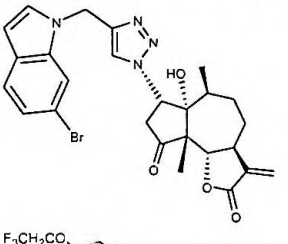
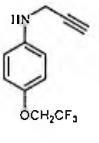
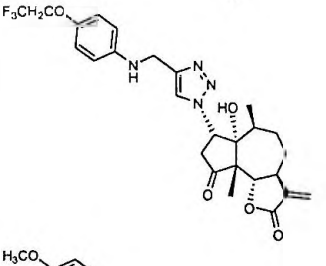
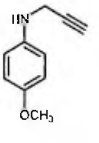
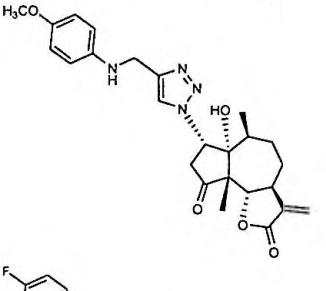
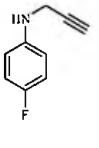
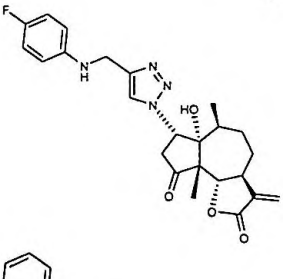
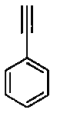
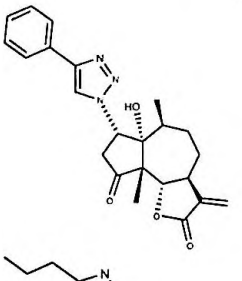
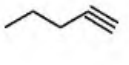
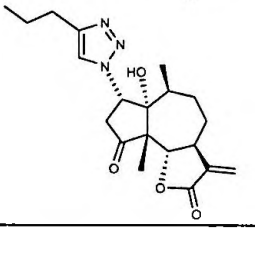
36			3(90)
37			3(97)
38			3(95)
39			3(95)
40			3(90)
41			3(94)
42			3(96)

a) All compounds were characterised by ^{1}H NMR, mass spectroscopy.
b) Yields obtained after column chromatography

Biological Screening Results

All the compounds were assayed for *in vitro* cytotoxicity against a panel of six human cancer cell lines including HCT-1 (colon), PC-3 (prostate), THP-1 (leukaemia), MCF-7 (breast), Hep-2 (liver) and A549 (lung). Mitomycin, Adriamycin and 5-FU were taken as reference compounds and the results are reported in terms of IC_{50} values (Table 6). From the IC_{50} values, it is clear that majority of the compounds have significant cytotoxic activity against prostrate, leukaemia, breast and cervix derived cancer cell lines. However, it may be noted that these compounds showed comparatively lesser activity against HCT-1, Hep-2 a colon and liver derived cancer cell lines respectively. Compound **24**, showed significant cytotoxicity against PC-3, THP-1, and MCF-7 cancer cell lines with *o*-chloro substitution on the aromatic ring, however, its activity was found to be maximum against PC-3 and hela cell line with IC_{50} value 3.1 μM. Majority of the compounds were also found to have promising activity against the above mentioned cell lines. All the compounds which were tested against hela (cervical) cancer cell line were found to show promising activity. From the IC_{50} values, it is clear that the compounds bearing halogen atom as substituent were more cytotoxic.

Table 4: Percentage Inhibition of Compounds at Higher Concentrations

Compd.	Conc.(μM)	Tissue Type (Cell Line Type)					
		Prostrate (PC-3)	Cervix (Hela)	Lung (A549)	Breast (MCF-7)	Leukaemia (THP-1)	Colon (HCT-15)
		% Growth inhibition					
24	100	100	100	100	100	97	89
	50	92	92	92	98	87	77
	30	78	87	79	74	65	68
	10	69	71	71	68	56	55
25	100	99	76	100	94	82	85
	50	88	62	92	77	71	74
	30	74	60	78	65	53	59
	10	56	56	64	54	44	46
26	100	83	94	94	91	88	95
	50	71	89	86	83	79	84
	30	63	83	67	73	64	76
	10	57	73	52	60	57	65
27	100	97	76	92	86	70	67
	50	82	63	83	76	50	52
	30	80	60	75	65	10	35
	10	75	60	60	52	0	8
28	100	79	88	89	89	73	46
	50	68	72	70	79	68	39
	30	45	70	63	65	45	23
	10	26	69	57	47	3	0
29	100	99	88	89	89	99	76
	50	88	72	70	79	86	67
	30	78	70	63	65	78	60
	10	73	69	57	47	67	27

Table 4 continued

	100	92	100	75	88	85	82
	50	88	93	69	73	76	78
30	30	68	86	63	55	56	67
	10	51	77	59	41	36	54
	100	92	87	71	74	70	77
	50	88	75	64	56	63	63
31	30	76	70	50	46	45	28
	10	64	62	39	25	20	10
	100	90	89	99	100	72	90
	50	76	79	85	93	67	83
32	30	60	60	80	78	60	61
	10	50	48	73	63	53	53
	100	89	64	81	83	99	70
	50	76	45	72	77	80	57
33	-	-	-	-	-	-	-
	30	65	40	59	69	51	43
	10	50	36	47	65	24	11
	100	99	90	72	82	90	81
	50	96	84	69	79	70	70
34	30	73	67	63	65	60	50
	10	61	51	52	55	39	46
	100	96	94	86	98	50	50
	50	86	80	70	87	28	31
35	30	73	73	47	79	22	29
	10	68	69	23	74	17	16
Mitomycin		1	55	-	-	-	-	-
5-FU		20	-	58	52	-	63	62
Adriamycin		1	-	-	-	62	-	-

The cytotoxicity data in (Table 4) clearly indicated that all the compounds were found active in primary screening.

Table 5: Percentage Inhibition of Compounds at Lower Concentrations

		Tissue type (Cell line type)					
Compd.	**Conc.(μM)**	**Prostrate (PC-3)**	**Cervix (Hela)**	**Lung (A549)**	**Breast (MCF-7)**	**Leukaemia (THP-1)**	**Colon (HCT-15)**
		% Growth inhibition					
24	1	67	69	49	54	13	40
25	1	20	55	27	41	0	0
26	1	9	65	67	40	3	11
27	1	0	55	17	11	0	0
28	1	64	51	53	18	0	0
29	1	61	58	61	32	10	0
30	1	57	59	33	0	0	2
31	1	29	34	65	54	0	0
32	1	29	34	65	54	46	38
33	1	34	27	5	58	15	0
34	1	46	46	0	12	23	43
35	1	38	62	17	60	13	0
Mitomycin	1	60	-	-	-	-	-
5-FU	20	-	-	55	-	63	62
Adriamycin	1	-	-	-	58	-	-
36	1	0	0	55	-	-	-
37	1	0	0	14	47	-	-
38	1	0	0	0	0	-	-
39	1	0	45	56	13	-	-
40	1	5	3	0	0	-	-
41	1	70	62	51	0	-	-
42	1	15	37	43	59	-	-

But certain molecules, that is, **24, 25, 27, 29, 30, 31** and **33** are the most active on all the four cancer cell lines that is lung, breast, colon and prostate. Subsequently all the molecules were further screened at lower micro molar concentrations (Table 5).

The IC_{50} values of synthesised compounds on the various cell lines of human *viz.,* lung, prostrate, cervix and breast cancer are given in the Table 6.

Table 6: IC_{50} Values (μM) of 1, 2, 3 Triazolyl Derivatives of Parthenin Against a Panel of Human Cancer Cell Lines

	Cell lines					
Compound	**PC-3**	**THP-1**	**HCT-15**	**Hela**	**A549**	**MCF-7**
3a	3.1	3.8	5.3	3.6	4.2	9.7
3b	4.3	25	23	3.9	6.3	4.3
3c	5.6	19	12	2.8	8.8	3.4
3d	4.7	>100	48	6.5	14	16
3e	6.4	48	>100	3.5	3.8	13
3f	5.2	12	27	3.1	4.1	7
3g	3.6	25	20	7.3	9	24
3h	7.5	33	45	6.8	21	32
3i	10	3.1	5.5	8.4	6.2	3.7
3j	7.6	26	39	>100	21	3.1
3k	4.6	17	8.5	3.9	23	15
3l	3.3	>100	>100	3.7	34	5.1
3m	>100	>100	20	4.8	>100	2.9
3n	>100	>100	26	7.2	>100	4.5
3o	>100	>100	>100	17	23	17
3p	>100	>100	0.5	15	>100	15
3q	>100	>100	15	28	23	28
3r	>100	>100	4.3	16	5.8	16
Parthenin	40.3	46.4	43.5	60	37.6	54.2
Mitomycin*	-	-	0.5	-	-	-
Adriamycin*	6	-	-	-	-	0.5
5-FU*	2.2	1	-	4.5	4.9	-

**Mitomycin, Adriamycin and 5-FU used as positive control for this study*

Out of these compound **24** is the most active on all the four cell lines that is prostrate, cervix, lung and breast with an IC_{50} value ranging from 3.1-9.7 μM.

General Structure-Activity Relationship (SAR) of Parthenin Derivatives

Overall the data obtained showed that the majority of the compounds were able to induce growth inhibition in all the cell lines tested and preliminary structure-activity relationships can be inferred. From the cytotoxicity data, it was established that, the exocyclic methylene group is also necessary for cytotoxicity. Since already it is known from literature that endocyclic double bond is necessary for cytotoxicity. Thus from the study SAR can be depicted that both double bonds are necessary for cytotoxicity of parthenin (both activated double bonds can act as Michael acceptors). Thus good lead compounds can be derived from parthenin when the double bonds are kept free while modifying at other positions.

From the results obtained; the cytotoxic efficacy of the synthesised compounds was analysed based on the SAR considering the following three structural parameters:

1. Hetero-atoms (O/N) conjugating the 1,2,3-triazollyl ring and aromatic ring
2. The nature of the substitution on aromatic ring (electron withdrawing/donating groups)
3. Position of the substitution over aromatic ring (o/m/p).

It was found that compounds containing the 'O' as heteroatom showed significant cytotoxicity compared to compounds containing 'N' as heteroatom. Also while varying the nature of substitution on the aromatic ring (electron withdrawing/donating groups), electron withdrawing groups showed a positive

effect on biological activity with halogens showing significant cytotoxicity. Among the halogens chloro- was more active than bromo- and fluoro while electron donating groups tend to decrease the activity both when phenols or anilines were taken as terminal alkynes. Similarly position of substitution over aromatic ring was affecting the cytotoxicity as *ortho* substituted compounds were more cytotoxic than *para*, which in turn were more cytotoxic than *meta* substituted derivatives. When compared with the biological activity obtained in α-methylene adducts our compounds in the present study were showing better activity, showing that *α*-methylene group also takes part in biological activity of the molecule as Michael acceptors. From the studies; most active product was the compound **24** (IC_{50} = 3.1-9.7 μM) which has chloro group at C-2 position of aromatic ring.

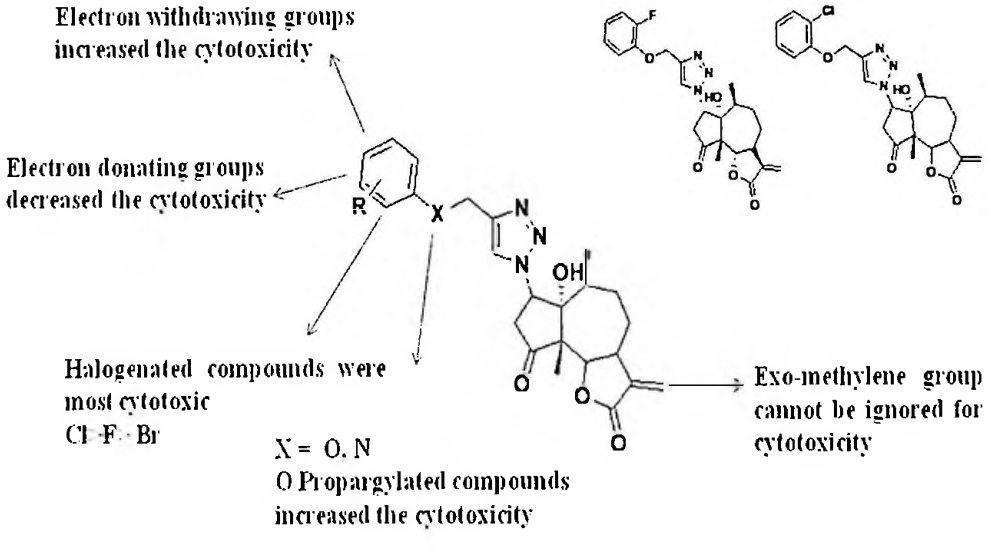


Figure 8: Structure-Activity Relationship (SAR) of parthenin analogues.

Drug-like (Pharmacokinetic) Properties

The description parameters of compounds that show the properties of drugs are outlined in Table 7.

Table 7: Drug-like Parameters of the Cytotoxic Compounds Derived from Parthenin Calculated Using Schrödinger Software

Entry	Mol.wt[a]	C logP[b]	nNO[c]	Rule of 5[d]	PSA[e]	dHB[f]	aHB[g]	Volume[h]	Rotor[i]
24	459.5	3.45	8	0	105	2	7	1207	**5**
25	493	3.493	8	1	105	2	7	1216	**5**
26	451	3.194	8	0	105	2	7	1178	**5**
27	451	3.592	8	1	105	2	7	1230	**5**
28	505	3.940	8	0	104	2	7	1265	**5**
29	471	3.726	8	0	104	2	7	1262	**5**
30	487	3.866	8	0	104	2	7	1304	**5**
31	455	4.294	8	0	104	2	7	1406	**6**
32	515	3.330	8	0	104	2	7	1235	**5**
33	515	3.339	8	0	105	2	7	11193	**5**
34	450	3.502	8	0	107	3	7.25	1320	**5**
35	454	3.028	8	0	108	3	7.25	1229	**5**
36	515	3.401	8	1	106	3	7.25	1268	**5**
37	538	4.78	8	1	98	2	6.25	1371	**4**
38	535	4.374	9	1	115	3	7.25	1395	**7**
39	466	3.146	8	0	108	3	7.25	1241	**5**
40	454	3.486	7	0	96	2	6.25	1218	**5**

The definitions of these various parameters and their range are given below.

a) Mol.wt: molecular weight of the molecule (130-600)

b) C Log P = Predicted octanoyl/water partition coefficient (2.0-6.5)

c) nNO = number of nitrogen and oxygen atoms (2-15)

d) Rule of 5= Number of violations in Lipinski rule of five. The five rules are M Wt <500, donor hydrogen bond ≤5, acceptor hydrogen bond ≤10,Clog P <5.Compounds that satisfy these rules are called as drug like

e) PSA = Vanderwaals surface area of polar nitrogen and oxygen atoms (7-200);

f) d HB = estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (0-6);

g) aHB =estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (2-20);

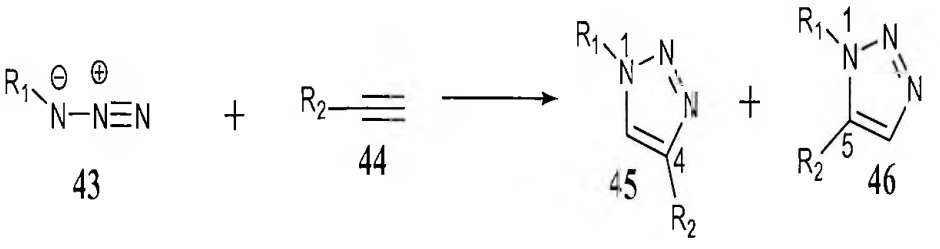
h) Volume = Total solvent-accessible volume in cubic angstrom using a probe with a 1.4A° radius

i) Rotor = Number of non-trivial (not CX_3),non-hindered (not alkene, amide, small ring) rotable bonds(0-15)

Keeping in view the biological activity and SAR of parthenin **13**, a research programme was initiated directed towards the design and synthesis of 1, 2, 3 triazolyl derivatives from this molecule. Thus compound **17** (2α-azido-coronophillin) was prepared from parthenin upon reaction with $TMSN_3$ and Et_3N while maintaining the pH to slightly alkaline. Structure of product **17** was confirmed through ^{1}H-NMR in which disappearance of peaks corresponding to double bond were observed and through IR

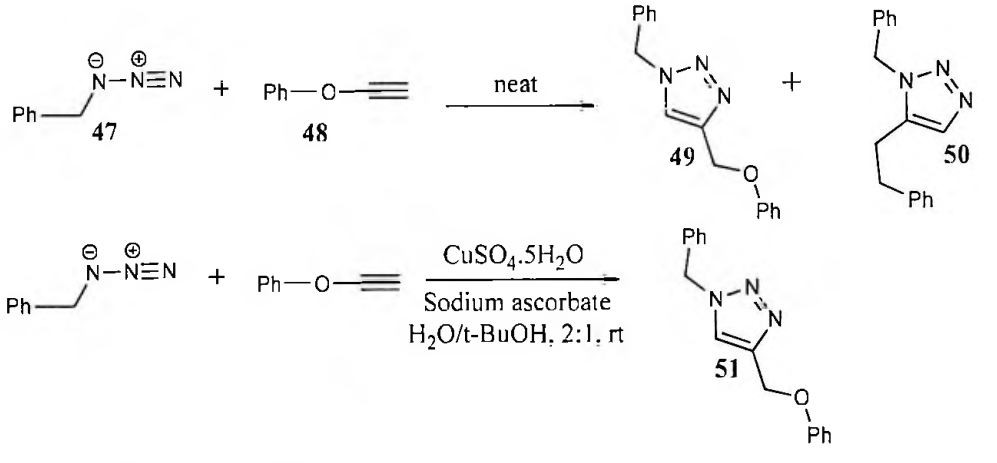
spectrophotometer wherein corresponding absorption of azido group was observed at 2168 cm^{-1}. Accordingly, a series of novel parthenin derivatives bearing bulky 4-substituted triazoles at the C-4 side chain has been synthesised by Cu(I) catalyzed 1,3 dipolar cycloaddition reaction of 2α-azido-coronophillin with arylpropargyl ether and arylpropargylamine obtained by the reaction of phenols and anilines respectively with propargyle bromide in presence of potassium carbonate in acetone under reflux conditions.

Click chemistry enables a modular approach to generate novel pharmacophores utilizing a collection of reliable chemical reactions which give products stereo selectively in high yields, produce inoffensive by-products, are insensitive to oxygen and water, utilise readily available starting materials, and have a thermodynamic driving force of at least 20 kcal mol^{-1}. Two types of click reactions that have influenced drug discovery are the nucleophilic opening of strained ring systems and 1,3-dipolar cycloaddition of particular interest is the Huisgen [3+2] cycloaddition between a terminal alkyne and an azide to generate substituted 1, 2, 3-triazoles. The formation of triazoles *via* the cycloaddition of azide and acetylene was first reported by Dimroth in the early 1900's but the generality, scope and mechanism of these cycloaddition was not fully realized until the 1960's (Huisgen, 1961). The convention cycloaddition generates a mixture of 1, 4- and 1, 5-disubstituted triazoles (Scheme 6).



Scheme 6: Synthesis of 1, 4 and 1, 5-disubstituted triazoles.

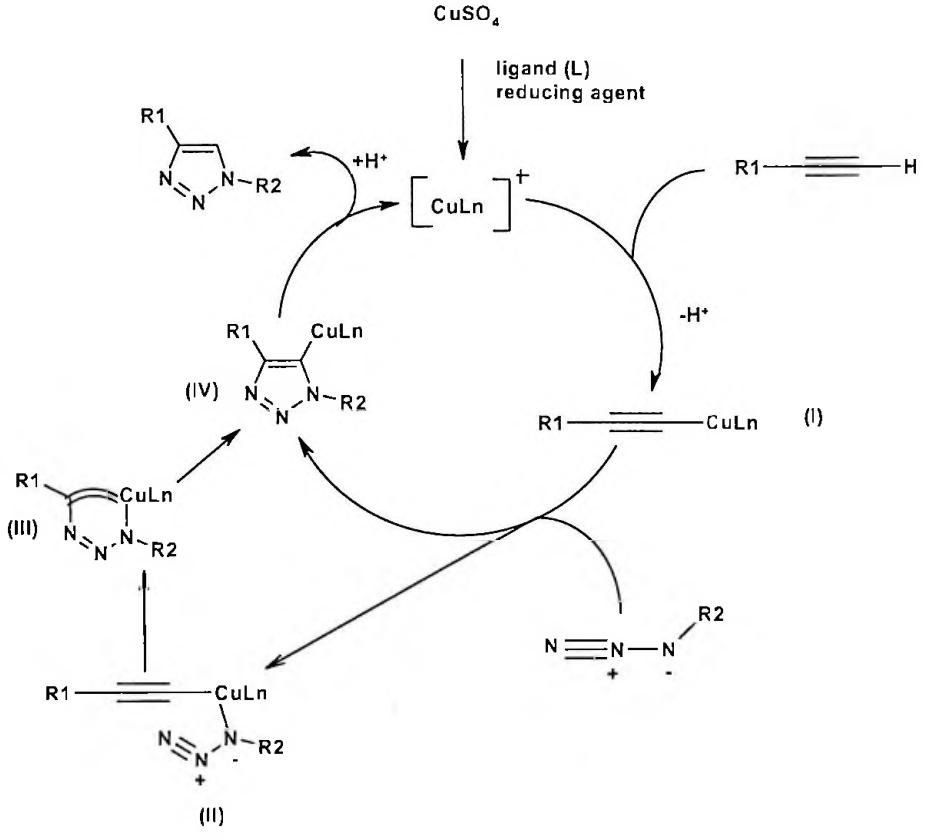
Various attempts to control the regioselectivity have been reported without much success until the discovery of the copper (I)-catalyzed reaction in 2002, which exclusively yields the 1, 4-disubstituted 1, 2, 3-triazole (Rostovtsev, Green, Fokin & Sharpless, 2002). The *in situ* reduction of copper (II) salts such as $CuSO_4 \cdot 5H_2O$ with sodium ascorbate in aqueous alcoholic solvents allows the formation of 1, 4-triazoles at room temperature in high yield (Scheme 7).



Scheme 7: Synthesis of 1, 4 di substituted triazoles.

The copper catalyzed reaction is thought to proceed in a stepwise manner starting with the generation of copper (I) acetylide **I** (Scheme 8). Density functional theory calculations show a preference for the stepwise addition **I – II – III - IV** over the concerted cycloaddition **I-IV** by approximately 12-15 kcal mol^{-1}, leading to the intriguing six membered metallocycle **III**. Comparison of the thermal reaction between benzyl azide and phenyl

propargyl ether with the copper-catalyzed reaction of the same substrates demonstrates the importance of copper catalysis. The thermal reaction leads to the formation of two disubstituted triazole isomers while the copper (I)-catalyzed reaction selectively produces the 1, 4-isomer in 91% yield.



Scheme 8: Mechanism of alkyne-azide click reaction.

So, using the above 1,3-dipolar cycloaddition reaction of 2α-azido-parthenin with arylpropargyl ether and arylpropargylamine in presence of $CuSO_4.5H_2O$ and sodium ascorbate, a series of 2α-[(4-substituted)-1,2,3-triazol-1-yl] derivatives differing in substitution at R_1 and R_2 have been synthesised in excellent yields as shown in (Table-3). The structure of the compound was confirmed through ^1H NMR in which peak at δ 7.7 confirmed the synthesis of triazole ring. The synthesised compounds were then examined for their anticancer activity against five human cancer cell lines *viz.*, PC-3,

MCF-7, A549, HCT-15, Hela cancer cell lines. Majority of the compounds exhibited significant activity on three cancer cell lines i.e., prostate, breast and cervix derived cell lines. Electron withdrawing groups were found to increase the cytotoxicity compared to electron donating groups. IC_{50} of the majority of compounds was found good. Compound **24** was showing better activity on all the four cell lines.

General Methods

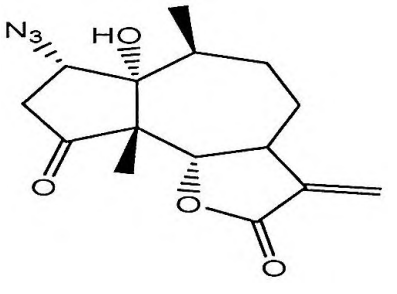
^{1}H and ^{13}C NMR spectra were recorded on 200 and 500 MHz spectrometers with TMS as the internal standard. Chemical shifts are expressed in parts per million (ppm). MS were recorded on Maldi mass spectrometer. Silica gel coated aluminium plates were used for TLC. Reagents and solvents used were mostly of LR grade. The chromatograms were visualised under UV-254-366 nm and $MeOH/H_2SO_4$.

General Procedure for the Michael Addition of $TMSN_3$ to Parthenin

Triethyl amine was added to a solution of $TMSN_3$ (1.2 equiv.) in dry methanol (3 ml) at room temperature and pH was maintained to 8.5. Parthenin (1 equiv.100 mg, 1 mmol) was separately dissolved in dry methanol (2 mL) and the solution was added to the methanolic solution of nucleophile at 0°C and kept for the required time. The progress and the completion of the reaction was monitored through TLC. The reaction mixture was dried completely, dissolved in water (5 mL) and extracted with $CHCl_3$ (10 mL) three times to obtain the product. The solvent was evaporated *in vacuo* and the crude was subjected for column chromatography (silica gel, 100-200 mesh, elution; *n*-hexane/EtOAc gradient) to afford pure product as colourless solid (0.06 g,

60%). The pure product was characterised on the basis of ^{1}H, ^{13}C and mass spectrometry.

(6S, 6αR, 7S, 9αS)-7-azido-octahydro-6α-hydroxy-6,9α-dimethyl methylene azuleno [4, 5-β]furan-2,9(9αH, 9βH)-dione



Crystalline white solid; m.p: 176-178 °C; $[\alpha]_D^{25}$ +29 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 3456.39, 2958.98, 2927.09, 2857.01, 2103.89, 1749.39, 1726.23; ^{1}H NMR (200 MHz, $CDCl_3$): 6.20 (d, 1H, *J* = 1.29 Hz), 5.57 (d, 1H, *J* = 2.72 Hz,), 5.00 (d, 1H, *J* = 8.8 Hz), 3.30 (t, 1H, *J* = 9.6 Hz), 2.28 (m, 1H), 2.40 (d, 2H, *J* =7.5), 2.48 (m, 1H), 2.20-1.64 (m, 4H), 1.18 (d, 3H, *J* = 7.0 Hz), 1.10 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.12, 16.30, 28.21, 31.23, 34.51, 38.54, 41.21, 50.45, 65.45, 70.98, 80.21, 123.31, 140.10, 170.01, 220.11; Maldi mass: 328 ($M + Na^+$).

General Procedure for Synthesis of Aromatic Terminal Alkynes

In a typical procedure, to a solution of any substituted phenol or aniline like *para* bromophenol (0.25 g, 1 mmol) was added potassium carbonate (1.2 g ,1.5 mmol) and propargyle bromide (0.65 g,1.2 mmol) with acetone as solvent. The reaction mixture was then refluxed at 80 °C for 4-5 hours under nitrogen atmosphere. The reaction mixture was then filtered and extracted with ethyl acetate and water. The ethyl acetate layer was then separated. The solvent was evaporated *in vacuo* and the crude was subjected for column chromatography (silica gel, 60-120 mesh, elution; *n*-hexane/EtOAc gradient)

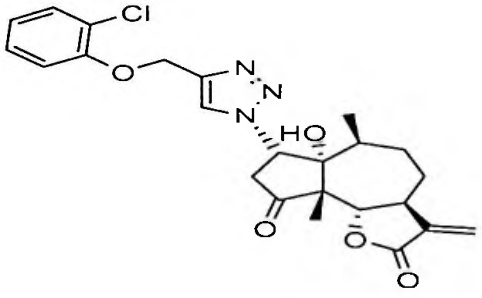
to afford pure product as colourless solid (0.3 g, 90%). The pure product was characterised on the basis of ^{1}H, ^{13}C and mass spectrometry.

General Procedure for the Azide-alkyne Click Reaction

In a typical procedure, to a solution of parthenin azide (0.05 g, 1 mmol) in t-BuOH: H_2O taken in 2:1 proportion was added terminal alkynes (0.09 g, 1.2 mmol), $CuSO_4.5H_2O$ (0.13 g, 2 mmol) and sodium ascorbate (0.09 g, 2 mmol). The reaction mixture was then stirred at room temperature for 3 hours, then filtered and extracted with ethyl acetate and water. The ethyl acetate layer was then separated. The solvent was evaporated in vacuo and the crude was subjected for column chromatography (silica gel, 100-200 mesh, elution; *n*-hexane/EtOAc gradient) to afford pure product as colourless solid. The pure product was characterised on the basis of ^{1}H, ^{13}C and mass spectrometry.

Compound Characterisation

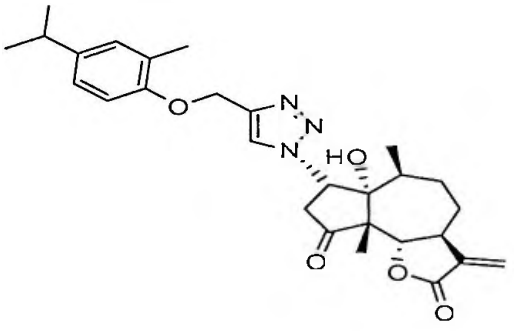
7-(4-[(2-Chlorophenoxy) methyl]-1H-1, 2, 3-triazol-1-yl)-octahydro-6-hydroxy-6α,9α-di Methyl-3-methyleneazuleno [4, 5-β] furan-2,9 (9αH, 9βH)-dione



Compound 24 (Yield 95%); Crystalline white solid; mp: 165-169 °C; $[\alpha]_D^{25}$ +29 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 681.49, 752.84, 997.96, 1118.39, 1162.79, 1241.73, 1277.09, 1446.84, 1484.51, 1588.92, 1753.62, 2925.18, 3400.23; ^{1}H NMR (200 MHz, CD_3OD): δ 8.25 (d, 1H, J = 11.58 Hz), 7.45 (d, 1H, J = 7.99 Hz), 7.30-7.25 (m, 2H), 6.95 (d, 1H, J = 1.29 Hz), 6.25 (d, 1H, J = 2.72 Hz), 6.00 (t, 1H, J = 9.77 Hz), 5.73 (d, 1H, J = 2.35 Hz), 5.25 (t, 2H, J

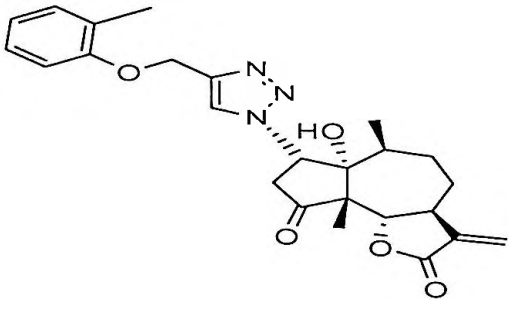
= 6.78 Hz), 5.10 (d, 1H, *J* = 8.28 Hz), 3.33-3.30 (m, 1H), 2.20 (d, 2H, *J* = 4.87 Hz), 1.75-1.53 (m, 4H), 1.30 (d, 3H, *J* = 5.6 Hz); 1.28 (s, 3H), 1.20 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): 10.9, 11.5, 20.4, 23.3, 27.7, 30.3, 38.0, 38.1, 42.4, 46.3, 60.1, 72.3, 73.6, 73.7, 115.7, 120.9, 123.7, 126.6, 129.9, 138.3, 142.4 158.8, 170.3, 220.2 ; ESI MS: 494 ($M + Na^+$), 510 ($M + K^+$); Anal. Calcd for $C_{24}H_{26}ClN_3O_5$: C, 61.08; H, 5.55; N, 8.90. Found: C, 61.13; H, 5.58; N, 8.93.

7-(4-[(4-Isopropyl-2-methylphenoxy)methyl]-1H-1, 2, 3-triazol-1-yl)-octahydro-6-hydroxy-6*α*, 9*α*-dimethyl-3-methyleneazuleno[4, 5-*β*]furan-2, 9(9*α*H, 9*β*H)-dione



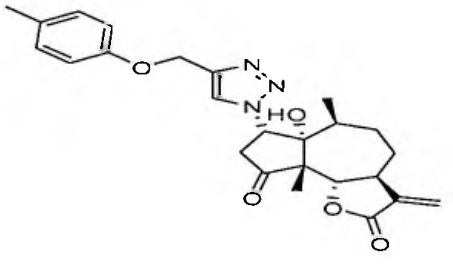
Compound 25 (Yield 92%); Colourless solid; mp: 134 °C; $[\alpha]_D^{25}$ +46 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 754.37, 815.66, 1033.51, 1125.94, 1160.82, 1250.87, 1383.35, 1611.03, 1752.43, 2925.17, 3391.67; ^1H NMR (200 MHz, $CDCl_3$): *δ* 8.12 (s, 1H), 7.10 (d, 1H, *J* = 3.8 Hz), 6.92 (d, 1H, *J* = 8.5 Hz), 6.75 (d, 1H, *J* = 7.6 Hz), 6.22 (d, 1H, *J* = 7.19 Hz), 6.00 (t, 1H, *J* = 9.59 Hz), 5.65 (d, 1H, *J* = 2.28 Hz), 5.25 (s, 2H), 5.00 (d, 1H, *J* = 8.2 Hz), 3.00 (t, 2H, *J* = 2.85 Hz), 2.10 (m, 1H), 1.84 (m, 3H), 1.75 (m, 4H), 1.25-1.20 (m, 9H), 0.95(m, 1H); ^{13}C(100 MHz, $CDCl_3$): 14.18, 14.58, 16.28, 16.50, 29.70, 37.44, 38.70, 44.55, 50.83, 59.55, 60.97, 62.29, 81.66, 84.97, 111.60, 111.82, 112.07, 121.38, 122.12, 124.21, 126.83, 127.66, 130.66, 136.97, 140.34, 156.11, 170.14, 210.97; Maldi mass: 516 ($M + Na^+$), 532 ($M + K^+$); Anal. Calcd for $C_{28}H_{35}N_3O_5$: C, 64.36; H, 6.48; N, 12.02. Found: C, 64.34; H, 6.45; N, 12.12

7-(4-[(o-Tolyloxy)methyl]-1H-1,2,3-triazol-1-yl)-octahydro-6-hydroxy-6*α*,9*α*-dimethyl-3-methyleneazuleno[4, 5-*β*]furan-2,9(9*α*H, 9*β*H)-dione



Compound 26 (Yield 95%); Colourless solid; mp: 134 °C; $[\alpha]_D^{25}$ +46 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 668.84, 754.80, 815.70, 989.38, 1121.27, 1162.34, 1189.07, 1237.92, 1273.08, 1336.93, 1391.95, 1494.14, 1601.60, 1752.83, 2941.26, 3434.86; ^1H NMR (200 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.25 (d, 2H, *J* = 5.8 Hz), 7.00 (d, 2H, *J* = 8.3 Hz), 6.25 (d, 1H, *J* = 8.5 Hz), 5.63 (t, 1H, *J* = 9.8 Hz), 5.25 (d, 2H, *J* = 9.7 Hz), 5.00 (d, 1H, *J* = 8.1 Hz), 3.75 (t, 1H, *J* = 5.6 Hz), 3.54-3.52 (m, 1H), 3.10 (t, 2H, *J* = 10.1 Hz), 2.55 (m, 1H), 2.25 (s, 3H), 1.52 (s, 3H), 1.25 (d, 3H, *J* = 3.4 Hz), 1.00 (m, 4H), 0.95 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): 14.11, 14.31, 16.31, 20.49, 27.42, 30.31, 32.11, 37.36, 38.34, 39.10, 45.05, 51.96, 59.25, 60.38, 61.54, 79.20, 83.54, 118.23, 120.12, 131.15, 140.35, 155.63, 170.51, 210.54; Maldi mass: 474 (M + Na^+), 490 (M + K^+); Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65. Found: C, 68.79; H, 6.75; N, 9.67.

7-(4-((p-Tolyloxy)methyl)-1H-1,2,3-triazol-1-yl)-octahydro-6-hydroxy-6*α*,9*α*-dimethyl-3-methyleneazuleno[4, 5-*β*]furan-2, 9 (9*α*H, 9*β*H)-dione



Compound 27 (Yield 90%); Colourless solid; mp: 134-138 °C; $[\alpha]_D^{25}$ +24 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 514.99, 565.72, 754.27, 815.98, 990.45, 1052.85, 1273.53, 1392.88, 1450.56, 1510.34, 1753.97, 2927.74, 3400.66; ^1H NMR (200 MHz, $CDCl_3$): δ 7.80 (d, 1H, J = 7.8 Hz), 7.24 (d, 2H, J = 8.2 Hz), 6.75 (d, 2H, J = 8.4 Hz), 6.25 (d, 1H, J = 12 Hz), 5.50 (t, 2H, J = 2.3 Hz), 5.25 (s, 2H), 5.00 (d, 1H, J = 7.9 Hz), 3.75 (t, 1H, J = 10.08 Hz), 3.50-3.25 (m, 1H), 3.00 (t, 2H, J = 9.58 Hz), 2.25 (d, 3H, J = 6.4 Hz), 1.50 (s, 3H), 1.25 (d, 3H, J = 3.4 Hz), 1.00 (m, 4H), 0.95 (m, 1H); ^{13}C (100 MHz, $CDCl_3$): 14.18, 14.58, 16.28, 16.50, 22.20, 29.70, 37.44, 38.70, 44.55, 50.83, 59.55, 84.66, 111.60, 111.82, 121.07, 121.38, 122.12, 124.23, 126.83, 127.06, 130.06, 140.34, 156.11, 170.84, 210.97; Maldi mass: 474 (M + Na^+), 490 (M + K^+); Anal. Calcd for $C_{25}H_{29}N_3O_5$: C, 66.50; H, 6.47; N, 9.31. Found: C, 66.54; H, 6.43; N, 9.34

Summary

A review of the various derivatives of thymol derivatives synthesised, indicates that, this is the first time a triazole derivative of thymol is been considered. Again, most biological nucleophiles had already been coupled to parthenin as alkylating agent. Thymol as a biological nucleophile and its ether derivatives can be coupled to parthenin, which will help discover several biological molecules as lead compounds or drug candidates.

CHAPTER THREE

MATERIALS AND METHODS

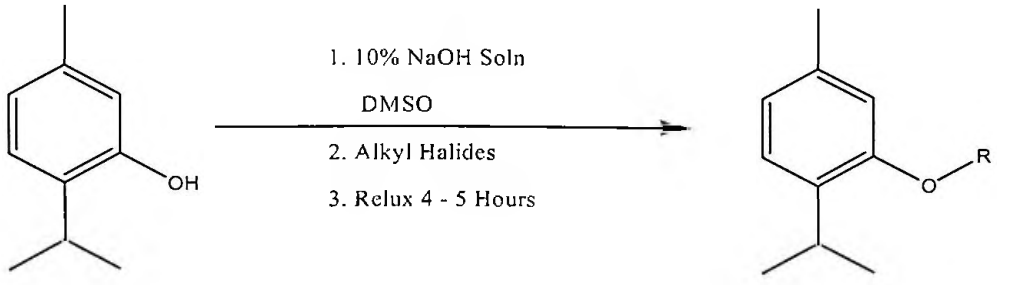
Introduction

All solvents (organic and inorganic), reagents and chemicals used for the research work were of analytical grade and obtained from Sigma-Aldrich Company Limited (USA) and supplied by Kobian Kenya Limited, Nairobi Kenya. The Parthenin used for the synthesis of the thymol-parthenin coupling products was extracted and isolated from *Parthenium hysterophorus*. Most of the chemical reactions were performed under nitrogen gas and in fume hood. The chemical processes used in the synthesis of the thymol derivatives are known chemical reactions with slight modifications where necessary to achieve the desired results.

Synthesis of Ether Derivatives of Thymol (Williamson Etherification Reaction)

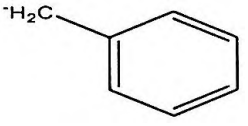
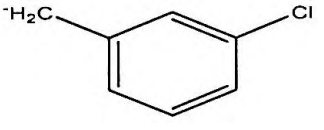
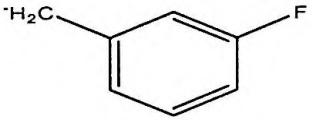
The sodium salt of thymol was prepared by dissolving thymol (1.00g, 0.005M) in 10% NaOH (10ml) and DMSO (10ml) successively with continuous stirring using a magnetic stirrer for about 10 minutes. The required stoichiometric amount of the various alkyl halides (0.005M) were added slowly with stirring and the reaction mixture was refluxed for 4-5 hours. Progress of the reaction was monitored by TLC and GC analysis. The reaction mixture was cooled to room temperature and the oily product was extracted with DCM (3x10ml), washed with saturated $NaHCO_3$ solution (2x10ml), followed by distilled water, (2x10ml) and dried over anhydrous Na_2SO_4. The DCM extract was filtered and evaporated under pressure (*en vacuo*) to obtain the crude ether derivatives which were purified by column chromatography (silica gel 60-120 mesh, hexane/ethyl acetate 21:1 v/v) as eluent in an

increasing polarity of the solvent system to afford pure ether derivatives in 80-90% yields.



Scheme 9: Synthesis of ether derivatives of thymol.

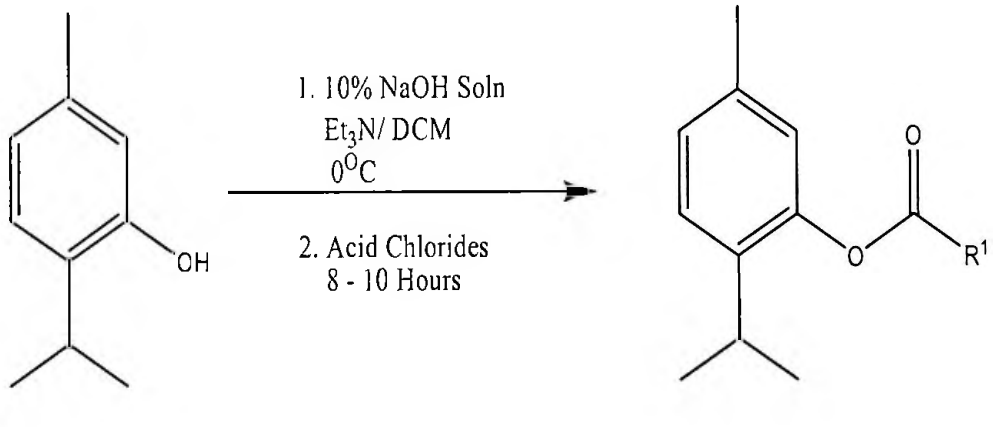
Table 8: Alkyl and Alkyl Substituted Ether Derivatives of Thymol

Name of ether derivative of thymol	- R
1. TM 2C	$-CH_2CH_2CH_3$
2. TM 2D	$-CH(CH_3)-CH_3$
3. TM 2E	$-CH_2CH_2CH_2CH_3$
4. TM 2F	$-CH(CH_3)-CH_2CH_3$
5. TM 2I	$-CH_2CH_2CH_2CH_2CH_2CH_3$
6. TM 2K	$-CH_2CH_2-Cl$
7. TM 2N	
8. TM 2O	
9. TM 2P	

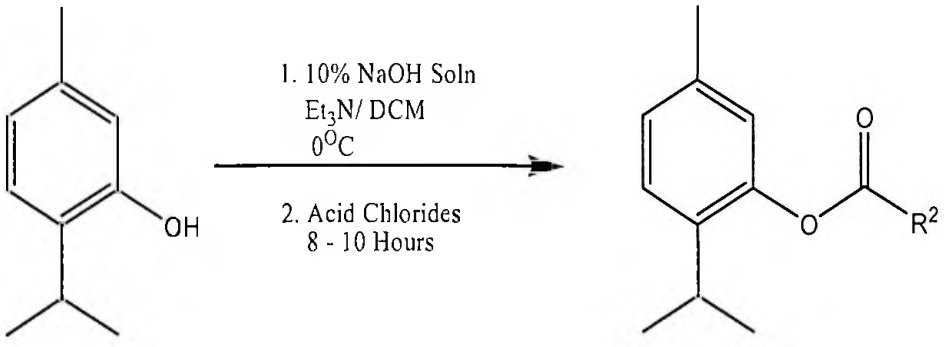
Synthesis of Ester Derivatives of Thymol (Esterification Reaction)

A solution of sodium salt of thymol (1.00g; 0.005M) was prepared by dissolving it in 10% NaOH (10ml) solution with continuous stirring using a magnetic stirrer for about ten minutes. To a solution of the prepared sodium salt of thymol (1.00equiv.) and trimethylamine (1.1equiv.) in anhydrous DCM, stoichiometric amount of acid chlorides (0.005M) were added at 0°C for about 1 hour. Stirring of the reaction mixture was continued at room temperature for about 10 hours. Progress of reaction was monitored by TLC and GC.

The reaction mixture was quenched with distilled water and extracted with DCM (3x 10ml), washed with distilled water, brine and dried over anhydrous sodium sulphate, Na_2SO_4. The DCM extract was filtered and evaporated under pressure (*en vacuo*) to obtain the crude ester derivatives which were purified by column chromatography (silica gel 60-120 mesh, hexane/ethyl acetate 19:1 v/v) as eluent to afford pure ester derivatives 90-95% yields.

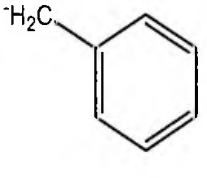


Scheme 10: Synthesis of alkyl ester derivatives of thymol.



Scheme 11: Synthesis of aromatic ester derivatives of thymol.

Table 9: Alkyl and Alkyl Substituted Ester Derivatives of Thymol

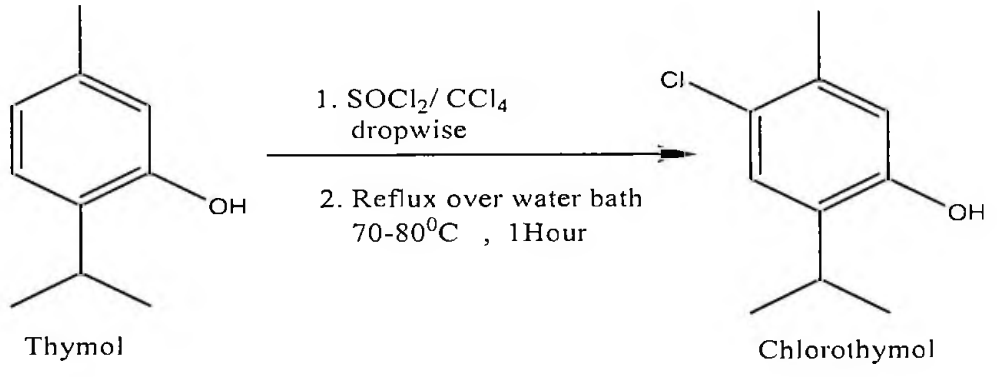
Name of ester derivative of thymol	- R^1
1. TM 1A	- CH_3
2. TM 1B	- CH_2-CH_3
3. TM 1C	- CH(-CH_3)-CH_3
4. TM 1D	- CH_2-CH_2-CH_3
5. TM 1E	- CH(-CH_3)-CH_2-CH_3
6. TM 1F	- CH_2-CH_2-CH_2-CH_3
7. TM 1G	- CH_2-CH_2-CH_2-CH_2-CH_3
8. TM 1I	
9. TM 1N	- $CHCl_2$

Chlorination of Thymol

The chlorination of thymol can be achieved by the reaction of thymol with thionyl chloride by refluxing of the mixture over water bath. This is very important with regards to the activity of thymol, as the substitution of chlorine at para position to the hydroxyl group on thymol enhances its activity.

Synthesis of 4-Chloro-2-isopropyl-5-methyl-phenol

To a solution of thymol (2.0g, 13.33mmol) in carbon tetrachloride (20ml), thionyl chloride (2.5g, 21.2mmol) was added and refluxed over water bath 60-70°C temperature. The reaction mixture was cooled to room temperature, diluted with water and extracted with DCM (3x10ml), washed with water, dried over anhydrous sodium sulphate an evaporated to dryness. The crude reaction product was purified by silica gel column chromatography and eluted with Hexane / Ethyl acetate. It crystallized from hexane as colourless needles.



Scheme 12: Synthesis of chlorothymol (TM 3A) from thymol.

Extraction and Isolation of Parthenin

Parthenin can be obtained from the extraction and isolation of the plant material of *Parthenium hysterophorus*.

Sample Collection and Preparation of Plant Material

Aerial parts of *P. hysterophorus*, including flowers, were collected locally. These were air dried in an open space for one week. The dried plant material was milled into powdered form, weighed and stored in a cold dry place in paper bags at the Behavioural and Chemical Ecology Unit of the International Centre of Insect Physiology and Ecology (ICIPE), Kenya.

Extraction of Powdered Sample

About (1kg) of dry, pulverized plant material of *P. hysterophorus* was extracted with methanol (2 L) by cold maceration for eight hours. The methanol extract was filtered and concentrated using the rotary evaporator. The crude methanol extract after concentration (86 g) was defatted with n-hexane (1 L), filtered and concentrated. The defatted material (29 g) was extracted with chloroform (500 mL). The chloroform extract residue (15 g) obtained after concentration was subjected to hot water extraction (10x 20 mL) for the isolation of a mixture composed of parthenin and coronopilin. The hot water extract was freeze dried and the residue weighed (2.2 g).

Isolation of Parthenin

This mixture was subjected to column chromatography over a silica gel column. Elution was conducted in Hexane–Ethyl acetate in increasing proportions of Ethyl acetate. The eluents were collected in fractions of 20 mL each. Elution of the components from the column was monitored by using thin-layer chromatography (TLC). Parthenin was obtained as white needle-like

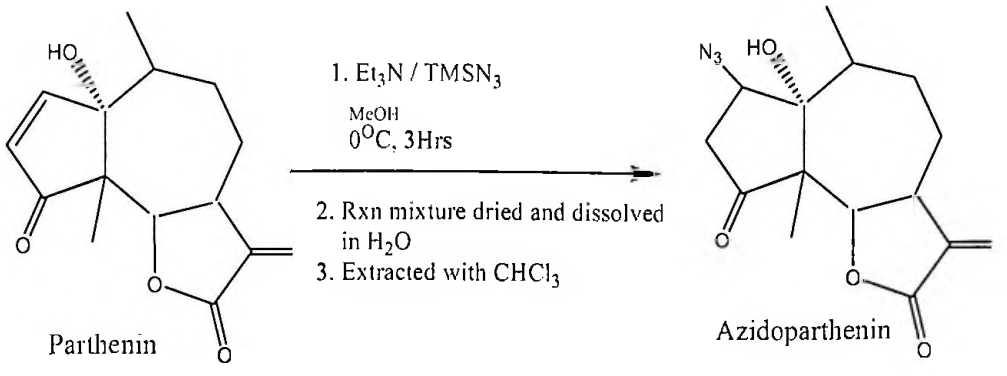
crystals, and the total content recovered in the 1kg plant material used was 750mg. The purity of the isolated Parthenin was established by HPLC, and the compound characterised on the basis of LC-QTOF and mass spectral data. The spectral data obtained from the isolated parthenin was in agreement with the data reported in literature (Hernández, Y. S. *et al.*, 2011).

Azide-Alkyne "Click Reaction" of Thymol and its Derivatives with Parthenin

The azide-alkyne click reaction was employed to synthesise the various triazole derivatives of thymol.

Synthesis of Azido Parthenin

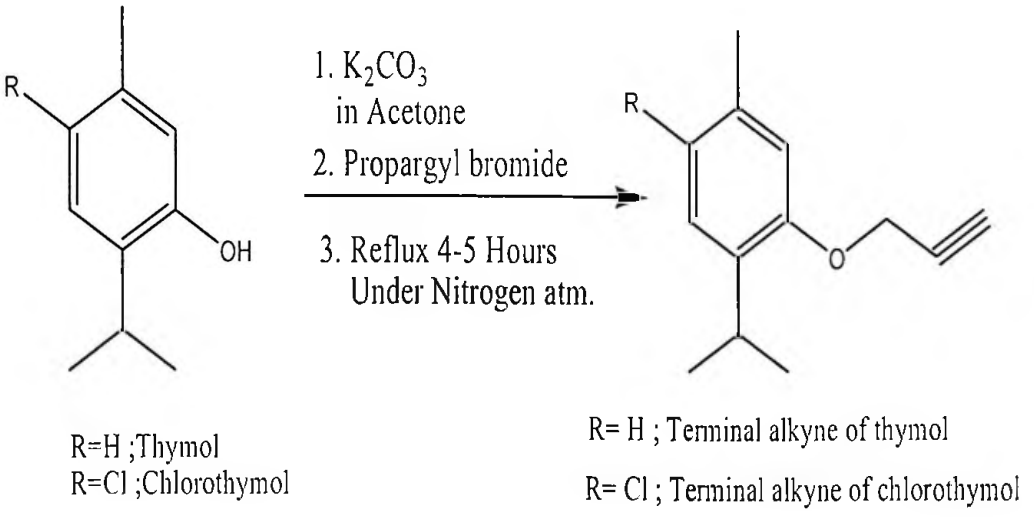
Triethyl amine (12 ml) was added to a solution of Azidotrimethylsilane, $TMSiN_3$ (1.2 equiv.) in dry methanol (8 ml) at room temperature and p^H of 8.7 was maintained. Parthenin (1.0equiv. 100 mg, 1mmol) was separately dissolved in dry methanol (4 ml) and the solution was added to the methanolic solution of the nucleophile at 0°C and kept for 4 hours. The progress and completion of the reaction was monitored through TLC analysis. The reaction mixture was dried completely, dissolved in water (5 ml) and extracted with Chloroform (10 ml) three times to obtain the product. The solvent was evaporated in vacuo, using nitrogen gas and the crude product was subjected for column chromatography (silica gel 100-200 mesh, elution; Hexane/Ethyl acetate gradient) to afford pure product as colourless jelly-like solid (0.07g, 70%).



Scheme 13: Synthesis of azidoparthenin intermediate from parthenin.

Synthesis of Aromatic Terminal Alkynes of Thymol and Chlorothymol

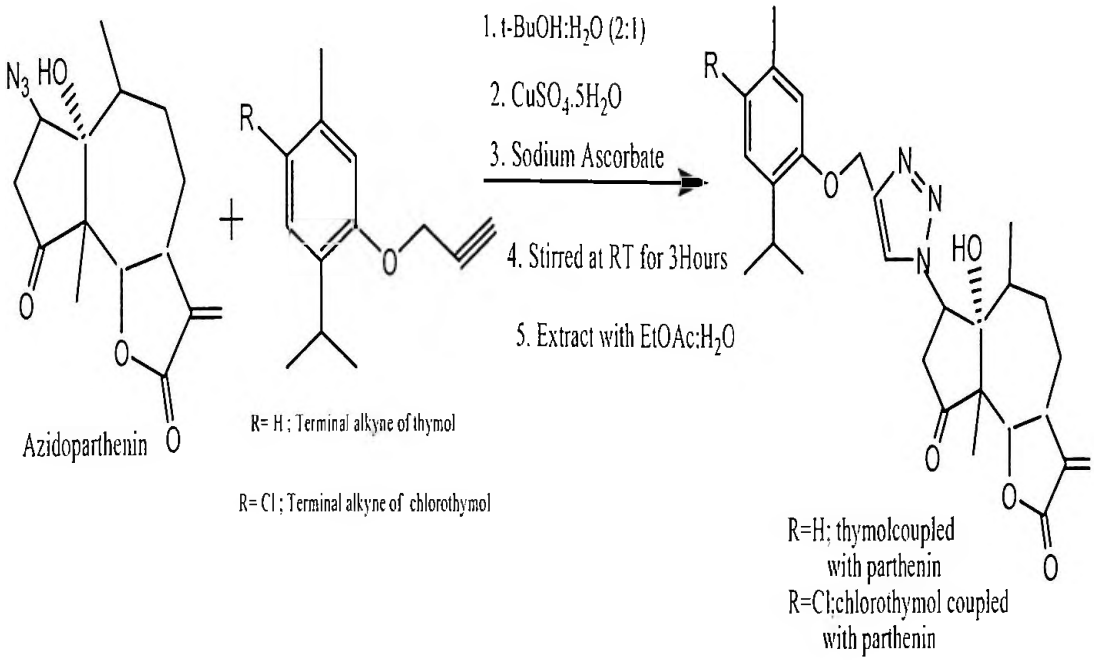
The sodium salt of thymol/chlorothymol was prepared by dissolving thymol/chlorothymol (1.00 g, 1 mmol) in 10% NaOH (10 ml), followed by addition of potassium carbonate(5.00 g, 1.5 mmol) and Propargyl bromide (1.90 g, 1.2 mmol) with acetone as solvent. Reaction mixture was refluxed at 80^oC for 3-4 hours under nitrogen atmosphere. Progress of reaction was monitored by TLC and GC analysis. The reaction mixture was filtered and extracted with ethyl acetate and water. The ethyl acetate layer was separated, dried over anhydrous sodium sulphate and filtered. The solvent was evaporated in vacuo and the crude product was subjected to column chromatography (silica gel 60-120 mesh, elution; Hexane/ethyl acetate gradient) to afford pure product as pale yellowish oily liquids.



Scheme 14: Synthesis of terminal alkynes of thymol and chlorothymol.

Azide-Alkyne "Click Reaction"

The terminal alkyne (0.10 g, 1.2 mmol) was added to a solution of azidoparthenin (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 hours using a magnetic stirrer. The progress of reaction was monitored through TLC analysis. The reaction mixture was filtered and extracted with ethyl acetate and water. The ethyl acetate layer was separated and the solvent was evaporated in vacuo, using nitrogen gas. The crude product was subjected to column chromatography (silica gel 100-200 mesh, elution; Hexane/ethyl acetate gradient) to afford pure product as colourless solid (0.05 g, 50%).



Scheme 15: Synthesis of 1, 2, 3-triazole derivatives of thymol-parthenin coupling compounds and their derivatives.

Synthesis of 1, 2, 3-Triazole Derivatives of Thymol from Aromatic Alkyl Azides

Different aromatic alkyl halides were converted to their azides and were reacted with the terminal alkynes of thymol and chlorothymol.

Synthesis of Aromatic Alkyl Azides

Various aromatic azides were prepared in excellent yield from aromatic alkyl chlorides by the use of sodium azide. These aromatic azides served as precursor for the synthesis of the triazole derivatives.

Preparation of 3- Chlorobenzyl Azide from 3-Chlorobenzyl Chloride

3-Chlorobenzyl Chloride (0.5 g) was dissolved in 10 ml DMSO. Sodium azide (0.25 g) was added as solid and the reaction was stirred overnight at ambient temperature. Water was added and the reaction product extracted into diethyl ether (3x10 ml). The combined ether layer was washed with brine (2x10 ml), dried and the solvent removed in vacuo to afford clear product. The

product was further purified by column chromatography with silica gel of dimension 100 nm and solvent gradient of Hexane/Ethyl acetate in an increasing polarity.

Preparation of 3- Fluorobenzyl Azide from 3-Fluorobenzyl Chloride

3-Chlorobenzyl Chloride (0.5 g) was dissolved in DMSO (10ml). Sodium azide (0.25 g) was added as solid and the reaction was stirred overnight at ambient temperature. Water was added and the reaction product extracted into diethyl ether (3x10ml). The combined ether layer was washed with brine (2x10ml), dried and the solvent removed in vacuo to afford clear product. The product was further purified by column chromatography with silica gel of dimension 100 nm and solvent gradient of Hexane/Ethyl acetate in an increasing polarity.

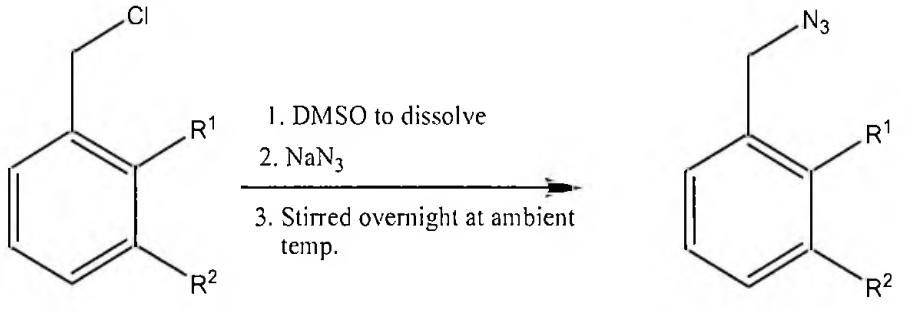
Preparation of Benzyl Azide from Benzyl Chloride

Benzyl Chloride (0.5 g) was dissolved in DMSO (10 ml). Sodium azide (0.25 g) was added as solid and the reaction was stirred overnight at ambient temperature. Water was added and the reaction product extracted into diethyl ether (3x10 ml). The combined ether layer was washed with brine (2x10 ml), dried and the solvent removed in vacuo to afford clear product. The product was further purified by column chromatography with silica gel of dimension 100 nm and solvent gradient of Hexane/Ethyl acetate in an increasing polarity.

Preparation of 2- Nitrobenzyl Azide from 2-Nitrobenzyl Chloride

2-Nitrobenzyl Chloride (0.5 g) was dissolved in DMSO (10 ml). Sodium azide (0.25 g) was added as solid and the reaction was stirred overnight at ambient temperature. Water was added and the reaction product extracted into diethyl ether (3x10 ml). The combined ether layer was washed with brine

(2x10 ml), dried and the solvent removed in vacuo to afford clear product. The product was further purified by column chromatography with silica gel of dimension 100 nm and solvent gradient of Hexane/Ethyl acetate in an increasing polarity



1. 3-Chlorobenzylchloride
2. 3-Fluorobenzylchloride
3. Chlorobenzylchloride
4. 2-Nitrobenzylchloride

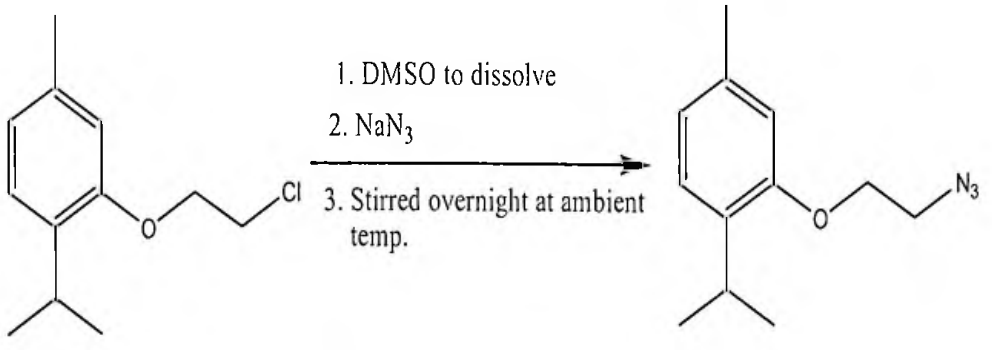
1. 3-Chlorobenzyl azide
2. 3-Fluorobenzyl azide
3. Chlorobenzyl azide
4. 2-Nitrobenzyl azide

1. $R^1 = H$, $R^2 = Cl$
2. $R^1 = H$, $R^2 = F$
3. $R^1 = H$, $R^2 = H$
4. $R^1 = NO_2$, $R^2 = H$

Scheme 16: Synthesis of aromatic alkyl azides from benzyl chloride and substituted benzyl chlorides.

Preparation of 2-isopropyl-5-methylphenoxyazidoethane from 2-isopropyl-5-methylphenoxy chloroethane

2-isopropyl-5-methylphenoxyChloroethane (0.5 g, 1 mmol) was dissolved in DMSO (10 ml). Sodium azide (0.25 g, 0.5 mmol) was added as solid and the reaction was stirred overnight at ambient temperature. Water was added and the reaction product extracted into diethyl ether (3x10 ml). The combined ether layer was washed with brine (2x10 ml), dried and the solvent removed in vacuo to afford clear product. The product was further purified by column chromatography with silica gel of dimension 100 nm and solvent gradient of Hexane/Ethyl acetate in an increasing polari



Scheme 17: Synthesis of 2-isopropyl-5-methylphenoxy azido ethane.

Synthesis of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-chloro-methylbenzene (TM 8A)

The terminal alkyne of thymol (0.10 g, 1.2 mmol) was added to a solution of 3- Chlorobenzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature for 3hours using a magnetic stirrer. The progress of reaction was monitored through TLC analysis. After completion, the reaction mixture was filtered and extracted with ethyl acetate and water. The ethyl acetate layer was separated and the solvent was evaporated in vacuo, using nitrogen gas. The crude product was subjected to column chromatography (silica gel 100-200 mesh, elution; Hexane/ethyl acetate gradient) to afford pure product as pale greenish waxy liquid. The pure product was characterised on the basis of GC-MS (EI & CI), LC-QTOF, FTIR, ^{1}H-NMR & ^{13}C-NMR.

Synthesis of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-fluoro-methylbenzene(TM 8B)

The terminal alkyne of thymol (0.10 g, 1.2 mmol) was added to a solution of 3- Fluorobenzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate

(0.10 g, 2 mmol) were added to the reaction mixture. The process was followed as in TM 8A above and the product recovered as pale greenish waxy liquid.

Synthesis of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene (TM 8C)

The terminal alkyne of thymol (0.10 g, 1.2 mmol) was added to a solution of Benzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The process was followed as in TM 8A above and the product recovered as pale yellowish oily liquid.

Synthesis of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro- methylbenzene (TM 8G)

The terminal alkyne of thymol (0.10 g, 1.2 mmol) was added to a solution of 2-Nitrobenzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The process was followed as in TM 8A above and the product recovered as pale yellow solid.

Synthesis of 1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-chloro-methylbenzene (TM 8D)

The terminal alkyne of Chlorothymol (0.10 g, 1.2 mmol) was added to a solution of 3- Chlorobenzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 hours using a magnetic stirrer. The progress of reaction was monitored through TLC analysis. After completion, the reaction mixture was filtered and extracted with ethyl acetate and water. The ethyl acetate layer was separated and the solvent was

evaporated in vacuo, using nitrogen gas. The crude product was subjected to column chromatography (silica gel 100-200 mesh, elution; Hexane/ethyl acetate gradient) to afford pure product as pale greenish waxy liquid. The pure product was characterised on the basis of GC-MS (EI & CI), LC-QTOF, FTIR, ^{1}H-NMR & ^{13}C-NMR.

Synthesis of 1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-fluoro-methylbenzene (TM 8E)

The terminal alkyne of Chlorothymol (0.10 g, 1.2 mmol) was added to a solution of 3- Fluorobenzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The process was followed as in TM 8D above and the product was recovered as dark brownish waxy liquid.

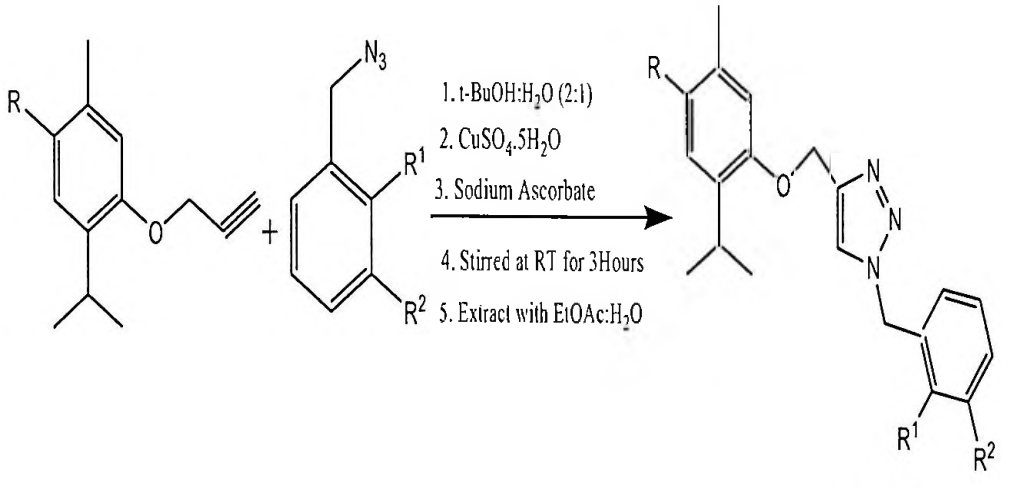
Synthesis of 1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene (TM 8F)

The terminal alkyne of Chlorothymol (0.10 g, 1.2 mmol) was added to a solution of Benzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The process was followed as in TM 8D above and the product was recovered as pale yellowish solid.

Synthesis of 1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro-methylbenzene (TM 8H)

The terminal alkyne of Chlorothymol (0.10 g, 1.2 mmol) was added to a solution of 2- Nitrobenzyl azide (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The process

was followed as in TM 8D above and the product was recovered as pale yellowish solid.



1. TM 8A R = H , R^1 = H , R^2 = Cl 2. TM 8B R = H , R^1 = H , R^2 = F

3. TM 8C R = H , R^1 = H , R^2 = H 4. TM 8D R = Cl , R^1 = H , R^2 =Cl

5. TM 8E R = Cl , R^1 = H , R^2 = F 6. TM 8F R = Cl , R^1 = H , R^2 = H

7. TM 8G R = H , R^1 = NO_2 , R^2 = H 8. TM 8H R = Cl , R^1 = NO_2 , R^2 = H

Scheme 18: Synthesis of 1, 2, 3-triazole derivatives of thymol with aromatic /aromatic substituted nucleus.

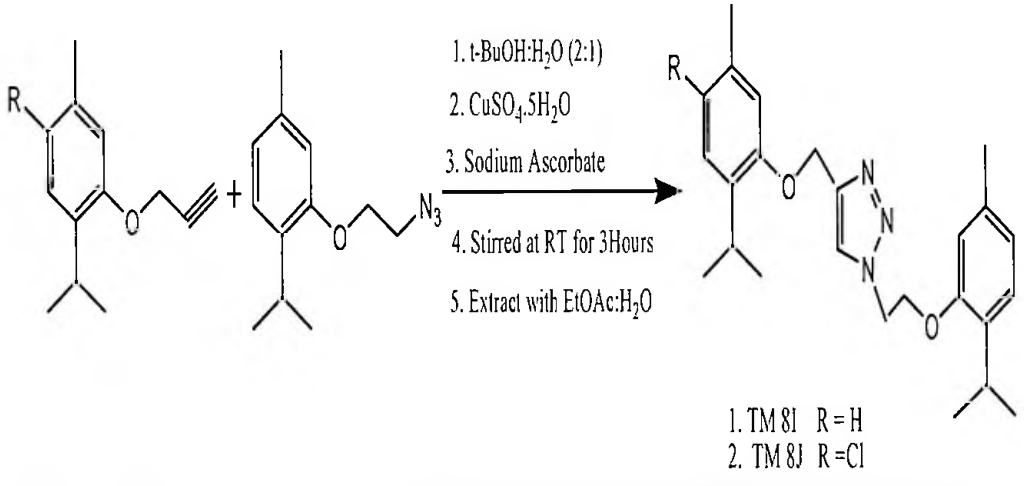
Synthesis of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-isopropyl-5-methyl-phenoxyethane (TM 8I)

The terminal alkyne of thymol (0.10 g, 1.2 mmol) was added to a solution of 2-isopropyl-5-methylphenoxyazidoethane (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 hours using a magnetic stirrer. The progress of reaction was monitored through TLC analysis. After completion, the reaction mixture was filtered and extracted with ethyl acetate and water. The ethyl acetate layer was separated and the solvent was evaporated in vacuo, using nitrogen gas. The crude product was subjected to column chromatography (silica gel 100-200 mesh, elution;

Hexane/ethyl acetate gradient) to afford pure product as yellowish viscous oily liquid. The pure product was characterised on the basis of GC-MS (EI & CI), LC-QTOF, FTIR, ^{1}H-NMR & ^{13}C-NMR.

Synthesis of 1-|4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-isopropyl-5-methyl-phenoxyethane (TM 8J)

The terminal alkyne of Chlorothymol (0.10 g, 1.2 mmol) was added to a solution of 2-isopropyl-5-methylphenoxyazidoethane (0.05 g, 1 mmol) in t-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and Sodium Ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The process was followed as in TM 8I above and the product was recovered as yellowish viscous liquid.



Scheme 19: Synthesis of 1, 2, 3-triazole derivatives of thymol coupled with an ether thymol derivative.

Synthesis of 6-Methyl-3-isopropyl-2-hydroxybenzaldehyde(TM 3B)

This procedure is an adaptation of the Duff reaction. A 2-L, three-necked, round-bottomed flask equipped with a mechanical overhead stirrer, reflux condenser, and thermometer was charged with (5 g, 0.033 mol) of thymol, (9.3 g, 0.065 mol, 2 eq) of hexamethylenetetramine, and 15 mL of glacial acetic acid. Complete dissolution results within minutes after stirring is initiated. The reaction mixture is heated to 130°C over a period of 60 min and

the temperature was diligently maintained within a range of 125-135°C for 2 hours with continuous stirring. The reaction mixture was then cooled to 75-80°C and aqueous sulfuric acid [15 mL of 33% (w/w)] was added with stirring while the temperature was maintained below 100°C. The resulting mixture was then heated to reflux (105-110°C) for 30-60 min, cooled to 75-80°C and transferred to a 1-L separatory funnel wrapped with electrical heating tape. The phases were allowed to separate while the temperature is maintained at 75-80°C; the lower aqueous phase was drawn off. The organic layer was transferred to an Erlenmeyer flask and cooled to 50°C, at which point methanol (10 mL) was added with stirring. The mixture was cooled to room temperature, then to ≤5°C with an ice bath and maintained at that temperature for 1 hr with continued stirring. The product was recovered was a yellowish oily liquid which was collected and washed with 30 mL of cold (≤5°C) methanol. This was dried under nitrogen gas for about 30 minutes to remove most of the solvent.

Screening for Biological Activity of Ether and Ester Derivatives of Thymol

Larvicidal Assay

Materials/Items for Test

1. Laboratory-reared mosquito larvae of known age or instar (reference strains or F1 of field-collected mosquitoes)
2. One pipette delivering 100–1000 µl.
3. Disposable tips (100 µl, 500 µl) for measuring aliquots of dilute solutions.
4. Five 1 ml pipettes for insecticides and one for the control.
5. Three droppers with rubber suction bulbs.

6. Data recording forms
7. Disposable cups (preferred as they avoid contamination) or Beakers of capacity 200 ml (holding 100 ml)
8. Graduated measuring cylinder.
9. Log–probit software
10. Pipettes with disposable tips.

Preparation of Stock Solutions or Suspensions and Test Concentrations for larvicidal assay

The technical materials were dissolved in dimethylsulphoxide (DMSO) to prepare dilute solutions for laboratory testing. About 10 ml of 1% stock solution of the technical materials were prepared by dissolving 100 mg of the technical materials in 10ml of DMSO.

These solutions were kept in a screw-cap vial, with aluminium foil over the mouth of the vial. These were then shaken vigorously to dissolve or disperse the materials in the solvent.

The stock solution is then serially diluted (ten-fold) in DMSO (2 ml stock solution to 18 ml DMSO). Test concentrations were obtained by the addition of the appropriate volume of the diluted stock solution to 100ml chlorine-free or distilled water in a 200 ml beakers.

Larvicidal Activity

Larvicidal activity was carried out as described by (WHO 2005; 2013) with minor modifications as described by Rahuman,Venkatesan & Gopalakrishnan, 2008; Wachira *et al.*, 2014 using third instar larvae of the female *Anopheles gambiae s.s.*

Four different concentrations 12.5, 25.0, 50.0 and 100.0 mg/L were tested, after the mosquito larvae were initially exposed to a wide range of test

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7. Disposable cups (preferred as they avoid contamination) or Beakers of capacity 200 ml (holding 100 ml)
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Four different concentrations 12.5, 25.0, 50.0 and 100.0 mg/L were tested, after the mosquito larvae were initially exposed to a wide range of test

concentrations and a control to find out the activity range of the materials under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range of four concentrations (12.5, 25.0, 50.0 and 100.0 mg/L), yielding between 10% and 95% mortality in 12 hours or 24 hours was used to determine LC_{50} and LC_{90} values.

An initial concentration of the individual test compounds were prepared by dissolving 100 mg of the material in 10ml of DMSO resulting in a stock solution of 10 mg/ml. The DMSO used as a solvent system for the dissolution of the test compounds served as a control.

Batches of 20 third instar larvae were transferred by means of droppers to 200ml beakers, each containing 100 ml of water. Small, unhealthy or damaged larvae were removed and replaced.

The appropriate volume of dilution was added to 100ml water in the beakers to obtain the desired target dosage starting with the lowest concentration. Four replicates were set up for each concentration for the various test compounds and controls were also set up simultaneously with tap water with which DMSO was added and the other tap water only. The test containers were held at 25-28°C and preferably a photoperiod of 12 hour light followed 12 hour dark (12L: 12D).

Larval mortality was recorded, whereby the number of dead larvae in each test was counted and removed after 12 h, 24 h, 48 h and 72 h of exposure. Moribund larvae were counted and added to dead larvae for calculating percentage mortality.

Adulticidal Assay

Materials/Items for test

1. 40 Cages of size ((15 × 15 × 15 cm)
2. filter paper (Whatman No. 1)
3. 6% Glucose solution
4. Laboratory-reared mosquitoes of known age of 3-5days (reference strains or F1 of field-collected mosquitoes)
5. One pipette delivering 100–1000 µl.
6. Disposable tips (100 µl, 500 µl) for measuring aliquots of dilute solutions.
7. Five 1 ml pipettes for insecticides and one for the control.
8. Three droppers with rubber suction bulbs.
9. Data recording forms
10. Graduated measuring cylinder.
11. Log–probit software
12. Pipettes with disposable tips

Preparation of Stock Solutions or Suspensions and Test Concentrations for adulticidal assay

The technical materials were dissolved in dimethyl sulfoxide (DMSO) to prepare dilute solutions for laboratory testing. About 10 ml of 1% stock solution of the technical materials were prepared by dissolving 100 mg of the technical materials in 10 ml of DMSO. These solutions were kept in a screw-cap vial, with aluminium foil over the mouth of the vial. These were then shaken vigorously to dissolve or disperse the materials in the solvent.

The stock solution is then serially diluted (ten-fold) in DMSO (2ml stock solution to 18ml DMSO). Test concentrations were obtained by the addition of

the appropriate volume of the diluted stock solution to 20ml 6% sugar in a 20ml vial.

Adulticidal Activity

Mosquitoes used for the experiments were obtained from established laboratory-reared colonies of *An. gambiae s.s.* (Mbita strain) at the International Centre of Insect Physiology and Ecology (ICIPE) Duduville campus, Nairobi, Kenya. The strain was initially collected as larvae from anopheline pools at Mbita Point, Suba District, Nyanza County in Western Kenya in April 2016. Larvae were reared in plastic trays (39 × 28 × 14 cm deep) in an insectary at a density of about 500 larvae per 3 L of distilled water. The rearing room was maintained at 32 ± 2°C, and 52% relative humidity (R.H.). The larvae were fed daily on (3 mg/larvae/day) Tetramin® fish food (Tetra, Germany). The adult mosquitoes were kept in cubic cages (30 × 30 × 30 cm) in a separate room maintained at 26 ± 2°C, 70–80% R.H. with a photoperiod of LD 12:12 h, the light being provided by a fluorescent lamp (40 watt). Both male and female mosquitoes were kept together after emerging and were separated during the assay. Mosquitoes were fed on 6% glucose solution *ad libitum* after emergence. The conditions in the bioassay rooms were the same as those of the rearing room.

Adulticidal tests were carried out using feeding assays as described by Wachira *et al.*, 2014 with minor modification. The female *An. gambiae s.s.* (3–5 days old) previously starved for 12 h were released into the experimental cages (15 × 15 × 15 cm) and left to acclimatize for 1 h. 20 adult mosquitoes were placed in a single cage. The prepared concentrations of test compounds and controls were then introduced into the centre of their respective cages and

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the mosquitoes fed on the test and control solutions through an immersed rolled up filter paper (Whatman No. 1) with 5 cm of it exposed above the top of the 20 mL vial. The female An. gambiae s.s were fed on the test compounds dissolved in dimethyl sulfoxide (DMSO) with the various concentrations prepared in 6% sugar solution contained in a 20 mL vial, while the control groups fed on a similar test compound-free solution, water alone, no food and water and lastly food with DMSO.

Four different concentrations; 12.5, 25.0, 50.0 and 100.0 mg/L of the test compounds were tested in three replicates per dose. The control solutions were 6% sugar solution only, 6% sugar solution with 1% DMSO, Again, to find out whether the mosquitoes were feeding, two other groups of cages were prepared with 6% sugar solution without water and another without food (6% sugar solution) and water. Daily mortality was recorded in all the mosquito groups for seven consecutive days.

Data Analysis/ Statistical Analysis

Data from all replicates were pooled together for analysis. The mortality data were subjected to probit analysis to calculate lethal concentration values (LC_{50}, LC_{90}) and lower and upper 95% fiducial limits. LC_{50}, LC_{90} and Chi-Square values were calculated using the EPA (U.S Environmental Protection Agency) computer probit analysis program (version 1.5). The potency of the test chemicals against the larvae of a particular vector and strain as well as the adult female mosquitoes can then be compared with the LC_{50} or LC_{90} values of other insecticides.

General Instrumentation Experimental Procedure

Mass Spectral Analysis (MS)

The mass spectra analysis were obtained at the Chemical Ecology Unit of the International Centre of Insects Physiology and Ecology (ICIPE), Nairobi-Kenya.

The synthesised compounds were analysed by coupled gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was carried out in the splitless injection mode using an Agilent Technologies 7890 gas chromatograph coupled to a 5975C inert XL EI/CI mass spectrometer (EI, 70 eV, Agilent, Palo Alto, CA) equipped with an HP-5 column (30 m × 0.25 mm ID × 0.25 μm film thickness, Agilent, Palo Alto, CA). Helium was used as the carrier gas at a flow rate of 1.2 ml/min. The oven temperature was held at 35°C for 3 min, then programmed to increase at 10°C/min to 280°C and maintained at this temperature for 10 min.

A high resolution (Q-TOF) mass spectroscopy instrument, SYNAPT G2-S. (Thermo Fisher Scientific, UK), with an electrospray ionization probe was used for accurate measurement over the full mass range of m/z 50 -2000. Nano-electrospray analyses were performed in positive ionization mode by using NanoMate to deliver samples diluted into MeOH + 10% NH_4OAc.

Infra-Red Spectra Analysis (IR)

The Thermo-Nicolet Avatar 370 Fourier Transform Infra-Red Spectrophotometer was used for obtaining all the Infra-Red spectra data over the range of 4000 – 500 cm^{-1}.

Nuclear Magnetic Resonance Analysis (NMR)

The NMR Spectra were determined using the Bruker FT-NMR Spectrometer at 500 and 125 MHz for ^{1}H-NMR and ^{13}C-NMR respectively. The chemical shifts are quoted in parts per million (ppm) relative to the signal of tetramethylsilane (TMS), which was used as an external standard for proton (^{1}H-NMR) and carbon-13 (^{13}C-NMR). Standard and coupling constants (J) values were measured in hertz (Hz). All NMR were run in deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulphoxide (DMSO) as solvent unless stated otherwise.

Summary

The hydroxyl (-OH) group attached to thymol was successfully modified into its ether and ester functional groups by Williamson etherification and esterification reactions respectively. Again, the incooperation of the triazole moiety as an attractive linker was achieved by the click reaction between O-Propargyl terminal alkyne of thymol with various substituted benzyl azide and an azido ether derivative of thymol. Their structures were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, LC-QTOF/MS and GC-MS-EI/CI. Lastly, thymol as an alkylating agent and its derivative, chlorothymol were coupled to parthenin. These were characterised on the basis of IR and LC-QTOF/MS.

The larvicidal and adulticidal potency of the ester and ether derivatives of thymol were screened against the larvae and the adult mosquito of *Anopheles gambiae s.s* at different concentrations ranging from 0.0125mg/ml to 0.1mg/ml.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

The various synthetic method employed, esterification reaction, Williamson etherification reaction, the Click Reaction were all successful. These synthesised derivatives of thymol were all characterised with the appropriate specral data. Thus the thymol derivatives were recovered in moderate to excellent yields and of high purity. The biological activity of the synthesised ester and ether derivatives of thymol were examined on the larvae and adult mosquito of *Anopheles gambiae s.s.* Most of the derivatives showed an enhanced activity compared to the reference compound, thymol.

Results

The synthesised ester derivatives of thymol were recovered in high yields and purity. Most of the ester derivatives, TM 1A (yield: 1.30g; 93%), TM 1B (yield: 1.25g; 91%), TM 1C (yield: 1.30g; 92%), TM 1D (yield: 1.80g; 96%), TM 1E (yield: 1.70g; 95%), TM 1I (yield: 1.20g; 96%), TM 1K (yield: 2.00g; 98%), TM 1M (yield: 1.20g; 92%), TM 1P (yield: 1.45g; 94%), M 1R (yield: 2.20g; 96%) and TM 1U (yield: 1.20g; 92%) were obtained as colourless, oily liquids at room temperature with the exception of TM 1Q (yield: 2.00g; 97%) which was obtained as an oily semi-solid substance. Compounds TM 1F (yield: 1.00g; 90%), TM 1G (yield: 1.10g; 90%), TM 1L (yield: 0.90g; 91%) and TM 1N (yield: 1.00g; 90%) were obtained as pale yellowish to yellowish oily liquids.

The synthesised ether derivatives of thymol were also recovered in high yields and purity. All the ether derivatives TM 2C (yield: 0.78g; 92%), TM 2D

(yield: 0.82g; 93%), TM 2E (yield: 1.00g; 95%), TM 2F (yield: 0.95g; 92%), TM 2I (yield: 1.20g; 94%), TM 2K (yield: 0.80g; 88%), TM 2N (yield: 0.75g; 85%), TM 2O (yield: 1.30g; 93%) and TM 2P (yield: 1.00g; 90%) were obtained as colourless, oily liquids at room temperature.

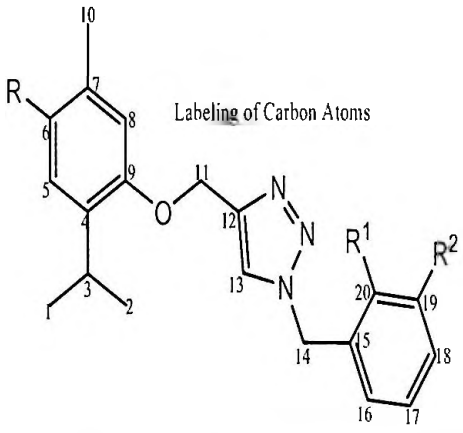
The parthenin-thymol coupling products TM 10A (yield: 0.30g; 63%) and TM 10B (yield: 0.38g; 65%) were obtained as solids and of moderate yields. The triazole derivatives of thymol were also recovered in moderate to high yields and were of high purity. TM 8A (Yield: 0.65g; 60%), TM 8B (yield: 0.60g; 67%), TM 8C (yield: 0.54g; 75%), TM 8D (Yield: 0.56g; 55%), TM 8E (Yield: 0.53g; 70%), TM 8F (Yield: 0.78g; 92%), TM 8G (Yield: 1.2g; 90%), TM 8H (Yield: 0.95g; 88%), TM 8I (Yield: 0.22g; 65%), TM 8J (Yield: 0.25g; 68%)

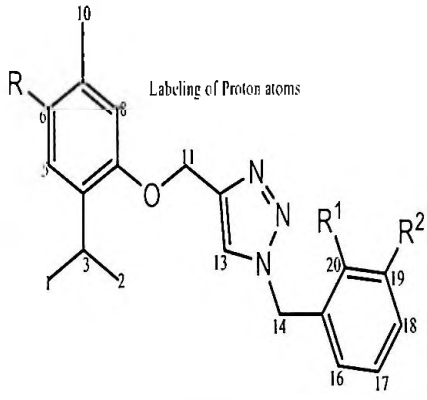
The characterisation of the synthesised compounds as well as the larvicidal and adulticidal potency of the ester and ether derivatives of thymol are reported and discussed in this section.

Table 11: IR Spectral Data for the Synthesised 1, 2, 3 - Triazole Derivatives of Thymol

cm^{-1}	Ar-H Stretching	C-H in alkyl region CH_3 and $-CH_2$	-N=N-	-N-N-	-CH	-C-O	C=O	-OH
TM 8A	3158.2, 3085.4	2957.7,2927.7,2885.6, 2867.0	1885.5	1610.1	1432.8	1241.9		
TM 8B	3156.4, 3080.0	2963.4, 2938.2,2872.9	1793.0,1730.7	1617.3	1450.2	1249.1		
TM 8C	3150.2,3087.5, 3031.3	2961.6, 2928.5, 2868.8	1733.2,1698.8	1610.1	1456.0	1256.0		
TM 8D	3155.7, 3073.7	2982.5, 2959.5,2927.8, 2868.3	1738.8	1601.9	1497.2, 1460.1	1248.2		
TM 8E	3117.1, 3075.5	2967.7, 2924.9, 2872.2	1729.3	1603.4	1489.5, 1451.0	1245.4		
TM 8F	3124.4, 3076.5	2962.2, 2926.6,2875.0	1762.8	1603.4	1495.8, 1457.4	1243.9		
TM 8G	3132.4, 3088.8	2981.0,2926.6, 2886.6, 2867.2	1733.3	1609.7	1454.4, 1444.5	1251.9		
TM 8H	3152.8, 3068.7 3011.8	2980.9, 2960.2, 2918.5,2864.2	1745.5	1615.5 1607.2	1463.7, 1436.5	1248.7		
TM 8I	3159.2, 3031.5	2958.6, 2923.6, 2868.9	1880.4, 1698.9	1611.4	1455.5, 1413.3	1252.6		
TM 8J	3060.3, 3033.7	2959.3, 2924.1, 2868.3	1742.7,1704.1	1610.8	1451.5	1245.1		
TM 10A		2926.5, 2870.9	1639.5, 1716.9	1611.9	1448.6	1287.7, 1246.4	1750.8	3274.1
TM 10B		2926.7, 2871.2	1721.8, 1637.5	1605.2	1445.4	1242.7	1750.5	3354.9

Source: Laboratory work (2016)





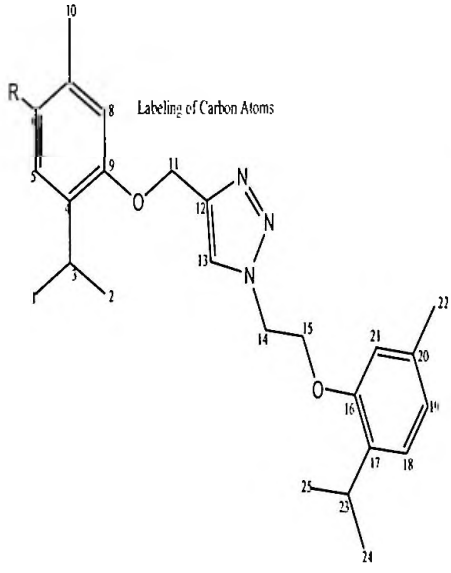
1. TM 8A R = H , R^1 = H , R^2 = Cl 2. TM 8B R = H , R^1 = H , R^2 = F

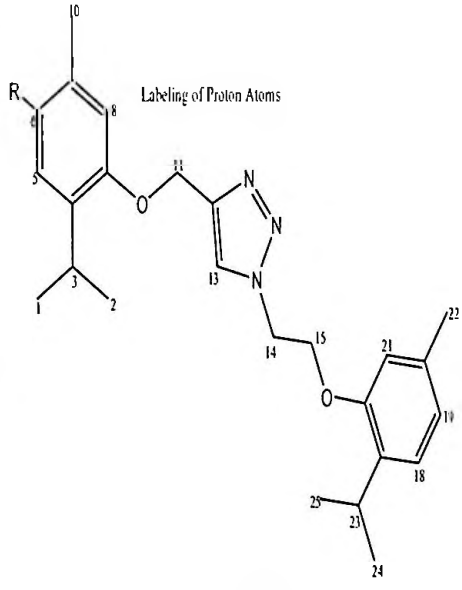
3. TM 8C R = H , R^1 = H , R^2 = H 4. TM 8D R = Cl , R^1 = H , R^2 = Cl

5. TM 8E R = Cl , R^1 = H , R^2 = F 6. TM 8F R = Cl , R^1 = H , R^2 = H

7. TM 8G R = H , R^1 = NO_2 , R^2 = H 8. TM 8H R = Cl , R^1 = NO_2 , R^2 = H

Figure 9: Labels of carbon and proton of 1, 2, 3-triazole derivatives of thymol (TM 8A - TM 8H).





TM 8I R = H **2. TM 8J R = Cl**

Figure 10: Labels of carbon and proton of 1, 2, 3-triazole derivatives of thymol (TM 8I and TM 8J).

Table 12: ^{13}C-NMR Spectral Data for Compounds TM8A, TM8B, TM8C, TM8G and TM 8I

^{13}C (ppm)	TM 8A	TM 8B	TM 8C	TM 8G	TM 8I
1	21.36	21.32	22.76	21.26	21.36[a]
2	21.36	21.32	22.76	21.36	21.36[a]
3	26.57	26.63	26.54	26.62	26.52[b]
4	134.32	134.29	136.47	130.71	133.98
5	130.44	129.96	126.03	129.70	126.12
6	116.49	123.51	121.88	125.43	122.22
7	136.50	136.50	134.65	134.43	136.59
8	112.94	115.89	112.95	112.88	112.30
9	155.26	155.22	155.34	155.21	155.33
10	22.81	22.77	22.80	22.79	22.81[c]
11	62.52	64.38	62.58	62.48	66.49
12	136.59	136.98	145.34	136.49	144.97
13	121.95	121.94	127.80	121.95	123.45
14	53.61	53.59	54.21	50.97	53.47
15	135.01	136.92	134.33	130.40	62.48
16	128.99	130.78	129.14[a]	130.25	154.18
17	128.04	126.05	128.78[b]	123.51	134.24
18	126.05	114.85	122.34	126.09	126.01
19	135.01	163.99	128.01[b]	134.32	121.82
20	126.06	116.48	129.04[a]	147.40	136.50
21					112.71
22					22.74[c]
23					26.48[b]
24					21.29[a]
25					21.29[a]

Source: Laboratory work (2016)
Those high-lighted with the same letter are interchangeable

Table 13: ^{13}C-NMR Spectral Data for Compounds TM8D, TM8E, TM8F, TM8H and TM8J

^{13}C (ppm)	TM 8D	TM 8E	TM 8F	TM 8H	TM 8J
1	22.61	22.63	22.59	20.91	22.74[a]
2	22.61	22.72	22.59	20.91	22.74[a]
3	26.61	26.60	26.58	26.53	26.58[b]
4	135.03	130.84	133.74	134.35	133.79
5	126.75	126.75	126.68	129.67	126.15
6	126.35	126.49	126.24	126.56	126.73
7	133.80	130.77	133.74	133.64	133.94
8	114.66	114.78	114.74	114.72	114.44
9	153.80	153.68	153.90	153.82	154.77
10	20.06	20.33	20.02	14.07	20.06[c]
11	62.72	64.60	62.72	62.48	66.47
12	136.69	136.69	136.73	144.53	136.62
13	118.03	122.22	116.55	125.29	123.55
14	53.62	NS	54.16	50.87	53.45
15	136.50	133.85	134.65	130.71	62.71
16	128.03	123.73	129.12	130.62	153.89
17	130.46	130.10	128.77[a]	126.06	133.79
18	126.04	113.78	122.67	130.14	126.27
19	133.80	163.93	128.00[a]	136.69	122.27
20	129.03	116.12	129.12	147.42	133.94
21					112.30
22					21.29[c]
23					26.48[b]
24					22.62[a]
25					22.62[a]

Source: Laboratory work (2016)

NS= not seen ; those high-lighted with the same letter are interchangeable.

Table 13: ^{13}C-NMR Spectral Data for Compounds TM8D, TM8E, TM8F, TM8H and TM8J

^{13}C (ppm)	TM 8D	TM 8E	TM 8F	TM 8H	TM 8J
1	22.61	22.63	22.59	20.91	22.74[a]
2	22.61	22.72	22.59	20.91	22.74[a]
3	26.61	26.60	26.58	26.53	26.58[b]
4	135.03	130.84	133.74	134.35	133.79
5	126.75	126.75	126.68	129.67	126.15
6	126.35	126.49	126.24	126.56	126.73
7	133.80	130.77	133.74	133.64	133.94
8	114.66	114.78	114.74	114.72	114.44
9	153.80	153.68	153.90	153.82	154.77
10	20.06	20.33	20.02	14.07	20.06[c]
11	62.72	64.60	62.72	62.48	66.47
12	136.69	136.69	136.73	144.53	136.62
13	118.03	122.22	116.55	125.29	123.55
14	53.62	NS	54.16	50.87	53.45
15	136.50	133.85	134.65	130.71	62.71
16	128.03	123.73	129.12	130.62	153.89
17	130.46	130.10	128.77[a]	126.06	133.79
18	126.04	113.78	122.67	130.14	126.27
19	133.80	163.93	128.00[a]	136.69	122.27
20	129.03	116.12	129.12	147.42	133.94
21					112.30
22					21.29[c]
23					26.48[b]
24					22.62[a]
25					22.62[a]

Source: Laboratory work (2016)

NS= not seen ; those high-lighted with the same letter are interchangeable.

Table 15: ^{1}H-NMR Assignments for Compounds TM8D, TM8E, TM8F, TM8H and TM8J

^{1}H (δ) ppm	TM 8D	TM 8E	TM 8F	TM 8H	TM 8J
1-H	1.15 3H d	1.17 3H d	1.13 3H d	1.07 3H d	1.20 3H d
2-H	1.17 3H d	1.17 3H d	1.15 3H d	1.08 3H d	1.20 3H d
3-H	3.23 1H m	3.26 1H m	3.21 1H m	3.14 1H m	3.30[a] 1H m
4	-	-	-	-	-
5-H	7.16 1H d	7.00 1H s	6.84 1H s	7.04 1H s	5.32 1H s
6-H	-	-	-	-	-
7	-	-	-	-	-
8-H	7.31 1H s	7.29 1H s	7.13 1H s	7.29 1H s	7.29 1H s
9	-	-	-	-	-
10-H	2.33 3H s	2.35 3H s	2.31 3H s	2.25 3H s	2.34[b] 3H s
11-H	5.55 2H s	5.67 2H s	5.54 2H s	5.89 2H s	5.21 2H s
12	-	-	-	-	-
13-H	7.63 1H s	7.63 1H s	7.56 1H s	7.73 1H s	7.80 1H s
14-H	5.20 2H s	5.63 2H s	5.16 2H s	5.12 2H s	4.40 2H d
15	-	-	-	-	4.84 2H s
16-H	7.28 1H d	7.38 1H d	7.35 1H d	-	-
17-H	7.33 1H t	7.06 1H t	7.37 1H t	6.80 1H s	-
18-H	NS	7.34 1H d	7.28 1H t	7.49 1H t	7.11 1H d
19	-	-	7.39 1H t	7.56 1H t	6.81 1H d
20-H	6.84 1H s	6.95 1H s	7.26 1H d	6.96 1H d	-
21-H					6.63 1H s
22-H					2.33[b] 3H s
23-H					3.20[a] 1H m
24-H					1.30 3H d
25-H					1.30 3H d

Source: Laboratory work (2016); NS= not seen
Those high-lighted with the same letter are interchangeable

H); 5.56 (s, 2H, O-CH_2); 5.22 (s, 2H, N-CH_2); 3.24 (m, 1H, CH); 2.32 (s, 3H, Ar-CH_3), 1.17 (d, 3H, J = 6.9 Hz, -CH_3); 1.12 (d, 3H, J = 6.8 Hz, -CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 163.99 (Ar-C), 155.22 (Ar-C), 136.98 (triazole Ar-C; r), 136.92 (Ar-C), 136.50 (Ar-C), 134.29 (Ar-C), 130.78 (Ar-C), 129.96 (Ar-C), 126.05 (Ar-C), 123.51(Ar-C), 121.94(triazole Ar-C;), 116.48 (Ar-C), 115.89 (Ar-C), 114.85 (Ar-C), 64.38 (-CH_2), 53.59 (-CH_2), 26.63 (-CH), 22.77 (-CH_3), 21.32 (-CH_3), 21.32 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 339, 340 and 378 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 339, 340, 354 and 368 respectively. MS (EI): $[M]^+$ 339, 296, 268, 191, 162, 109.

1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene (TM 8C)

Reovered as a pale yellowish oily liquid with a yield 0.54g (75%). IR (KBr, cm^{-1}): 3150.2, 3087.5, 3031.3, 2961.6, 2928.5, 2868.8, 1733.2, 1698.8, 1610.1, 1579.0, 1557.5, 1456.0, and 1256.8. ^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.54 (s, 1H, trizole H); 7.41 (t, 1H, Ar-H); 7.40 (t, 1H, Ar-H); 7.31 (t, 1H, J = 1.8 Hz, Ar-H); 7.29 (d, 1H, J = 1.2 Hz, Ar-H), 7.12 (d, 1H, J = 8.0 Hz, Thymol-Ar-H); 6.80 (d, 1H, J = 5.8 Hz, Thymol-Ar-H); 6.79 (s, 1H, Thymol-Ar-H); 5.57 (s, 2H, O-CH_2); 5.22 (s, 2H, N-CH_2); 3.25 (m, 1H, CH); 2.34 (s, 3H, Ar-CH_3), 1.18 (d, 3H, J = 6.9 Hz, -CH_3); 1.13 (d, 3H, J = 7.0 Hz, -CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$,, 500 MHz): δ_C 155.34 (Ar-C), 145.34 (Ar-C), 136.47 (Ar-C), 134.65 (Ar-C), 134.33 (Ar-C), 129.14 (Ar-C), 129.04 (Ar-C), 128.78 (Ar-C), 128.01 (Ar-C), 127.80 (Ar-C), 126.03 (Ar-C), 122.34 (Ar-C), 121.88 (Ar-C), 112.95 (Ar-C), 62.58 (-CH_2), 54.21 (-CH_2), 26.54 (-CH), 22.80(-CH_3), 22.76 (-CH_3), 22.76 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 321, 322 and 360 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and

$[M+C_2H_5]^+$ 321, 322, 336 and 350 respectively. MS (EI): $[M]^+$ 321, 278, 250, 173, 144, 91.

1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro-methylbenzene (TM 8G)

Recovered as pale yellow solid with a yield 1.2g, (90%). IR (KBr, cm^{-1}): 3132.4, 3088.8, 2981.0, 2951.3, 2921.7, 2886.6, 2867.2, 1733.3, 1609.7, 1577.3, 1520.1, 1504.1, 1454.4, 1444.5 and 1251.9 ^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.78 (s, 1H, triazole H); 7.64 (d, 1H, Ar-H); 7.63 (t, 1H, J = 6.7 Hz, Ar-H); 7.58 (t, 1H, J = 7.2 Hz, Ar-H), 7.29 (s, 1H, Thymol-Ar-H); 7.12 (d, 2H, J = 7.5 Hz, Ar-H); 7.08 (d, 2H, J = 7.6 Hz, Ar-H); 6.80 (d, 1H, J = 7.3 Hz, Thymol-Ar-H); 5.29 (s, 2H, O-CH_2); 5.99 (s, 2H, N-CH_2); 3.28 (m, 1H, CH); 2.34 (s, 3H, Ar-CH_3), 1.20 (d, 3H, J = 7.0 Hz, -CH_3); 1.19 (d, 3H, J = 7.0 Hz, -CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 155.21 (Ar-C), 147.40 (Ar-C), 136.49 (triazole Ar-C;), 134.43 (Ar-C), 134.32 (Ar-C), 130.71 (Ar-C), 130.40 (Ar-C), 130.25 (Ar-C), 129.70 (Ar-C), 126.09 (Ar-C), 125.43 (Ar-C), 123.51 (Ar-C), 121.95 (triazole Ar-C;), 112.88 (Ar-C), 62.48 (-CH_2), 50.97 (-CH_2), 26.62 (-CH), 22.97 (-CH_3), 21.36 (-CH_3), 21.36 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 366, 367 and 405 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 366, 367, 381 and 395 respectively. MS (EI): $[M]^+$ 366, 218, 189, 159, 136, 105

1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-isopropyl-5-methyl-phenoxyethane (TM 81)

Recovered as yellowish viscous oily liquid with a yield 0.22g (65%). IR (KBr, cm^{-1}): 3159.2, 3031.5, 2958.6, 2923.6, 2868.9, 1880.4, 1698.9, 1611.4, 1578.0, 1504.6, 1455.5, 1413.3 and 1252.6 ^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.80 (s, 1H, triazole-H); 7.29 (s, 1H, Thymol-Ar-H); 7.13 (s,1H, Thymol-Ar-

H); 7.11 (q, 2H, J = 7.8 Hz Thymol-Ar-H); 6.81 (d, 1H, *J* = 9.8 Hz, Thymol-Ar-H); 6.63 (s, 1H, Thymol-Ar-H), 5.54 (s, 2H, O-CH_2); 5.32 (t, 2H, *J* = 4.9 Hz, -CH_2-O); 5.23 (t, 2H, *J* = 5.0 Hz, N-CH_2-); 3.30 (m, 1H, -CH); 3.20 (m, 1H, -CH); 2.34 (s, 3H, Ar-CH_3); 2.33 (s, 3H, Ar-CH_3); 1.20 (d, 6H, *J* = 7.0 Hz, -CH_3); 1.20 (d, 6H, *J* = 7.0 Hz, -CH_3). $^{13}C\{^1H\}$ NMR $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 155.33 (Ar-C), 154.80 (Ar-C), 144.97 (Ar-C; triazole Ring), 136.59 (Ar-C), 136.50(Ar-C), 134.24 (Ar-C), 133.98 (Ar-C), 126.12 (Ar-C), 126.01 (Ar-C), 123.45 (Ar-C), 122.22(Ar-C; triazole Ring), 121.82 (Ar-C), 112.71 (Ar-C), 112.30 (Ar-C), 66.49 (-CH_2) 62.48 (-CH_2), 53.47 (-CH_2), 26.52 (-CH), 26.48 (-CH), 22.81 (-CH_3), 22.74 (-CH_3), 21.36 (-CH_3), 21.36 (-CH_3), 21.29 (-CH_3), 21.29 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 407, 408 and 446 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 407, 408, 422 and 436 respectively. MS (EI): $[M]^+$ 407, 364, 336, 258, 230, 188, 163, 135.

1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-chloro-methylbenzene (TM 8D)

Recovered as pale greenish waxy liquid with a yield 0.56 g (55%). IR (KBr, cm^{-1}): 3155.7, 3073.7, 2982.5, 2959.5, 2927.8, 2868.3, 1738.8, 1601.9, 1581.3, 1572.4, 1513.9, 1497.2, 1460.1, 1431.1 and 1248.2 ^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.63 (s, 1H, triazole H); 7.33 (t, 1H, *J* = 7.9 Hz, Ar-H); 7.28 (d, 1H, *J* = 9.4 Hz Ar-H) ; 7.16 (d, 1H, *J* = 8.9 Hz, Ar-H); 6.84 (s, 1H, Ar-H); 5.55 (s, 2H, O-CH_2); 5.20 (s, 2H, N-CH_2); 3.23 (m, 1H, CH); 2.33 (s, 3H, Ar-CH_3). 1.17 (d, 3H, *J* = 6.6 Hz, -CH_3), 1.15 (d, 3H, *J* = 6.6 Hz, -CH_3); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 153.80 (Ar-C), 136.69 (triazole Ar-C;), 136.50 (Ar-C), 135.03 (Ar-C), 133.80 (Ar-C), 133.80 (Ar-C), 130.46 (Ar-C), 129.03

(Ar-C), 128.03 (Ar-C), 126.75 (Ar-C), 126.35 (Ar-C), 126.04(Ar-C), 118.03 triazole (Ar-C;), 114.66 (Ar-C), 62.72 (-CH_2), 53.62(-CH_2), 26.61 (-CH), 22.61 (-CH_3), 22.61 (-CH_3), 20.06 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 389, 390 and 428 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 389, 390, 404 and 418 respectively. MS (EI): $[M]^+$ 389, 354, 318, 207, 178, 125, 91.

1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-fluoro-methylbenzene (TM 8E)

Recovered as pale brownish waxy liquid with a yield 0.53g, (70%). IR (KBr, cm^{-1}): 3117.1, 3075.5, 2967.7, 2924.9, 2872.2, 1729.3, 1603.4, 1590.2, 1513.3, 1489.5, 1451.0 and 1245.4 ^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.63 (s, 1H, triazole H); 7.38 (d, 1H, J = 5.8 Hz, Ar-H); 7.34 (d, 1H, J = 6.2 Hz Ar-H) ; 7.29 (s, 1H, Ar-H); 7.06 (t, 1H, J = 8.0 Hz Ar-H) 7.00 (s, 1H, Ar-H); 6.95 (s, 1H, Ar-H); 5.67 (s, 2H, O-CH_2); 5.63 (s, 2H, N-CH_2); 3.26 (m, 1H, CH); 2.35 (s, 3H, Ar-CH_3). 1.17 (d, 3H, J = 6.6 Hz, -CH_3), 1.17 (d, 3H, J = 6.6 Hz, -CH_3); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 163.93 (Ar-C), 153.68 (Ar-C), 136.69 (triazole Ar-C; Ring), 133.85 (Ar-C), 130.84(Ar-C), 130.77 (Ar-C), 130.10 (Ar-C), 126.75 (Ar-C), 126.49 (Ar-C), 123.73 (Ar-C), 122.22 (triazole Ar-C;), 116.12 (Ar-C), 114.78 (Ar-C), 113.78 (Ar-C), 64.60 (-CH_2), 54.80 (-CH_2), 26.60 (-CH), 22.72 (-CH_3), 22.63 (-CH_3), 20.33 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 373, 374 and 412 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 373, 374, 388 and 402 respectively. MS (EI): $[M]^+$ 373, 356, 338, 302, 184, 162, 109.

1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene (TM 8F)

Recovered as pale yellowish semi-solid with a yield 0.78g, (92%). IR (KBr, cm^{-1}): 3124.4, 3076.5, 3041.6, 2962.2, 2926.6, 2875.0, 1762.8, 1603.4, 1560.4, 1495.8, 1457.4, 1438.5 and 1243.9 ^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.56 (s, 1H, triazole H); 7.39 (t, 1H, J = 3.5 Hz, Ar-H); 7.37 (t, 1H, J = 7.0 Hz, Ar-H); 7.35 (d, 1H, J = 1.4 Hz, Ar-H); 7.28 (t, 1H, J = 5.6 Hz, Ar-H); 7.26 (d, 1H, J = 1.5 Hz, Ar-H); 7.13 (s, 1H, Thymol- Ar-H); 6.84 (s, 1H, Thymol-Ar-H) ; 5.54 (s, 2H, O-CH_2); 5.16 (s, 2H, N-CH_2); 3.21 (m, 1H, CH); 2.31 (s, 3H, Ar-CH_3). 1.15 (d, 3H, J = 7.0 Hz, -CH_3), 1.13 (d, 3H, J = 7.0 Hz, -CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 153.90(Ar-C), 136.73(triazole Ar-C;), 134.65(Ar-C), 133.74(Ar-C), 133.74(Ar-C), 129.12(Ar-C), 129.12 (Ar-C), 128.77 (Ar-C), 128.77 (Ar-C), 126.68 (Ar-C), 126.24 (Ar-C), 122.67 (Ar-C), 126.67 (triazole Ar-C;), 114.74 (Ar-C), 62.72 (-CH_2), 54.16 (-CH_2), 26.58 (-CH), 22.59 (-CH_3), 22.59 (-CH_3), 20.02 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 355, 356 and 394 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 355, 356, 370 and 384 respectively. MS (EI): $[M]^+$ 355, 320, 284, 169, 144, 91.

1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro-methylbenzene (TM 8H)

Recovered as pale yellowish solid with a yield 0.95g, (88%). IR (KBr, cm^{-1}): 3152.8, 3068.7, 3037.4, 3011.8, 2980.9, 2960.2, 2918.5, 2864.2, 1615.5, 1607.2, 1568.0, 1535.5, 1501.3, 1491.3, 1463.7, 1436.5 and 1248.7 ^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.73 (s, 1H, triazole H); 7.56 (d, 1H, J = 6.8 Hz, Ar-H); 7.49 (t, 1H, J = 7.4 Hz, Ar-H), 7.29 (s, 1H, Thymol-Ar-H); 7.04 (s, 1H, Thymol-Ar-H); 6.96 (t, 1H, J = 7.7 Hz, Ar-H); 6.80 (d, 1H, Ar-H); 5.89

(s, 2H, O-CH_2); 5.12 (s, 2H, N-CH_2); 3.14 (m, 1H, CH); 1.96 (s, 3H, Ar-CH_3), 1.08 (d, 3H, J = 6.9 Hz, -CH_3); 1.07 (d, 3H, J = 6.9 Hz, -CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 153.82(Ar-C), 147.42(Ar-C), 144.53 (triazole Ar-C;), 136.69 (Ar-C), 134.35 (Ar-C), 130.71 (Ar-C), 133.64 (Ar-C), 130.62 (Ar-C), 130.14 (Ar-C), 129.67 (Ar-C), 126.56 (Ar-C), 126.06 (Ar-C), 125.29 (Ar-C), 123.96 (triazole Ar-C;), 114.72 (Ar-C), 62.48 (-CH_2), 50.87 (-CH_2), 26.53 (-CH), 20.91 (-CH_3), 20.91 (-CH_3), 14.07 (-CH_3). HRMS (ESI): *m/z* $[M]^+$, $[M+1]^+$ and $[M+K]^+$ 400, 401 and 439 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 400, 401, 415 and 429 respectively. MS (EI): $[M]^+$ 400, 357, 329, 218, 189, 169, 136.

1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2- Isopropyl-5-methyl-phenoxyethane (TM 8J)

Recovered as yellowish viscous liquid with a yield 0.25g, (68%). IR (KBr, cm^{-1}): 3160.3, 3033.7, 2959.3, 2924.1, 2868.3, 1742.7, 1704.1, 1610.8, 1579.2, 1451.5 and 1245.1 .^1H NMR ($CDCl_3$, 500 MHz): δ_H 7.80 (s, 1H, triazole-H); 7.29 (s, 1H, Thymol-Ar-H); 7.11 (d, 1H, Thymol-Ar-H); 6.81 (d, 1H, J = 7.7 Hz, Thymol-Ar-H); 6.63 (s, 1H, Thymol-Ar-H); 5.32 (s, 1H, Thymol-Ar-H); 5.21 (s, 2H, O-CH_2); 4.84 (t, 2H, J = 4.9 Hz, -CH_2-O); 4.40 (t, 2H, J = 5.1 Hz, N-CH_2-); 3.30 (m, 1H, -CH); 3.20 (m, 1H, -CH); 2.34 (s, 3H, Ar-CH_3); 2.33 (s, 3H, Ar-CH_3); 1.30 (d, 6H, J = 7.0 Hz, -CH_3); 1.20 (d, 6H, J = 7.3 Hz, -CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$,, 500 MHz): δ_C 154.77(Ar-C), 153.89(Ar-C), 136.62 (triazole Ar-C;), 133.94 (Ar-C), 133.94 (Ar-C), 133.79(Ar-C), 133.79 (Ar-C), 126.73(Ar-C), 126.27 (Ar-C), 126.15(Ar-C), 123.55 (triazole Ar-C;), 122.27(Ar-C), 114.44 (Ar-C), 112.30 (Ar-C), 66.47(-CH_2) 62.71 (-CH_2), 53.45 (-CH_2), 26.58 (-CH), 26.48(-CH), 22.74 (-CH_3),

22.74 (-CH_3), 22.62 (-CH_3), 22.62 (-CH_3), 21.29 (-CH_3), 20.06 (-CH_3). HRMS (ESI): *m/z* $[M-1]^+$, $[M]^+$ and $[M+1]^+$ 440, 441 and 442 respectively. MS (CI): *m/z* $[M]^+$, $[M+H]^+$, $[M+CH_3]^+$ and $[M+C_2H_5]^+$ 441, 442, 456 and 470 respectively. MS (EI): $[M]^+$ 441, 406, 370, 258, 230, 186, 135, 105.

7-(4-|(2-Isopropyl-5-methylphenoxy) methyl]-1H-1, 2, 3-triazol-1-yl)-octahydro-6-hydroxy-6*α*, 9*α*-dimethyl-3-methyleneazuleno [4, 5-*β*] furan-2, 9(9*α*H, 9*β*H)-dione (TM 10A)

Recovered as colourless solid with a yield 0.01g, (55%). IR (KBr, cm^{-1}): 3274.1, 2926.5, 2870.9, 1750.8, 1716.9, 1611.9, 1448.6, 1374.6, 1287.7 and 1246.4 HRMS (ESI): *m/z* $[M+H]^+$, $[M+H+CH_3]^+$ and $[M+K+CH_3]^+$ 494, 509 and 547 respectively.

7-(4-[(4-chloro-2-Isopropyl-5-methylphenoxy) methyl]-1H-1, 2, 3-triazol-1-yl)-octahydro-6-hydroxy-6*α*, 9*α*-dimethyl-3-methyleneazuleno [4, 5-*β*] furan-2, 9(9*α*H, 9*β*H)-dione (TM 10B)

Recovered as colourless solid with a yield 0.01g, (50%). IR (KBr, cm^{-1}): 3354.9, 2959.3, 2926.7, 2871.2, 1750.5, 1721.8, 1637.5, 1605.2, 1445.4, 1389.5 and 1242.7. HRMS (ESI): *m/z* $[M+H]^+$ and $[M+H+K+H_2O]^+$ 528 and 585 respectively.

Table 17: Relative Toxicity of Thymol, its Alkyl and Alkyl Substituted Ether Derivatives after 24 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi-square
TM 2C	$Y = -3.417 + 3.$	10.71	26.10	7.96– 12.82	22.20–33.37	3.730
TM 2D	$Y = -2.567 + 2.$	8.97	26.80	5.82– 11.47	22.30–34.89	5.312
TM 2F	$Y = -1.876 + 2.$	6.26	21.93	2.72– 9.11	17.60–29.07	4.808
TM 2I	$Y = -1.860 + 1.$	9.43	44.22	5.70– 12.67	34.90–63.57	3.363
TM 2K	$Y = -2.227 + 2.$	10.21	38.91	6.76– 13.13	31.50–53.58	8.833
TM 2N	$Y = -0.692 + 2$	1.89	6.02	0.02 – 4.01	1.19 – 9.00	2.986
THYMOL	$Y = -6.862 + 4.$	37.18	73.03	33.5-41.10	63.80–87.15	12.287

Source: Laboratory work (2016)

Table 18: Relative Toxicity of Thymol, its Alkyl and Alkyl Substituted Ether Derivatives after 48 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi-square
TM 2D	$Y = -4.612 + 4.892x$	8.80	16.02	5.08–10.63	14.–20.46	4.456
TM 2F	$Y = -1.100 + 2.194x$	3.71	12.19	0.01–6.60	4.78–16.78	3.023
TM 2I	$Y = -1.231 + 2.781x$	2.76	7.96	0.00–6.03	0.01–11.00	6.094
TM 2K	$Y = -1.982 + 2.503x$	6.19	20.14	2.54–9.01	16.14–26.48	6.813
THYMOL	$Y = -8.667 + 6.506x$	21.49	33.83	18.60–24.70	28.90–43.94	31.020

Table 19: Relative Toxicity of Thymol, its Alkyl and Alkyl Substituted Ether Derivatives after 72 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi- square
TM 2D	$Y = -2.218 + 3.229x$	4.86	12.12	0.05–8.15	5.02–15.65	2.932
TM 2F	$Y = -1.231 + 2.789x$	2.76	7.96	0.00–6.03	0.01– 11.00	2.219
TM 2K	$Y = -2.015 + 3.181x$	4.30	10.87	0.00–7.92	0.01–14.30	2.135
THYMOL	$Y = -11.87 + 10.093x$	15.00	20.11	14.10– 16.20	18.20–23.49	2.287

Source: Laboratory work (2016)

The larvicidal activity of all the ether derivatives are higher in comparison to the parent compound, Thymol. Tables 16-19 revealed that all the ether derivatives showed significant activity to the larvae of the *An. gambiae s.s* over the exposure time period of 12 hours, 24 hours, 48 hours and 72 hours respectively. It was observed that among the nine ether derivatives of thymol, TM 2O was the most effective recording a proportion of about 0.99 dead larvae after the first 12 hours with LC_{50} value of 1.90 mg/L after 12 hours of exposure in the experiment. This was closely followed by TM 2P with LC_{50} value of 4.61 mg/L also after 12 hours of exposure, TM 2N with LC_{50} values 2.11, and 1.89 mg/L after 12 and 24 hours respectively, and TM 2E with LC_{50} values 14.07 and 2.23 mg/L after 12 and 24 hours respectively. These ether derivatives of thymol were found to be more effective than the parent compound, thymol and the control neem powder (32% azadirachtin) which recorded a proportion of about 0.9 after the 24 hour period into the experiment.TM 2C recorded LC_{50} values of 24.36, 10.71 mg/L after 12 and 24 hours of exposure respectively. The LC_{50} values recorded for TM 2I is 15.65, 9.43, and 2.76 mg/L after 12, 24 and 48 hours of exposure respectively. The LC_{50} values recorded for TM 2F 8.14, 6.26, 3.71 and 2.76 mg/L; TM 2K 12.50, 10.21, 6.19 and 4.30 mg/L; TM 2D 21.12, 8.97, 8.80 and 4.86 mg/L after 12, 24, 48 and 72 hours of exposure respectively. Thymol, the parent compound was the least toxic with LC_{50} values 84.90, 37.18, 21.49, and 15.01 mg/L after 12, 24, 48 and 72 hours of exposure respectively. The 1% DMSO solution and water recorded zero proportion of dead larvae over the study period (Figure 11).

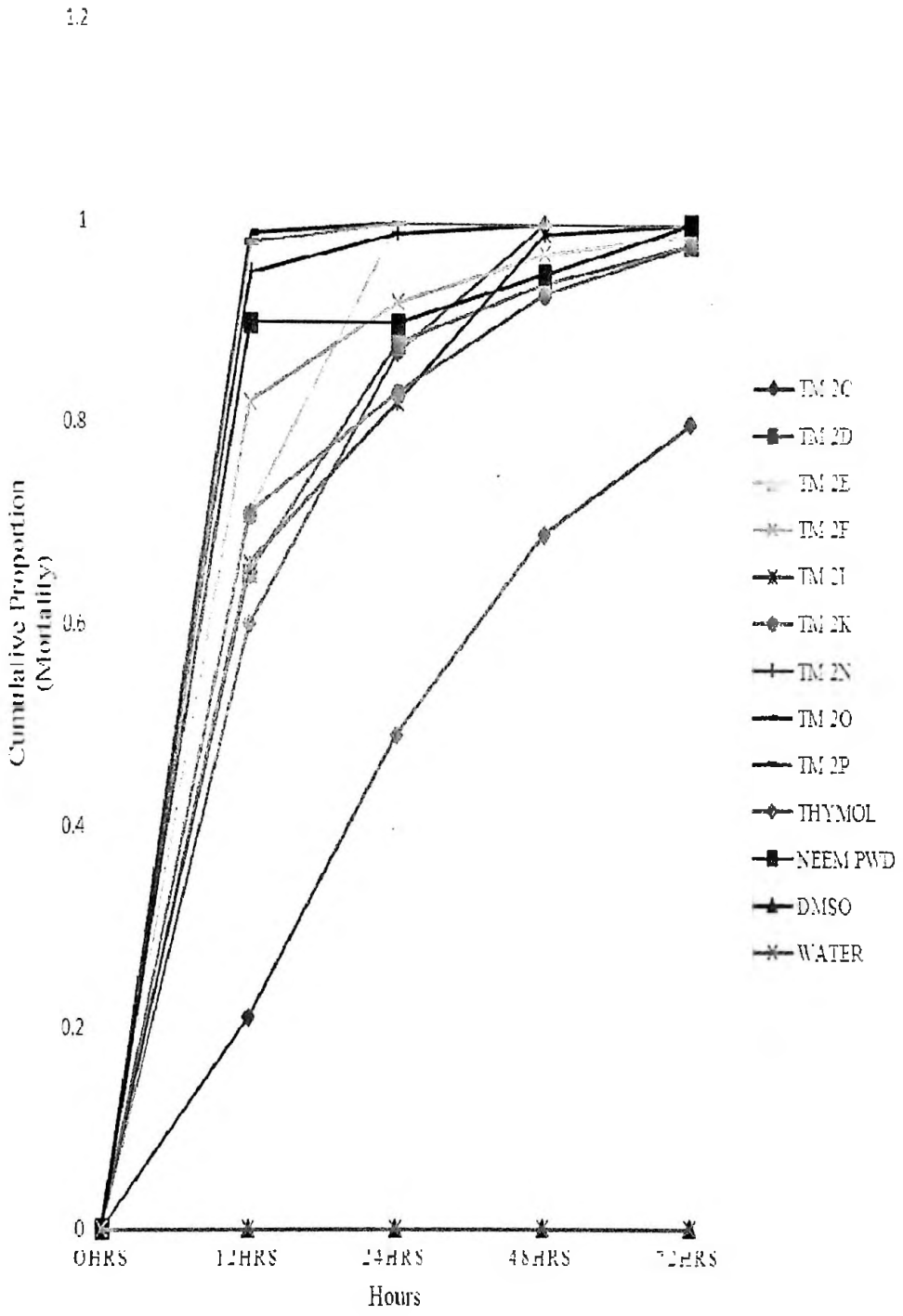


Figure 11: Cumulative proportion of dead larvae for thymol, its alkyl and alkyl substituted ether derivatives.

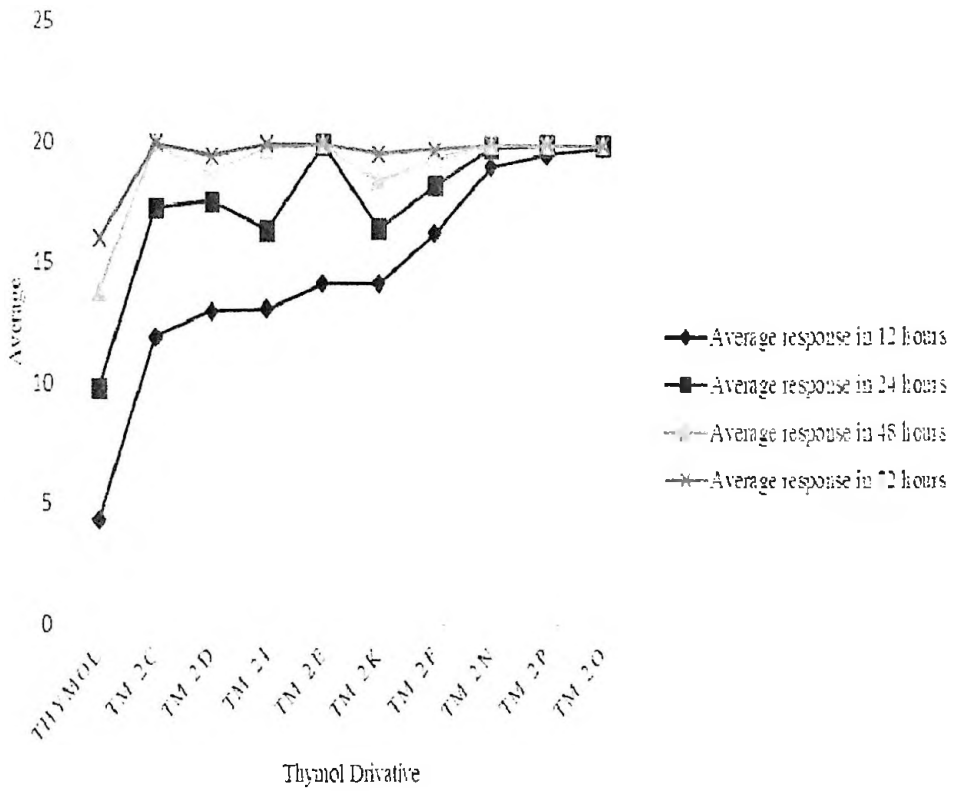


Figure 12: Average number of dead larvae over the four study periods for thymol, its alkyl and alkyl substituted ether derivatives.

The larval mortality rates recorded of most of the ether derivatives were found to be directly proportional to increasing exposure time as most of them showed the highest mortality rates after 72hours of treatment (Figure 12), with the exception of TM 2O and TM 2P, which recorded a 100% mortality at the first 12hours, TM 2N and TM 2E also recorded a 100% mortality at 24hours as well as TM 2C which recorded a 100% mortality at 48hours of exposure time (Figure 11). Again, the mortality rates of these ether derivatives increase with a corresponding increase in the concentration of the test compounds prepared.

From Figure 12, the average number of dead larvae recorded after the 12 hour period appears significantly different from that of 24, 48 and 72 hours. The superiority of TMs 2O, 2P, and 2N is also clearly indicated. An Analysis of Variance test was performed to ascertain a significant difference in the mean number of dead larvae recorded by the thymol and its derivatives across the four study period. This is presented in Table 20.

Table 20: Analysis of Variance (ANOVA) for Thymol and its Alkyl and Alkyl Substituted Ether Derivatives for Larvicidal Assay

Source	DF	Sum of Squares	Mean Squares	F	P – value
Treatment	3	28151111	9383704	1.00	0.403
Error	36	337190076	9366391		
Total	39	365341186			

The following hypothesis was tested;

$H_o : \mu_{12} = \mu_{24} = \mu_{48} = \mu_{72}$ versus $H_1 : \mu_i \neq \mu_j$ for some $i \neq j$

That is the average number of dead larvae recorded by thymol and its derivative was not significantly different after 12 hours, 24 hours, 48 hours and 72 hours. Since (p-value = 0.403 > α = 0.05) we fail to reject the null hypothesis and hence conclude that the average number of dead larvae observed after 12 hours was not significantly different from that of 24 hours through to 72 hours.

Bioassay Results on Larvicidal Activity of Alkyl and Alkyl Substituted Ester Derivatives of Thymol

The experimental data of the estimated lethal concentrations at 50% (LC_{50}) and 90% (LC_{90}) showing the larvicidal activity of the synthesised alkyl and alkyl substituted ester derivatives are represented in the tables 21-24 for specific hours as follows:

Table 21: Relative Toxicity of Thymol, its Alkyl and Alkyl Substituted Ester Derivatives after 12 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi– square
TM 1A	$Y = -1.977 + 0.651x$	109.15	1016.32	244 – 4425.7	333.6-7411.15	1.644
TM 1B	$Y = -4.263 + 2.312x$	69.84	250.20	58.71 – 87.20	175.76–430.59	18.560
TM 1C	$Y = -2.710 + 1.749x$	35.40	191.00	29.11 – 43.34	129.20–362.50	12.430
TM 1D	$Y = -4.069 + 2.169x$	75.21	293.35	62.30 – 96.82	197.61–547.70	17.876
TM 1E	$Y = -3.124 + 1.804x$	53.93	277.02	44.52–68.43	179.40–564.10	4.159
TM 1F	$Y = -3.191 + 1.460x$	153.11	1155.23	103.13-323.20	479.10–713.75	4.050
TM 1G	$Y = -2.956 + 1.276x$	207.40	2096.01	124.16-635.23	670.30-2982.30	5.625
TM 1I	$Y = -4.788 + 2.957x$	41.60	112.93	36.57– 47.43	92.00 –149.50	1.734
TM 1N	$Y = -5.086 + 3.049x$	46.50	122.51	41.11–53.23	99.50–163.30	9.090
THYMOL	$Y = -6.628 + 3.436x$	84.90	200.51	74.25–101.20	154.81–302.30	5.514

Source: Laboratory work (2016)

Table 22: Relative Toxicity of Thymol, its Alkyl and Alkyl Substituted Ester Derivatives after 24 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi– square
TM 1A	$Y = -2.686 + 1.522x$	58.20	404.01	46.30– 79.20	228.00–1125.0	7.865
TM 1B	$Y = -4.392 + 2.674x$	43.89	132.50	35.70– 55.61	94.22 – 237.14	26.451
TM 1C	$Y = -2.785 + 2.216x$	18.10	68.43	14.42– 21.50	54.10 – 97.10	10.163
TM 1D	$Y = -3.615 + 2.253x$	40.22	149.15	34.40– 47.50	111.60–229.20	16.283
TM 1E	$Y = -4.974 + 3.348x$	30.60	73.90	27.20– 34.41	62.30 – 93.20	9.939
TM 1F	$Y = -3.112 + 1.613x$	85.00	529.43	65.81-125.90	286.0-1618.02	3.761
TM 1G	$Y = -2.731 + 1.308x$	122.50	1170.02	84.40-246.20	468.0–7969.01	5.960
TM 1I	$Y = -1.626 + 1.664x$	9.51	55.90	5.30 – 13.10	42.31 – 88.90	6.767
TM 1N	$Y = -4.288 + 2.930x$	29.10	79.53	25.50– 33.02	65.53 – 103.92	13.332
THYMOL	$Y = -6.862 + 4.370x$	37.18	73.03	33.59– 41.10	63.80 – 87.15	12.287

Source: Laboratory work (2016)

Table 23: Relative Toxicity of Thymol, its Alkyl and Alkyl Substituted Ester Derivatives after 48 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi– square
TM 1A	$Y = -2.793 + 1.822x$	44.11	172.52	28.21– 41.33	112.70 –309.54	11.887
TM 1B	$Y = -4.438 + 2.891x$	34.31	95.14	28.78– 40.91	73.33 – 141.82	20.314
TM 1C	$Y = -4.052 + 3.435x$	15.10	35.70	12.82– 17.20	30.32 – 45.20	10.980
TM 1D	$Y = -3.389 + 2.426x$	25.00	84.20	21.20– 28.90	66.91 – 117.42	15.176
TM 1E	$Y = -4.551 + 3.431x$	21.21	50.11	18.61– 23.90	42.60 – 62.70	5.198
TM 1F	$Y = -3.786 + 2.247x$	58.40	179.90	41.30– 57.81	132.10–285.78	2.015
TM 1G	$Y = -2.167 + 1.167x$	71.92	900.20	52.71-120.60	368.0 –6183.00	3.137
TM 1N	$Y = -2.491 + 2.877x$	7.30	20.51	3.81 – 9.90	17.00 – 26.62	2.467
THYMOL	$Y = -8.667 + 6.506x$	21.49	33.83	18.60– 24.70	28.90 – 43.94	31.020

Source: Laboratory work (2016)

Table 24: Relative Toxicity of Thymol, its Alkyl and Alkyl Substituted Ester Derivatives after 72 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi – square
TM 1A	$Y = -3.139 + 2.266x$	29.30	89.22	25.56– 33.41	69.82– 127.64	14.144
TM 1B	$Y = -4.138 + 2.821x$	24.31	83.40	20.31– 28.42	68.11– 110.40	8.850
TM 1C	$Y = -3.179 + 3.022x$	11.30	29.91	8.46 – 13.48	25.20 – 38.83	7.900
TM 1D	$Y = -3.273 + 2.607x$	18.00	55.83	14.86– 21.01	45.73 – 74.31	8.271
TM 1E	$Y = -4.145 + 3.690x$	14.20	32.32	11.91– 16.23	27.60 – 40.74	6.732
TM 1F	$Y = -4.253 + 2.808x$	32.72	93.60	28.58– 37.30	76.31–123.90	1.903
TM 1G	$Y = -1.815 + 1.167x$	35.33	43.71	26.22– 47.60	214.1-1932.20	1.821
TM 1N	$Y = -2.056 + 2.600x$	4.61	11.90	0.07 – 7.91	4.70 – 15.41	4.588
THYMOL	$Y = -11.871 + 9.048x$	15.00	20.11	14.10– 16.20	18.20 – 23.49	2.287

Source: Laboratory work (2016)

Among the ester derivatives of thymol, TM 1I, TM 1N, TM 1C, and TM 1E showed better activity than the parent compound, thymol. TM 1I showed the strongest larvicidal activity and recorded a 100% mortality after 24 hours of exposure time towards the *An. gambiae s.s.* This was followed by TM 1N, TM 1C, TM 1E, while the others (TM 1D, TM 1B, TM 1A, TM 1F and TM 1G) showed lower activity compared to thymol (Tables' 21-24 & Figure 14). The LC_{50} values recorded of TM 1I is 41.60, 9.51 mg/L after 12 and 24 hours of exposure respectively. The LC_{50} values recorded for TM 1N (46.50, 29.11, 7.30, and 4.61 mg/L) ; TM 1C (35.40, 18.11, 15.10 , and 11.30 mg/L); TM 1E (53.90, 30.60, 21.21, and 14.20 mg/L) after 12,24,48 and 72 hours of exposure time and hence showed better larvicidal potency than the parent compound, thymol with LC_{50} values 84.90, 37.18, 21.49 and 15.10 mg/L after 12, 24, 48 and 72 hours exposure respectively. The LC_{50} values of TM 1D (75.20, 40.20, 25.10, and 18.00 mg/L); TM 1B (69.80, 43.90, 34.31, and 24.31 mg/L); TM 1A (109.15, 58.22, 44.11 and 29.30 mg/L); TM 1F (153.11, 85.00, 58.40 and 32.72 mg/L); TM 1G (207.40, 122.50, 71.92 and 35.33 mg/L) after 12, 24, 48 and 72 hours exposure respectively recorded much lower larvicidal activity compared to the parent compound, thymol. It was again realized that, the positive control neem powder (32% azadirachtin) was more potent than all the ester derivatives, except TM 1I which exhibited the highest toxicity at 24 hours post-treatment with an LC_{50} value of 9.51 mg/L. The 1% DMSO solution and water recorded zero proportion of dead larvae over the study period (Figure 13).

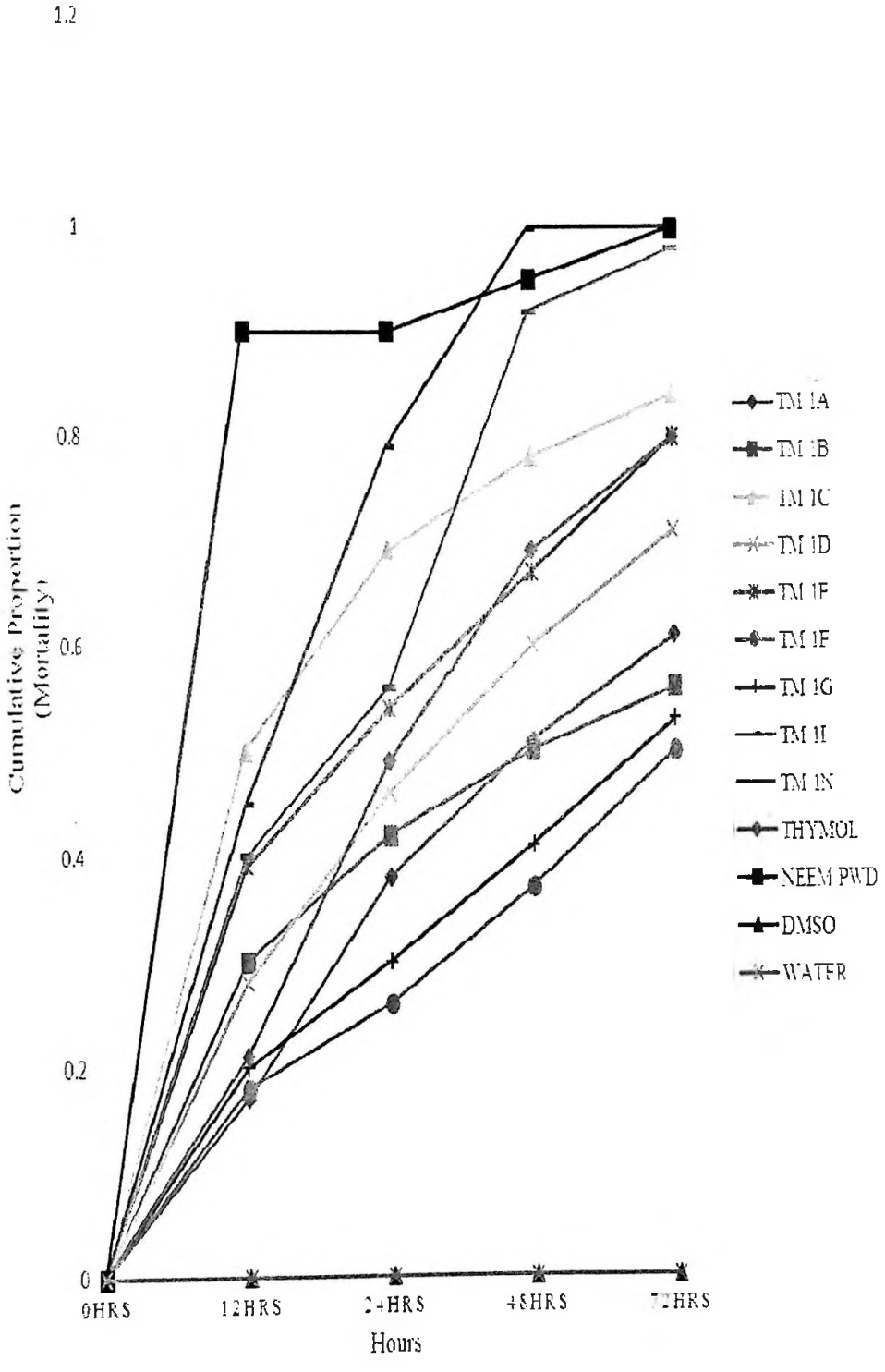


Figure 13: Cumulative Proportion of dead larvae for thymol, its alkyl and alkyl substituted ester derivatives.

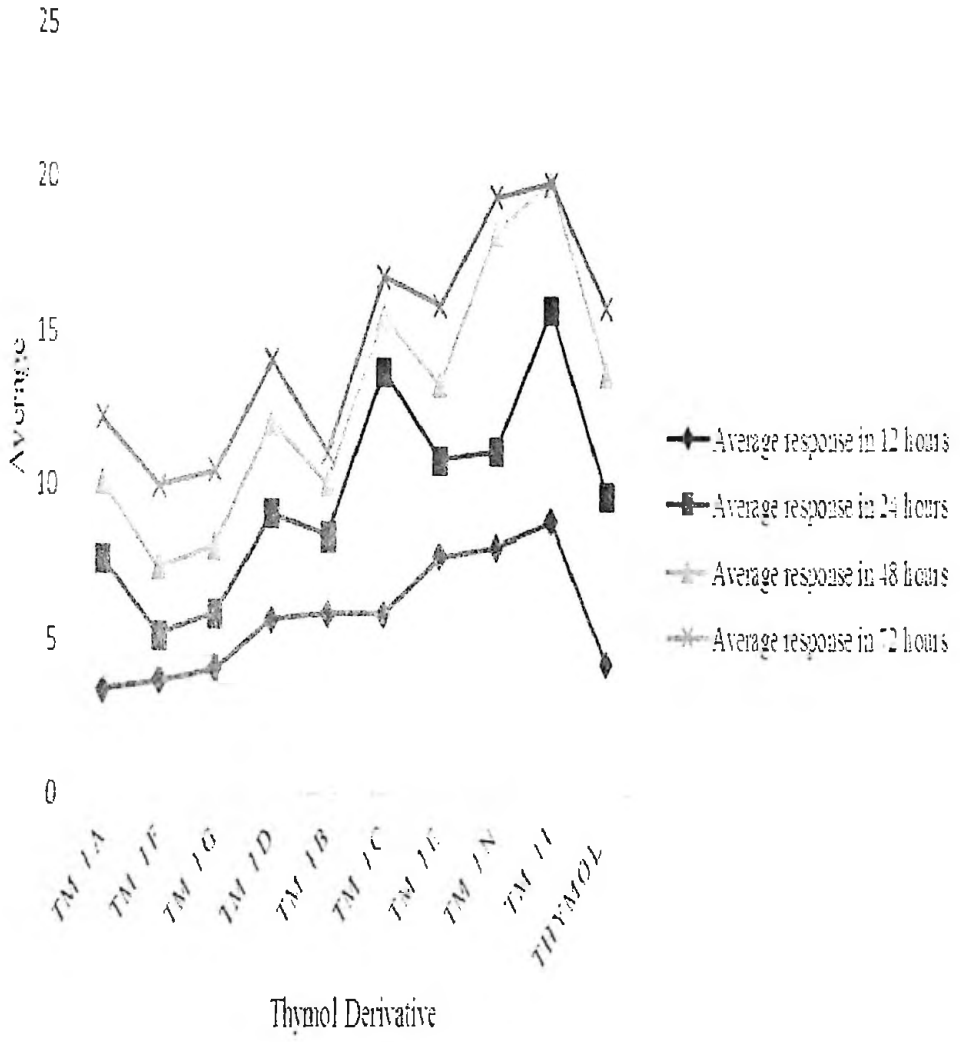


Figure 14: Average number of dead larvae over the four study periods for thymol, its alkyl and alkyl substituted ester derivatives.

The larval mortality rates recorded for all the ester derivatives were found to be directly proportional to increasing exposure time and concentration of the test materials prepared, as all of them showed the highest mortality rates after 72 hours of treatment, except TM 1I which recorded a 100% mortality after 48 hours of exposure time (Figure 14). The activity of all the test compounds were concentration dependant as the highest mortality rates for the various compounds were recorded at the highest concentration.

From Figure 14, the average number of dead larvae recorded after the 12 hour period appears significantly different from that of 24, 48 and 72 hours. An Analysis of Variance test was performed to ascertain a significant difference in the mean number of dead larvae recorded by the thymol and its derivatives across the four study period. This is presented in Table 25.

Table 25: Analysis of Variance (ANOVA) for Thymol and its Alkyl and Alkyl Substituted Ester Derivatives for Larvicidal Assay

Source	DF	Sum of Squares	Mean Squares	F	P – value
Treatment	3	453.1	151.0	13.23	0.000
Error	36	410.9	11.4		
Total	39	864.0			

The following hypothesis was tested; $H_o : \mu_{12} = \mu_{24} = \mu_{48} = \mu_{72}$ versus $H_1 : \mu_i \neq \mu_j$ for some $i \neq j$

A significant difference was observed in the number of dead larvae recorded over the four time period considered (p-value = 0.000 < α = 0.05). This implies that, the effectiveness of the chemicals was influenced by the duration considered.

Bioassay Results on Larvicidal Activity of Aromatic and Substituted Aromatic Ester Derivatives of Thymol

The experimental data of the estimated lethal concentrations at 50% (LC_{50}) and 90% (LC_{90}) showing the larvicidal activity of the synthesised aromatic and substituted aromatic ester derivatives as represented in the tables 26-29 for specific hours as follows:

Table 26: Relative Toxicity of Thymol, it's Aromatic and Aromatic Substituted Ester Derivatives after 12 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi– square
TM 1K	$Y = -1.516 + 1.028x$	29.81	524.70	20.3 – 41.1	226.9 –371.8	8.050
TM 1L	$Y = -5.588 + 3.838x$	28.62	61.71	25.6 – 31.8	53.1 – 75.3	6.026
TM 1M	$Y = -6.268 + 3.284x$	81.01	199.10	70.9 – 96.5	153.4 –298.0	2.700
TM 1P	$Y = -2.535 + 0.968x$	416.30	878.10	176.1 – 634.6	1324 –4.5E5	2.159
TM 1Q	$Y = -3.946 + 1.882x$	125.11	600.30	93.9 – 201.2	326.2–1.8E3	9.689
TM 1R	$Y = -1.852 + 1.050x$	58.10	966.71	42.4 – 95.5	362.2 –951.7	2.031
TM 1U	$Y = -8.524 + 4.874x$	56.10	102.80	51.0 – 62.0	89.4 – 124.5	11.574
THYMOL	$Y = -6.628 + 3.436x$	84.90	200.50	74.2 – 101.2	154.8 –302.3	5.514

Source: Laboratory work (2016)

Table 27: Relative Toxicity of Thymol, it's Aromatic and Aromatic Substituted Ester Derivatives after 24 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi – square
TM 1K	$Y = -2.273 + 1.917x$	15.40	71.60	11.3 –19.0	54.8 – 108.9	13.589
TM 1L	$Y = -4.823 + 5.666x$	10.20	19.00	7.7 – 11.9	16.6 – 24.4	1.718
TM 1M	$Y = -19.88 + 13.753x$	28.10	34.82	26.7 –64.1	29.7 – 383.4	0.564
TM 1P	$Y = -3.808 + 2.770x$	23.71	68.82	20.4 –27.1	56.5 – 90.7	11.892
TM 1Q	$Y = -1.517 + 1.100x$	23.90	35.00	15.7 –32.2	174.0 – 163.0	2.707
TM 1R	$Y = -2.711 + 1.971x$	23.74	106.11	17.6 –30.1	73.0 – 210.7	21.314
TM 1U	$Y = -5.458 + 3.804x$	27.20	59.12	24.3 –30.3	50.8 – 72.3	4.010
THYMOL	$Y = -6.862 + 4.370x$	37.18	73.01	33.6 –41.1	63.8 – 87.2	12.287

Source: Laboratory work (2016)

Table 28: Relative Toxicity of Thymol, it's Aromatic and Aromatic Substituted Ester Derivatives after 48 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi – square
TM 1K	$Y = -3.348 + 4.72x$	6.81	14.12	1.4 – 9.4	11.2 – 18.3	1.350
TM 1L	$Y = -3.029 + 4.597x$	6.78	14.01	1.2 – 9.3	11.0 – 18.1	0.850
TM 1M	$Y = -13.939 + 9.054x$	14.72	18.92	13.8–16.5	16.8 – 24.3	4.090
TM 1P	$Y = -1.265 + 2.121x$	4.01	15.90	0.57 – 7.1	10.4 – 21.3	4.452
TM 1Q	$Y = -3.491 + 3.430x$	10.41	24.60	7.7 – 12.5	21.0 – 31.5	3.317
TM 1R	$Y = -3.773 + 3.410x$	12.82	30.41	10.3– 14.8	25.9 – 38.6	6.203
TM 1U	$Y = -4.711 + 5.635x$	9.91	18.53	7.2 – 11.6	16.2 – 23.9	1.973
THYMOL	$Y = -8.667 + 6.506x$	21.49	33.81	18.6– 24.6	28.9 – 43.9	31.020

Source: Laboratory work (2016)

Table 29: Relative Toxicity of Thymol, it's Aromatic and Aromatic Substituted Ester Derivatives after 72 hours of Exposure for Larvicidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi- square
TM 1K	$Y = -1.605 + 2.366x$	3.41	9.23	0.00 – 5.91	0.43-13.60	3.684
TM 1M	$Y = -8.733 + 8.712x$	12.60	18.30	11.4 – 13.6	16.3–23.0	0.935
TM 1Q	$Y = -4.736 + 5.439x$	8.90	16.12	5.1 – 10.7	14.1–21.1	1.507
TM 1R	$Y = -2.218 + 2.623x$	4.82	12.10	0.051 – 8.2	5.0–15.6	2.932
TM 1U	$Y = -2.218 + 2.623x$	4.94	12.13	0.081 – 8.4	5.2–15.8	2.935
THYMOL	$Y = -11.871 + 9.048x$	15.10	20.11	14.1 – 16.2	18.2–23.5	2.097

Source: Laboratory work (2016)

All of the aromatic and substituted aromatic ester derivatives of thymol, showed higher larvicidal activity in comparison to the parent compound, thymol. Tables 26-29 revealed these class of derivatives showed significant activity to the larvae of the *An. gambiae s.s* over the exposure time period of 12 hours, 24 hours, 48 hours and 72 hours respectively. It was observed that among the seven aromatic and aromatic substituted ester derivatives of thymol, TM 1P showed the most potent larvicidal activity and recorded a 100% mortality after 48 hours of exposure time towards the *An. gambiae s.s.* This was followed by TM 1L, TM 1K, TM 1R, TM 1U, TM 1Q, TM 1M respectively compared to the parent compound, thymol (Figure 16). The LC_{50} values recorded of TM 1P is 416.30, 23.71, 4.01 mg/L and TM 1L is 28.62, 10.20, 6.78 mg/L after 12, 24 and 48 hours of exposure respectively. The LC_{50} values recorded for TM 1K (29.81, 15.40, 6.81, and 3.41 mg/L) ; TM 1R (58.10, 23.74, 12.82 , and 4.82 mg/L); TM 1U (56.10, 27.20, 9.91, and 4.94 mg/L) TM 1Q (125.11, 23.90, 10.41, and 8.90 mg/L) TM 1M (81.01, 28.10,14.72, and 12.60 mg/L) after 12, 24, 48 and 72 hours of exposure time, hence showed better larvicidal potency than the parent compound, thymol with LC_{50} values 84.90, 37.18, 21.49 and 15.10 mg/L after 12, 24, 48 and 72 hours exposure respectively. It was again realized that, the positive control neem powder (32% azadirachtin) was more potent than all the ester derivatives, except TM 1P and TM 1L which exhibited the highest toxicity at 48 hours post-treatment with an LC_{50} values of 4.01 mg/L and 6.78 mg/L respectively.

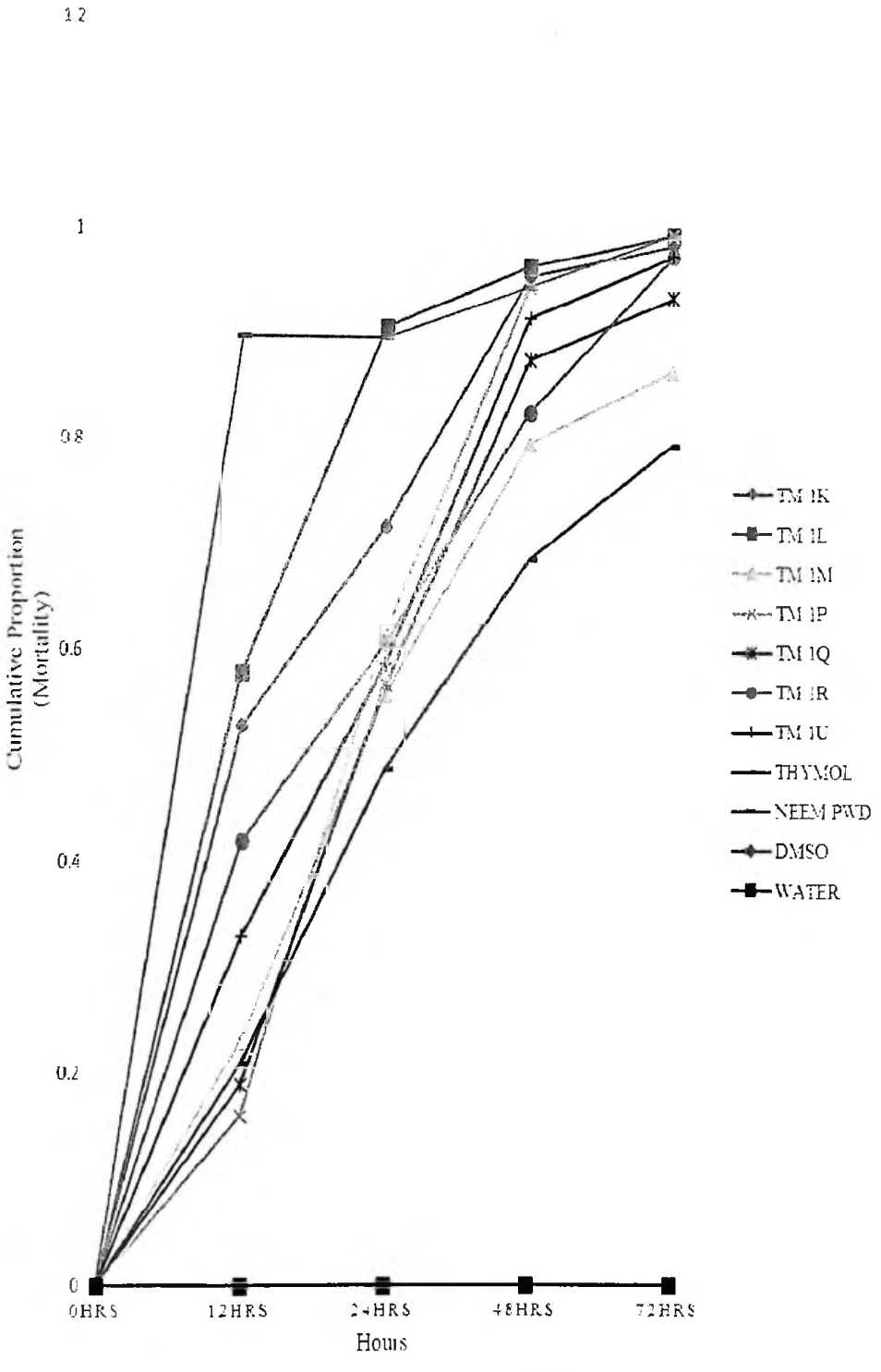


Figure 15: Cumulative Proportion of dead larvae for thymol, its aromatic and aromatic substituted ester derivatives.

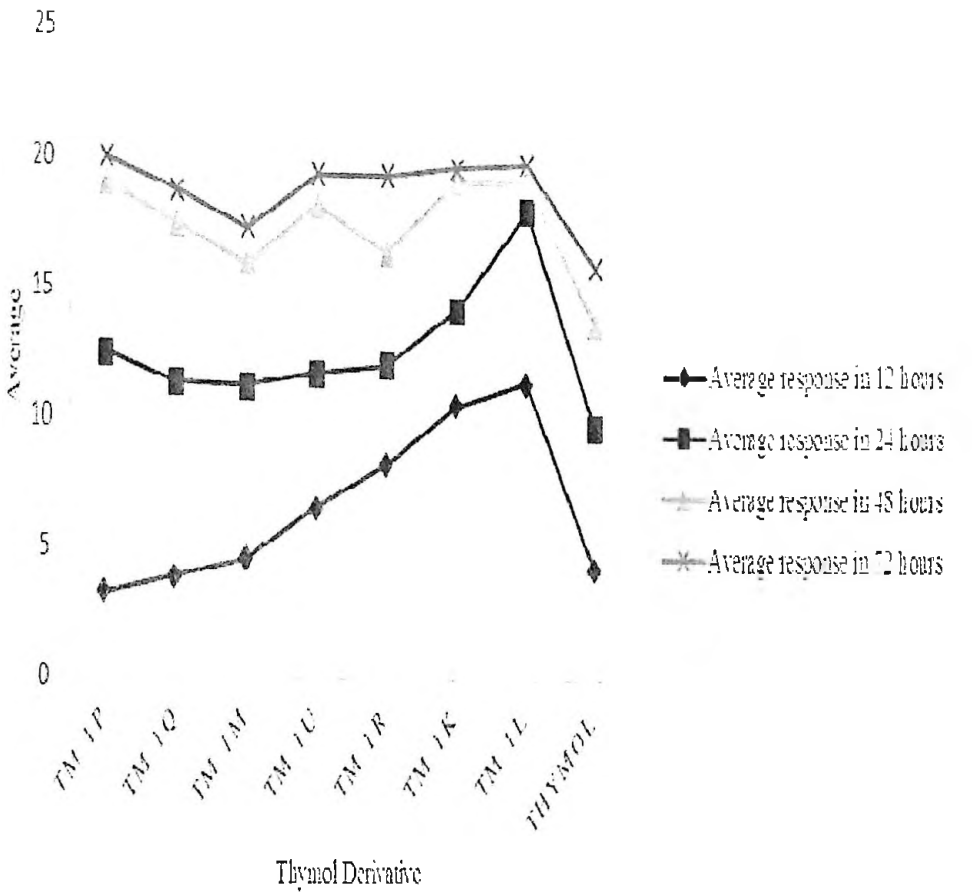


Figure 16: Average number of dead larvae over the four study periods for thymol, its aromatic and aromatic substituted ester derivatives.

The larval mortality rates recorded for all these ester derivatives were found to be directly proportional to increasing exposure time and concentration of the test materials prepared, since all of them showed the highest mortality rates after 72 hours of treatment (Figure 15), except TM 1P and TM 1L which recorded a 100% mortality after 48 hours of exposure time. The activity of all the test compounds were concentration dependant as the highest mortality rates for the various compounds were recorded at the highest concentration. From Figure 16, the average number of dead larvae recorded after the 12 hour period appears significantly different from that of 24, 48 and 72 hours. An Analysis of Variance test was performed to ascertain a

significant difference in the mean number of dead larvae recorded by the thymol and its derivatives across the four study period. This is presented in Table 30.

Table 30: Analysis of Variance (ANOVA) for Thymol, it's Aromatic and Aromatic Substituted Ester Derivatives for Larvicidal Assay

Source	DF	Sum of Squares	Mean Squares	F	P – value
Treatment	3	706.16	235.39	35.60	0.000
Error	28	184.67	6.60		
Total	31	890.83			

The following hypothesis was tested;

$H_o : \mu_{12} = \mu_{24} = \mu_{48} = \mu_{72}$ versus $H_1 : \mu_i \neq \mu_j$ for some $i \neq j$

A significant difference was observed in the number of dead larvae recorded over the four time period considered (p-value = 0.000 < α = 0.05). This implies the effectiveness of the chemicals was influenced by the duration considered.

Boiassay Results on Adulticidal Activity of Alkyl and Alkyl Substituted Ether Derivatives of Thymol

The experimental data of the estimated lethal concentrations at 50% and 90% showing the adulticidal activity of the synthesised alkyl and alkyl substituted ether derivatives are represented in the tables 31-37 for specific days as follows:

Table 31: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ether Derivatives after One Day of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi-square
TM 2C	$Y = -2.709 + 1.016x$	464.00	8464.22	175– 25676	1083 – 5.9E7	2.885
TM 2D	-	-	-	-	-	-
TM 2E	$Y = -3.486 + 1.486x$	221.11	1611.01	128 – 832	524 – 30074	3.216
TM 2F	$Y = -9.421 + 3.956x$	241.21	507.13	131 – 851	211 – 3152	0.756
TM 2I	$Y = -3.932 + 1.565x$	326.03	2147.11	163 – 2829	574 – 1.6E5	3.665
TM 2K	$Y = -3.985 + 1.579x$	334.10	2168.20	166 – 3111	574 – 1.8E5	4.775
TM 2N	$Y = -4.361 + 1.975x$	162.06	720.22	111 – 352	336 – 4020	2.633
TM 2O	$Y = -5.104 + 1.960x$	401.05	1809.12	182–32052	449 – 5.4E6	3.084
TM 2P	$Y = -3.670 + 1.169x$	1380.10	17238.00	285-2.4E13	1121–2.5E22	3.068
THYMOL	$Y = -5.785 + 2.224x$	400.12	1507.00	178 –1.1E6	376 – 2.1E9	2.546

Source: Laboratory work (2016) (-) = No response shown by test compound

Table 32: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ether Derivatives after Two Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 2C	$Y = -2.633 + 1.069x$	291.10	4599.10	136 – 3485	837 – 1.7E6	2.084
TM 2D	$Y = -5.048 + 1.984x$	350.20	1549.00	172 – 7977	435 – 5.3E5	3.917
TM 2E	$Y = -3.612 + 1.701x$	133.12	753.00	93 – 265	345 – 3967	0.692
TM 2F	$Y = -7.067 + 3.025x$	217.00	576.00	140 – 1493	258 - 24290	1.519
TM 2I	$Y = -2.813 + 1.156x$	271.01	3484.03	134 – 2220	747 – 4.7E5	1.129
TM 2K	$Y = -3.718 + 1.443x$	377.04	2909.01	174 – 5140	663– 5.4E5	3.308
TM 2N	$Y = -4.191 + 2.066x$	107.22	445.15	82 – 168	251 – 1337	2.271
TM 2O	$Y = -4.940 + 2.142x$	202.31	802.00	131 – 583	349 – 7099	2.288
TM 2P	$Y = -5.417 + 1.988x$	531.11	2341.02	195–4.2E10	436 – 1.3E17	2.943
THYMOL	$Y = -2.686 + 0.825x$	179.70	641.90	307–3.9E12	2182–1.3E23	1.363

Source: Laboratory work (2016)

Table 33. Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ether Derivatives after Three Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 2C	$Y = -2.928 + 1.286x$	189.10	1877.00	110 – 722	554 – 4.49E5	1.690
TM 2D	$Y = -5.356 + 2.281x$	223.00	813.11	139 – 867	336 – 1.24E4	4.175
TM 2E	$Y = -3.053 + 1.553x$	93.00	619.20	67.7 – 159	295 – 2831	3.030
TM 2F	$Y = -4.110 + 1.615x$	351.00	2183.00	171 – 3738	571 – 2.2E5	1.877
TM 2I	$Y = -3.149 + 1.466x$	141.00	1055.00	93 – 336	409 – 9516	0.512
TM 2K	$Y = -4.096 + 1.836x$	170.10	850.22	113 – 413	367– 6128	4.930
TM 2N	$Y = -4.380 + 2.376x$	70.00	241.10	58 – 90	165 – 456	4.408
TM 2O	$Y = -4.542 + 2.279x$	98.01	359.00	78 – 143	219 – 893	3.944
TM 2P	$Y = -2.316 + 1.226x$	78.00	861.10	55 – 147	332 – 8319	1.708
THYMOL	$Y = -2.586 + 1.217x$	133.11	506.10	84 – 377	480 – 2.78E4	2.622

Source: Laboratory work (2016)

Table 34: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ether Derivatives after Four Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 2C	$Y = -2.928 + 1.286x$	189.10	1877.00	110 – 722	554–4.49E5	1.690
TM 2D	$Y = -5.452 + 2.483x$	157.00	516.00	113 – 319	271 – 2391	6.218
TM 2E	$Y = -2.915 + 1.519x$	83.00	580.10	61 – 138	279 – 2594	2.766
TM 2F	$Y = -4.458 + 1.950x$	193.30	878.00	125 – 529	372 – 7450	1.335
TM 2I	$Y = -3.866 + 2.263x$	51.01	188.20	43 – 63	132 – 332	6.245
TM 2K	$Y = -4.411 + 2.014x$	155.12	670.11	108 – 324	321– 3496	4.105
TM 2N	$Y = -4.145 + 2.308x$	62.00	224.10	52 – 79	154 – 416	3.572
TM 2O	$Y = -3.030 + 1.674x$	65.00	377.20	50 – 92	210 – 1145	4.732
TM 2P	$Y = -2.508 + 1.519x$	45.00	313.15	35 – 61	175 – 966	1.673
THYMOL	$Y = -1.956 + 1.074x$	66.15	320.00	45.9 – 130	347 – 18804	1.363

Source: Laboratory work (2016)

Table 35: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ether Derivatives after Five Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 2C	$Y = -2.987 + 1.485x$	103.00	749.10	73.0 – 194.0	329.0 – 4439	3.025
TM 2D	$Y = -5.452 + 2.483x$	157.00	516.00	113.0 –319.0	271.0 – 2391	6.218
TM 2E	$Y = -3.283 + 1.859x$	58.20	285.10	46.8 – 78.1	176.0 – 672	3.043
TM 2F	$Y = -4.493 + 2.132x$	128.00	511.00	94.9 – 222.0	275.0–1824	2.581
TM 2I	$Y = -4.350 + 2.649x$	43.90	134.10	37.4 – 52.2	102.0 – 202	10.72
TM 2K	$Y = -4.328 + 2.359x$	68.40	239.10	56.5 – 87.9	163.0 – 452	2.894
TM 2N	$Y = -5.220 + 3.138x$	46.10	118.00	40.0 – 53.6	94.0 – 164	1.897
TM 2O	$Y = -2.883 + 1.661x$	54.40	322.10	42.9 – 74.3	186.0 – 899	3.582
TM 2P	$Y = -2.671 + 1.662x$	40.50	239.00	32.0 – 52.0	146.0 – 585	0.518
THYMOL	$Y = -1.956 + 1.074x$	66.15	320.00	45.9 – 130.0	347.0–1.9E4	1.363

Source: Laboratory work (2016)

Table36: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ether Derivatives after Six Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 2C	$Y = -3.321 + 1.739x$	81.00	443.00	62.0– 124.0	241 – 1431	2.751
TM 2D	$Y = -4.518 + 2.111x$	138.00	559.00	100.0–255.0	290 – 2246	2.725
TM 2E	$Y = -3.283 + 1.859x$	58.20	285.10	46.8 – 78.1	176 – 672	3.043
TM 2F	$Y = -3.405 + 1.637x$	120.00	731.10	85.0 – 233.0	334 – 3906	2.424
TM 2I	$Y = -4.253 + 2.622x$	42.00	129.00	36.0 – 49.7	98 – 194	5.535
TM 2K	$Y = -3.980 + 2.267x$	57.00	209.20	47.0 – 72.0	145 – 382	2.253
TM 2N	$Y = -5.730 + 3.625x$	38.00	86.10	33.0 – 43.0	71.4 – 111	4.261
TM 2O	$Y = -2.219 + 1.387x$	39.80	334.00	30.0 – 54.0	177 – 1243	1.439
TM 2P	$Y = -2.004 + 1.400x$	27.00	223.00	19.0 – 36.0	128 – 681	0.498
THYMOL	$Y = -1.983 + 1.251x$	38.12	306.01	28.0 – 54.0	194 – 2107	1.135

Source: Laboratory work (2016)

Table 37: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ether Derivatives after Seven Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 2C	$Y = -3.461 + 1.857x$	73.00	358.00	57.5 –104.0	209 – 953	2.285
TM 2D	$Y = -4.467 + 2.244x$	98.00	365.00	77.2– 143.0	221 – 927	4.687
TM 2E	$Y = -3.492 + 2.014x$	54.20	234.00	44.2 – 69.8	154 – 478	4.700
TM 2F	$Y = -2.848 + 1.548x$	69.10	465.10	52.7– 105.0	239 – 1753	1.860
TM 2I	$Y = -3.919 + 2.539x$	35.00	112.01	29.6 – 41.4	85.6 – 167	5.589
TM 2K	$Y = -4.791 + 2.870x$	46.70	131.50	40.2 – 55.1	101 – 190	5.386
TM 2N	$Y = -5.835 + 3.901x$	31.30	66.70	27.7 – 35.5	56.2 – 84.6	5.521
TM 2O	$Y = -1.725 + 1.343x$	19.30	174.00	12.0 – 26.0	102 – 529	2.223
TM 2P	$Y = -2.024 + 1.483x$	23.20	169.00	16.2 – 30.1	105 – 428	1.139
THYMOL	$Y = -2.578 + 1.789x$	27.60	144.00	21.5 – 34.4	97.4 – 283	4.146

Source: Laboratory work (2016)

The thymol derivative, TM 2D did not show any response within the first day of exposure of the adult female mosquitoes of *An. gambiae s.s* across all dosage levels (12.5 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L). When probit model is applied to measurements at the extremes of the curve, a number of problems arise. The model predicts zero response only at zero dose, even if the data strongly suggest the existence of a threshold. The model also suggests that a 100% response only occurs at infinite doses. Thus, the model for TM 2D could not be computed.The adulticidal activity of the parent compound, thymol was higher than most of the alkyl and alkyl substituted ether derivatives. Tables 31-37 revealed that thymol with LC_{50} value of 27.60 mg/L showed significant activity to the adult female mosquitoes of the *An. gambiae s.s* over the exposure time period of 7 days compared to all the alkyl and alkyl substituted ether derivatives with the exception of TM 20 and TM 2P which demonstrated a marginal superior activity to Thymol. It is observed that among the nine alkyl and alkyl substituted ether derivatives of thymol, TM 2O was the most effective with LC_{50} value of 19.30 mg/L after 7days of exposure in the experiment. This was closely followed by TM 2P with LC_{50} value of 23.20 mg/L after 7days of exposure in the experiment. The LC_{50} value of Thymol 27.60 mg/L was higher than the other ether derivatives TM 2N, TM 2I, TM 2K, TM 2E, TM 2F, TM 2C and TM 2D which recorded LC_{50} values of 31.30, 35.00, 46.70, 54.20, 69.10, 73.00 and 98.00 mg/L respectively after 7 days of exposure in the experiment. The 6% glucose in water with 0.1M DMSO solution and the 6% glucose in water as control recorded zero proportion of dead adult mosquito over the study period. When the adult mosquitoes were subjected to water alone without food (ie 6% glucose in

water) during the study period, there was 100% mortality recorded between day 3 and day 4. Again, when the adult mosquitoes were starved (ie no water and food, 6% glucose), 100% mortality was recorded within the first two days (Figure 17).

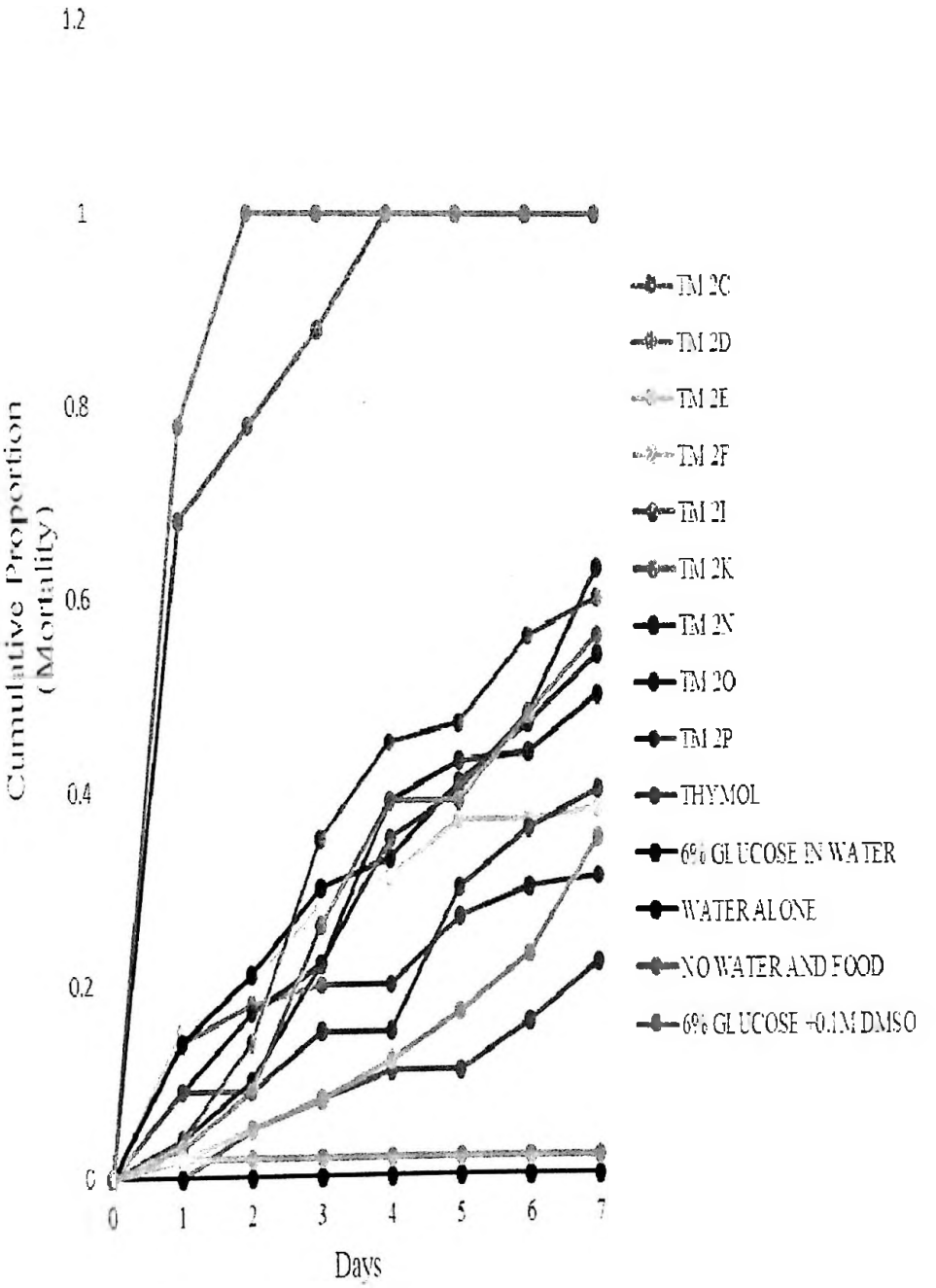


Figure 17: Cumulative Proportion of dead mosquitoes for thymol, its alkyl and alkyl substituted ether derivatives.

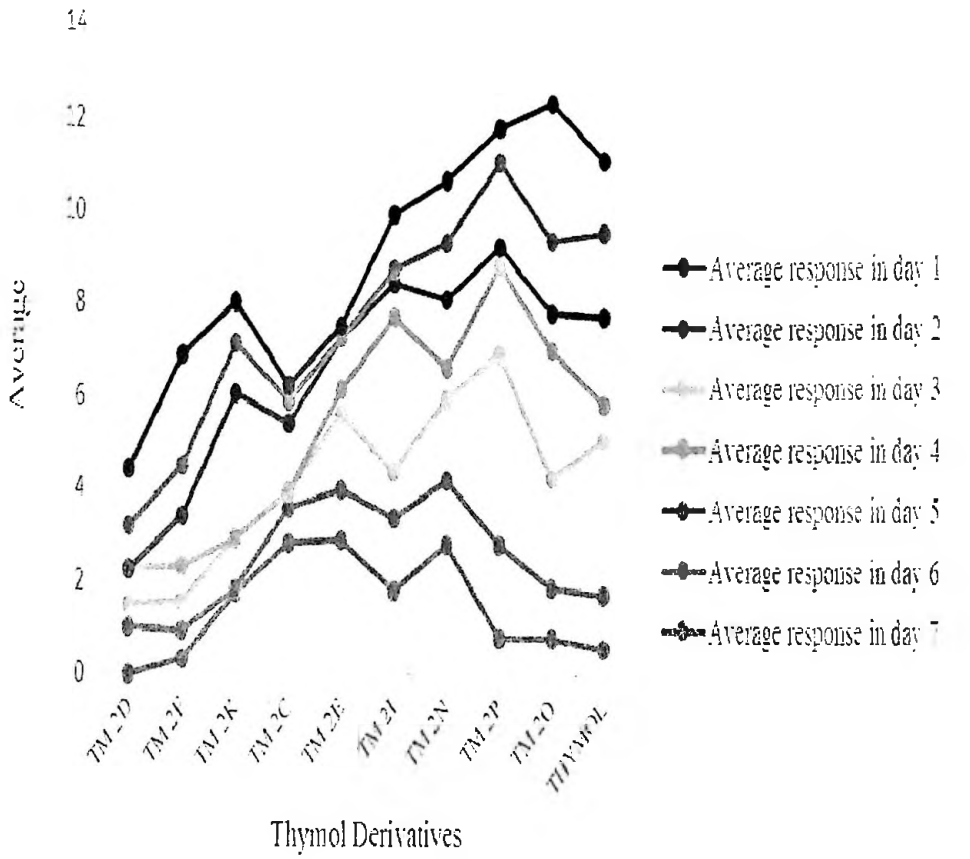


Figure 18: Average number of dead mosquitoes over the seven study periods for thymol, its alkyl and alkyl substituted ether derivatives.

The study further explored the average number of dead adult mosquito for each day over the seven day period considered in the study. This is illustrated in Figure 18.

The average number of dead adult mosquitoes recorded after day 1 appears significantly different from that of day 2 through day seven for all the thymol derivatives with the exception of TM 2C with LC_{50} values 464.00, 291.10, 189.10, 189.10, 103.00, 81.00 and 73.00 mg/L respectively from day 1 to day 7 in the experiment. This shows that, activity of TM 2C was the same for day 3 and day 4 (Figure 18). To ascertain whether there was a significant difference in the mean number of dead mosquitoes recorded across the period

considered in the study, an Analysis of Variance test was performed (Table 38).

Table 38: Analysis of Variance (ANOVA) for Thymol and its Alkyl and Alkyl Substituted Ether Derivatives for Adulticidal Assay

Source	DF	Sum of Squares	Mean Squares	F	P – value
Treatment	6	441.39	73.57	16.74	0.000
Error	63	276.83	4.39		
Total	69	718.23			

The following hypothesis was tested;

$H_o : \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7$ versus $H_1 : \mu_i \neq \mu_j$ for some $i \neq j$

That is the average number of dead female mosquitoes recorded by thymol and its derivative was not significantly different after day 1, day 2, through day 7. Since (p-value = 0.000 < α = 0.05) the null hypothesis was rejected and hence concluded that the average number of dead mosquitoes observed after day 1 was significantly different from that after day 2 through to 7 days after. Thus, the effect of thymol and it derivatives was influenced by length of time.The adulticidal mortality rates recorded of most of the ether derivatives were found to be directly proportional to increasing exposure time as most of them showed the highest mortality rates after 7 days of treatment, see (Figure 18). Again, the mortality rates of these ether derivatives increases with a corresponding increase in the concentration of the test compounds prepared.

Bioassay Results on Adulticidal Activity of Alkyl and Alkyl Substituted Ester Derivatives of Thymol

The experimental data of the estimated lethal concentrations at 50% (LC_{50}) and 90% (LC_{90}) showing the adulticidal activity of the synthesised alkyl and alkyl substituted ester derivatives are represented in the tables 39-45 for specific days as follows:

Table 39: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ester Derivatives after One Day of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 1A	$Y = -9.441 + 4.023x$	222.00	463.00	143 – 525	295 – 996	0.728
TM 1B	*	*	*	*	*	*
TM 1C	$Y = -3.547 + 1.405x$	334.10	2729.00	162 – 3298	653 – 310237	4.075
TM 1D	$Y = -3.782 + 1.746x$	146.01	793.00	100 – 316	353 – 4831	4.898
TM 1E	$Y = -3.199 + 1.231x$	397.00	4368.00	171 – 7865	808 – 2.3E6	5.570
TM 1F	$Y = -5.957 + 2.171x$	554.20	2155.00	312 – 1130	672 – 2.9E5	3.148
TM 1G	-	-	-	-	-	-
TM 1I	$Y = -5.010 + 2.130x$	225.10	900.20	139 – 859	360 – 13504	6.028
TM 1N	$Y = -4.445 + 1.602x$	596.00	3759.00	214 – 8.1E5	633 – 4.8E8	3.887
THYMOL	$Y = -5.785 + 2.224x$	400.12	1507.00	178 – 1.1E6	376 – 2.1E9	2.546

Source: Laboratory work (2016) (*) = Not Determined; (-) = No response shown by test compound

Table 40: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ester Derivatives after Two Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 1A	$Y = -3.677 + 1.621x$	186.11	1147.00	117 – 519	439 – 11442	2.573
TM 1B	*	*	*	*	*	*
TM 1C	$Y = -3.447 + 1.661x$	119.05	704.02	84.5 – 227	704 – 3644	4.660
TM 1D	$Y = -2.950 + 1.536x$	83.20	568.10	61.8 – 138	275 – 2557	5.194
TM 1E	$Y = -3.397 + 1.373x$	298.00	2561.00	150– 2274	641 – 2.0E5	4.744
TM 1F	$Y = -4.257 + 1.679x$	343.00	1992.10	170 – 3634	539 – 2.0E5	2.428
TM 1G	$Y = -6.487 + 2.503x$	391.15	1270.12	170 – 3.7E33	315 – 1.3E57	3.343
TM 1I	$Y = -4.800 + 2.085x$	211.00	882.10	134 – 676	364 – 9700	4.225
TM 1N	$Y = -3.868 + 1.589x$	272.11	1741.00	148 – 1485	527 – 58971	3.931
THYMOL	$Y = -2.686 + 0.825x$	179.70	641.90	307 – 3.9E12	2182–1.3E23	1.363

Source: Laboratory work (2016) (*) = Not Determined

Table 41: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ester Derivatives after Three Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 1A	$Y = -3.512 + 1.642x$	138.00	829.00	94.2 – 289	364 – 4975	1.649
TM 1B	*	*	*	*	*	*
TM 1C	$Y = -3.299 + 1.873x$	57.80	279.20	46.5 – 76.8	173 – 653	4.501
TM 1D	$Y = -3.274 + 1.921x$	50.60	235.20	41.1 – 65.1	152 – 499	2.909
TM 1E	$Y = -3.243 + 1.441x$	178.00	1376.00	109– 557	471 – 2.1E4	7.663
TM 1F	$Y = -4.044 + 1.595x$	343.00	2181.00	169 – 3414	574 – 2.0E5	1.406
TM 1G	$Y = -6.129 + 2.441x$	324.11	1085.00	164 – 6.7E4	333 – 7.2E6	1.914
TM 1I	$Y = -4.912 + 2.256x$	122.10	426.14	93.0 – 196	246 – 1280	2.400
TM 1N	$Y = -3.868 + 1.589x$	272.10	1741.00	148 – 1485	527 – 58971	3.931
THYMOL	$Y = -2.586 + 1.217x$	133.11	506.10	84 – 377	480 – 27801	2.622

Source: Laboratory work (2016) (*) = Not Determined

Table 42: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ester Derivatives after Four Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 1A	$Y = -3.401 + 1.597x$	135.05	857.00	92.1 – 286.0	367 – 5460	1.045
TM 1B	*	*	*	*	*	*
TM 1C	$Y = -6.035 + 4.210x$	27.10	54.70	24.0 – 30.6	46.6 – 68.4	1.602
TM 1D	$Y = -2.403 + 1.581x$	33.12	214.15	25.4 – 42.5	131 – 533	3.112
TM 1E	$Y = -3.243 + 1.441x$	178.00	1376.00	109.0 – 557.0	471 – 2.1E5	7.663
TM 1F	$Y = -3.758 + 1.554x$	262.03	1748.01	144.0 – 1336.0	532 – 5.4E4	4.076
TM 1G	$Y = -5.349 + 2.120x$	334.00	1342.00	169.0 – 8054.0	399 – 5.0E5	2.971
TM 1I	$Y = -5.366 + 2.678x$	101.00	303.02	81.6 – 140.0	198 – 664	1.472
TM 1N	$Y = -3.629 + 1.626x$	171.00	1050.00	110.0– 452.0	412 – 9911	4.935
THYMOL	$Y = -1.956 + 1.074x$	66.15	320.00	45.9 – 130.0	347 – 1.9E4	1.363

Source: Laboratory work (2016) (*) = Not Determined

Table 43: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ester Derivatives after Five Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 1A	$Y = -3.375 + 1.657x$	109.00	646.00	78.6 – 195.0	310.0 – 2920.0	2.514
TM 1B	*	*	*	*	*	*
TM 1C	$Y = -7.385 + 5.467x$	22.40	38.50	20.2 – 24.9	33.5 – 47.2	5.079
TM 1D	$Y = -2.777 + 1.890x$	29.50	140.00	23.3 – 36.3	97.5 – 258.0	1.713
TM 1E	$Y = -2.743 + 1.410x$	88.20	715.00	63.5 – 160.0	312.0 – 4434.0	4.346
TM 1F	$Y = -3.435 + 1.566x$	156.00	1029.00	102.0 – 383.0	409.0 – 8742.0	1.820
TM 1G	$Y = -5.816 + 2.420x$	253.10	857.20	150.0–1792.0	328.0–37165.0	1.166
TM 1I	$Y = -4.506 + 2.258x$	99.00	366.10	77.9– 145.0	223.0– 915.0	3.366
TM 1N	$Y = -3.523 + 1.666x$	130.00	765.00	90.6 – 263.0	344.0 – 4381.0	5.222
THYMOL	$Y = -1.956 + 1.074x$	66.15	320.00	45.9 – 130.0	347.0 – 1.9E4	1.363

Source: Laboratory work (2016) (*) = Not Determined

Table 44: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ester Derivatives after Six Days of Exposure for Adulticidal Assay

Thymoi Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 1A	$Y = -3.492 + 1.781x$	91.40	479.00	69.2–144.0	257–1591	1.934
TM 1B	*	*	*	*	*	*
TM 1C	$Y = -6.393 + 4.843x$	20.90	38.40	18.6–23.4	33.0–47.9	6.223
TM 1D	$Y = -2.894 + 2.063x$	25.30	106.00	20.1–30.7	77.6–173.0	0.850
TM 1E	$Y = -2.741 + 1.441x$	79.90	619.10	58.7–135.0	285.0-3245.0	3.055
TM 1F	$Y = -3.043 + 1.403x$	148.00	1211.00	95.0–384.0	44 –13584	1.481
TM 1G	$Y = -3.742 + 1.572x$	240.10	1570.00	137.0–999.0	511 – 32866	2.467
TM 1I	$Y = -3.967 + 2.044x$	87.30	370.00	68.6–126.0	221– 944	3.569
TM 1N	$Y = -2.848 + 1.434x$	96.90	759.10	68.9–182.0	328 – 4771	1.509
THYMOL	$Y = -1.9831 + 1.251x$	38.12	306.01	28.0–54.2	194 – 2107	1.135

Source: Laboratory work (2016) (*) = Not Determined

Table 45: Relative Toxicity of Thymol and its Alkyl and Substituted Alkyl Ester Derivatives after Seven Days of Exposure for Adulticidal Assay

Thymol Derivatives	Regression	LC_{50}	LC_{90}	CI for LC_{50}	CI for LC_{90}	Chi - square
TM 1A	$Y = -3.739 + 2.129x$	57.10	228.00	46.9 – 72.9	153 – 445	1.375
TM 1B	*	*	*	*	*	*
TM 1C	$Y = -4.891 + 4.060x$	16.02	33.10	13.7 – 18.2	28.1 – 42.8	3.692
TM 1D	$Y = -3.011 + 2.207x$	23.10	88.13	18.4 – 27.9	66.8 – 136	2.214
TM 1E	$Y = -3.032 + 1.708x$	59.60	336.00	47.1 – 82.6	194 – 935	3.989
TM 1F	$Y = -2.867 + 1.378x$	121.05	1027.10	81.0 – 273.0	394 – 9629	2.241
TM 1G	$Y = -3.576 + 1.562x$	195.00	1288.00	119 – 596.0	466 – 15841	2.005
TM 1I	$Y = -4.116 + 2.150x$	82.10	324.20	65.6 – 114.0	202– 741	3.573
TM 1N	$Y = -2.983 + 1.799x$	45.50	235.00	36.6 – 58.8	148 – 528	1.686
THYMOL	$Y = -2.578 + 1.789x$	27.60	144.00	21.5 – 34.4	97.4 – 283	4.146

Source: Laboratory work (2016); (*) = Not Determined

After one day of the mosquitoes exposure to the thymol derivatives, TM 1G, showed no response across all dosage levels (12.5 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L) and its model could not be computed.

Among the alkyl ester derivatives of thymol, TM 1C, and TM 1D showed better activity than the parent compound, thymol. TM 1C showed the strongest adulticidal towards the female mosquitoes of *An. gambiae s.s.* with an LC_{50} value of 16.02 mg/L after 7 days of exposure time. This was followed by TM 1D with an LC_{50} value of 23.11 mg/L after 7 days of exposure time. The other alkyl ester derivatives TM 1N, TM 1A, TM 1E, TM 1I, TM 1F and TM 1G with LC_{50} values 45.50, 57.10, 59.60, 82.10, 121.05 and 195.00 mg/L respectively after 7 days of exposure time, showed lower activity compared to thymol with an LC_{50} value of 27.60 mg/L after 7 days of exposure time, (Table 45). The 6% glucose in water with 0.1M DMSO solution and the 6% glucose in water as control recorded zero proportion of dead adult mosquito over the study period. When the adult mosquitoes were subjected to feeding on water alone without food (ie 6% glucose in water) during the study period, there was 100% mortality recorded between day 3 and day 4. Again, when the adult mosquitoes were starved (ie no water and food, 6% glucose), 100% mortality was recorded within the first two days (Figure 19).

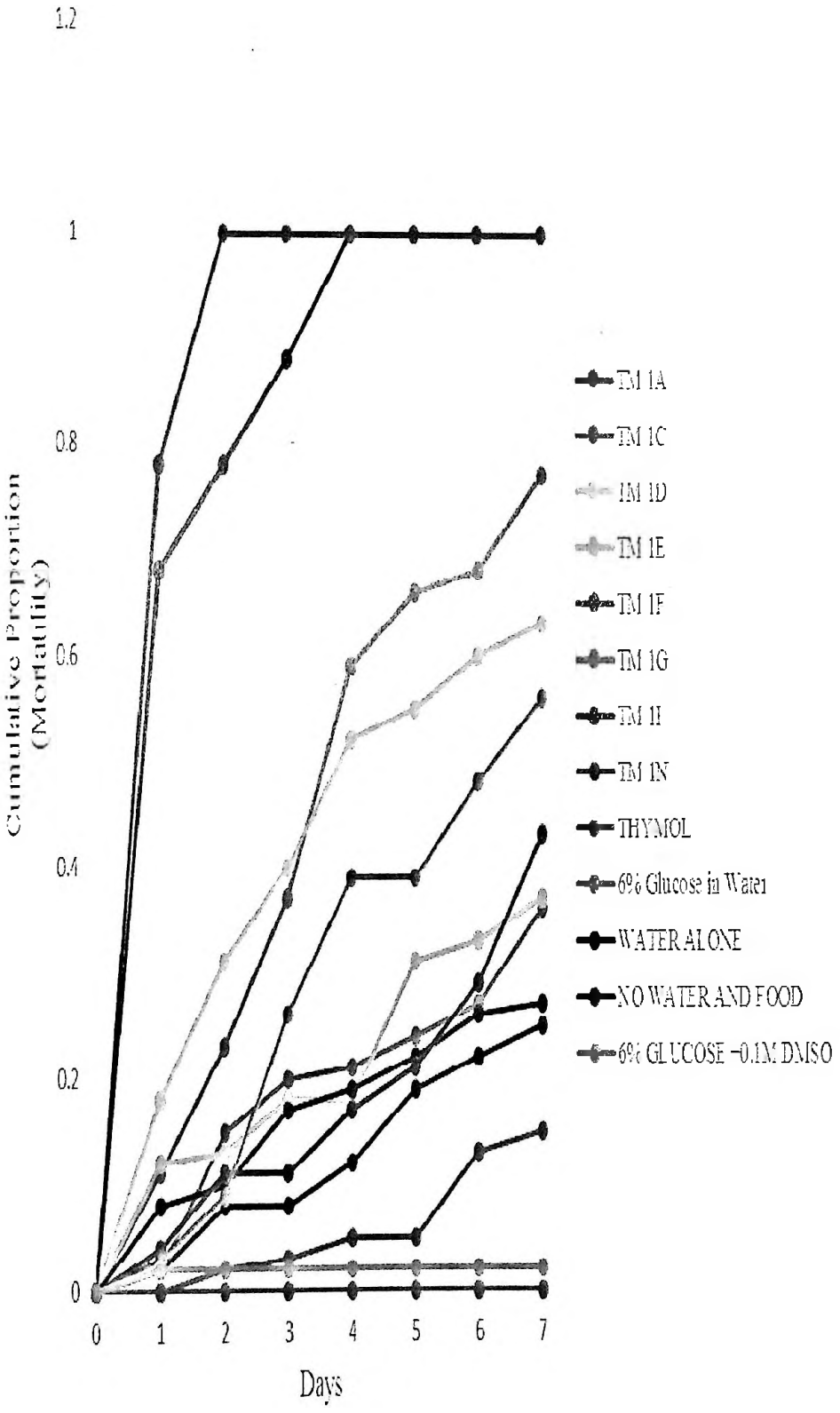


Figure 19: Cumulative proportion of dead mosquitoes for thymol, its alkyl and alkyl substituted ester derivatives.

The average number of dead adult mosquito recorded after day one appears significantly different from that of day two through day seven for all the thymol derivatives with the exception of TM 1E (397.00, 298.00, 178.00, 178.00, 88.20, 79.90 and 59.60 mg/L), TM 1F(554.20, 343.00, 343.00, 262.03, 156.00, 148.00 and 121.05 mg/L) and TM 1N(596.00, 272.10, 272.10, 171.00, 130.00, 96.90 and 45.50 mg/L) respectively from day 1 to day 7 in the experiment. The activity of TM 1E was the same for day 3 and day 4, whilst that of TM 1F and TM 1N was the same for day 2 and day 3 (Figures 19 & 20).

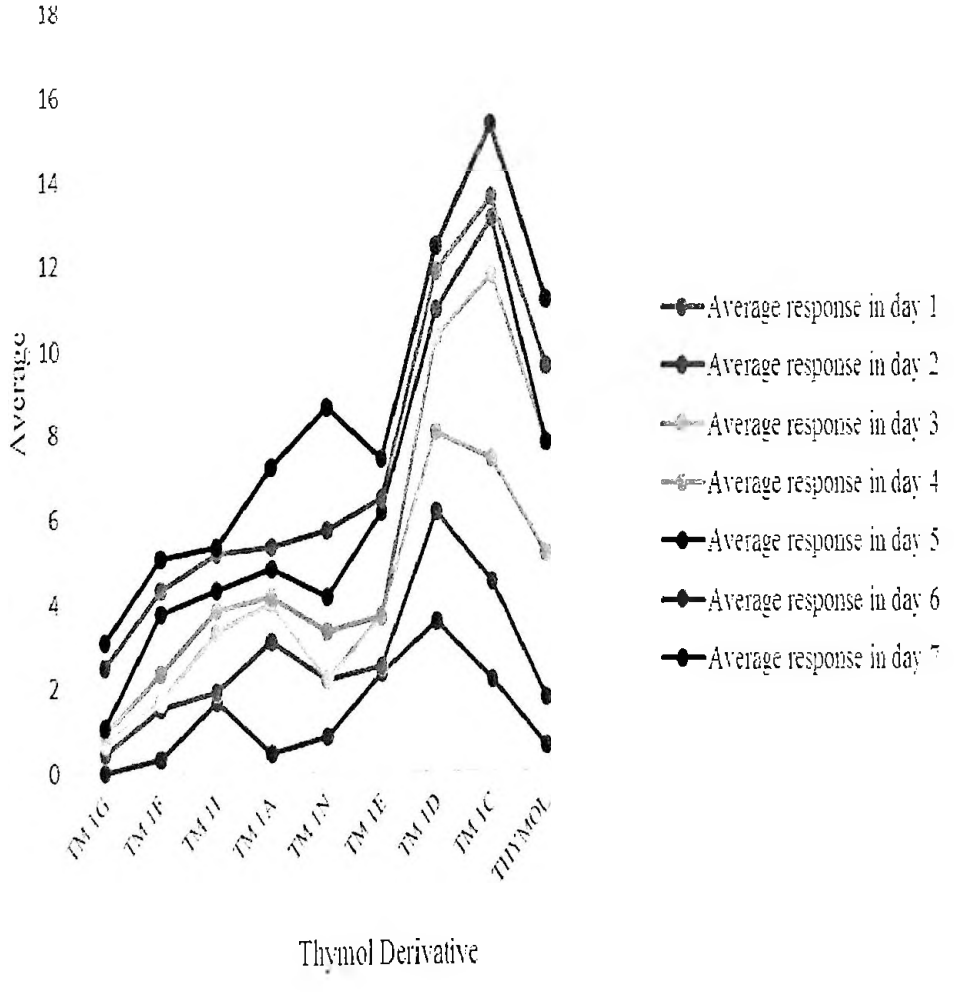


Figure 20: Average number of dead mosquitoes over the seven study periods for thymol, its alkyl and alkyl substituted ester derivatives.

To ascertain whether there was a significant difference in the mean number of dead mosquitoes recorded across the period considered in the study, Analysis of Variance test was performed (Table 46).

Table 46: Analysis of Variance (ANOVA) for Thymol and its Alkyl and Alkyl Substituted Ester Derivatives for Adulticidal Assay

Source	DF	Sum of Squares	Mean Squares	F	P – value
Treatment	6	345.10	57.52	5.86	0.000
Error	56	549.64	9.81		
Total	62	894.74			

The following hypothesis was tested;

$H_o : \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7$ versus $H_1 : \mu_i \neq \mu_j$ for some $i \neq j$

The null hypothesis of no difference in the mean number of dead adult female mosquitoes over the seven period was rejected hence the average number of dead mosquitoes observed after day 1 was significantly different from that after day two through to seven days after, (p-value = 0.000 < α = 0.05). Thus, the effect of thymol and it derivatives was influenced by length of time. The adulticidal mortality rates recorded for all the alkyl ester derivatives were found to be directly proportional to increasing exposure time and concentration of the test materials prepared, as all of them showed the highest mortality rates after 7 days of treatment. The activity of all the test compounds were concentration dependant as the highest mortality rates for the various compounds were recorded at the highest concentration (Figure 20).

Discussion

Characterisation of Synthesised Compounds

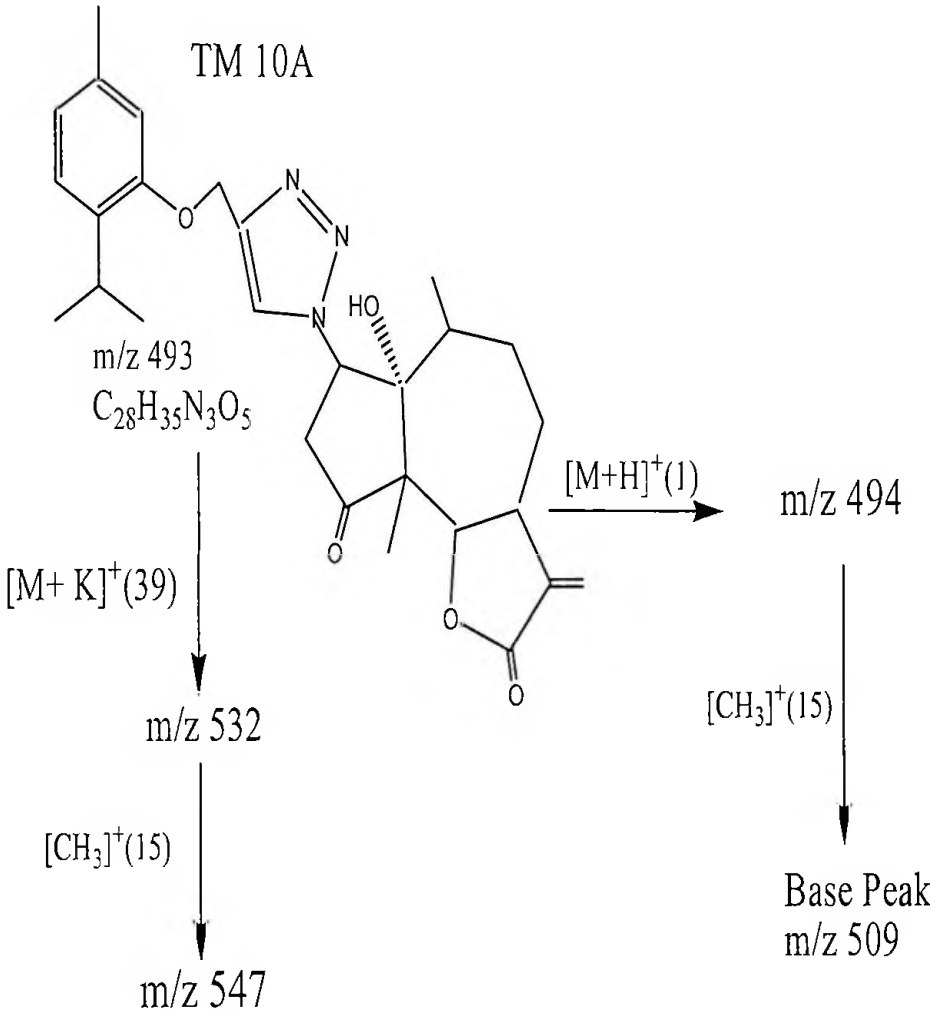
Structural elucidation of the various synthesised derivatives of thymol is discussed using their ^{1}HNMR, ^{13}C-NMR, IR and Mass spectral analysis. The mass spectra as a tool would be employed to chemical formula, characteristic fragment patterns and possible fragment ions. IR spectroscopy would be used to determine the presence or absence of a variety of functional groups in the synthesisied thymol derivatives. Finally, the NMR data would be used to establish the structure fully of the prepared compounds through the application of factors such as chemical shifts, spin multiplicity, coupling constants and integration.

Thymol-Parthenin Coupled Compounds (TM 10A and TM 10B)

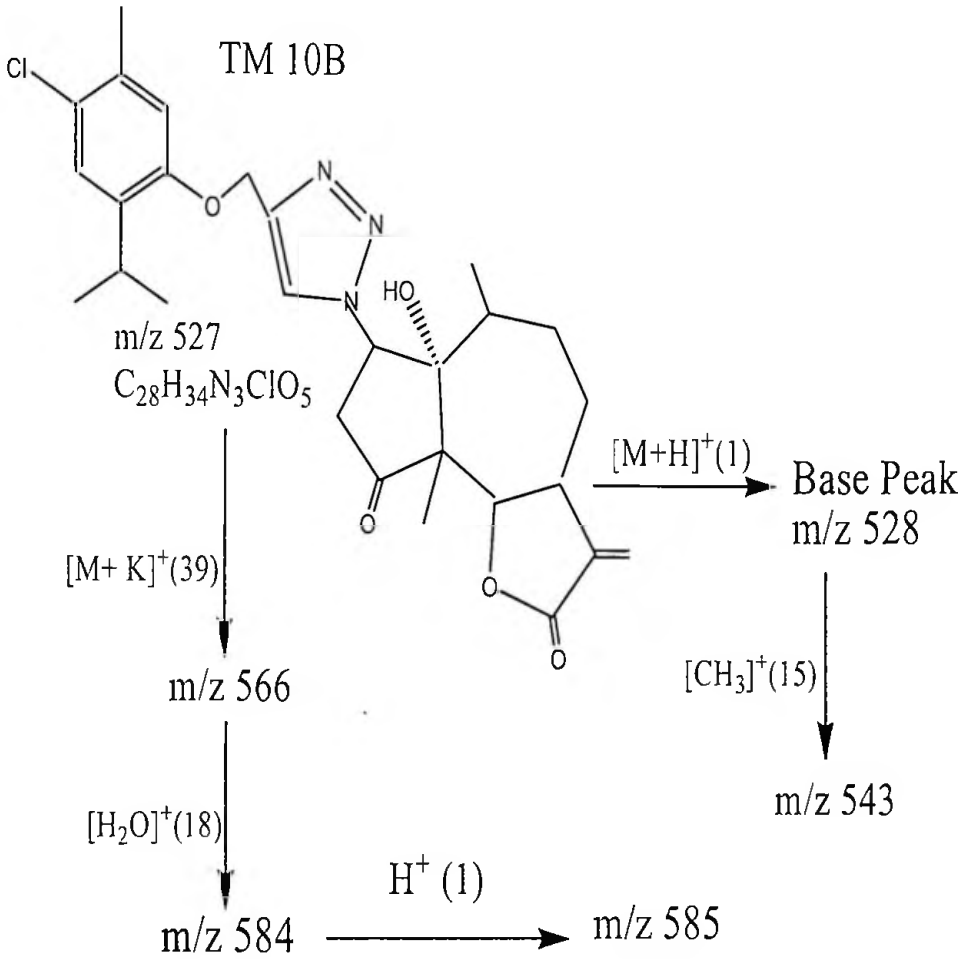
The infra-red spectrum of the thymol-parthenin coupled compounds were very revealing as it indicated all the major functional groups expected in the target molecules (TM 10A and TM 10B) (Appendices B-3 & B-4). A characteristic broad absorption peak was observed at 3274.1 and 3354.9 cm^{-1} for TM 10A and TM 10B respectively which is indicative of the hydroxyl (-OH) group on these compounds from the parthenin moiety. The C - H stretching in alkyl region was characterised by a strong absorption peak with a shoulder at 2926.5 and 2870.9 cm^{-1} for TM 10A as well as 2926.7 and 2871.2 cm^{-1} for TM 10B which is indicative of the aliphatic methylene ($-CH_2-$) and methyl ($-CH_3$) groups. A characteristic and pronounced carbonyl peak appeared at 1750.8 cm^{-1} for TM 10A and 1750.5 cm^{-1} for TM 10B with a corresponding shoulder peak to the carbonyl peak which is an indicative of an

azo group –N=N- was observed at 1716.9 cm^{-1} for TM 10A as well as 1721.8 cm^{-1} for TM 10B. The presence of the azido peaks in the carbonyl region and the absence of the representative absorption peak for azido group at 2100.0 cm^{-1} confirms the formation of the triazolyl ring moiety in all the compounds (TM 10A and TM 10B). The ether functional group linkage –C– O(aromatic) was characterised by an absorption in the region 1287.7 and 1246.4 cm^{-1} for TM 10A and 1242.7 cm^{-1} for TM 10B (Table 11).

The high resolution mass spectrum of the compounds (TM 10A and TM 10B) which led to a confirmation of their structure gave the molecular ion $[M+H]^+$ peak at m/z 528 for the isotopic mass of 35 for the chlorine and a corresponding peak at m/z 530 for the isotopic mass of 37 for the chlorine were observed for TM 10B. Again, a maldi mass of the $[M+H]^+$ peak with potassium and water adducts was observed at m/z 585. This confirm traces of water in the synthesised compound, TM 10B.The molecular ion $[M+H]^+$ with a methyl adduct peak at m/z 509 was observed for TM 10A, which was also a representative of the base peak of the compound. In addition to this, a maldi mass of $[M]^+$ with a potassium and a methyl adduct was observed at m/z 547 for TM 10A. The total ion chromatogram mass fragments for the compounds TM 10A and TM 10B are shown (Schemes 20 & 21; Appendices J-1 & J-2) respectively.



Scheme 20: The TIC mass fragmentation details of TM 10A.



Scheme 21: The TIC mass fragmentation details of TM 10B.

Triazoles with thymol moiety (TM 8A, TM8B, TM 8C AND TM 8G)

Analysis of the ^{1}H-NMR indicated the presence of fourteen major signals for all the derivatives without any substitution on the aromatic thymol nucleus (TM 8A, 8B and 8G), except TM 8C which showed fifteen major signals because the other aromatic nucleus other than the thymol nucleus is non-substituted. The signal at 7.67, 7.34, 7.54 and 7.78 ppm (1proton) is due to the triazolyl proton in the triazole ring which is found between the two aromatic rings for TM 8A, 8B, 8C and 8G respectively. The signal at 3.29, 3.24, 3.25 and 3.28ppm (1proton) for TM 8A, 8B, 8C and 8G respectively is a multiplet which is an indicative of the methyne carbon of the isopropyl group with two

adjacent methyl protons on these compounds. The multiplet is a combination of two doublets (doublets of doublets) at 1.19 and 1.20 ppm for TM 8A, 1.12 and 1.17ppm for TM 8B, 1.13 and 1.18ppm for TM 8C, and 1.19 and 1.20 ppm for TM 8G respectively of the two methyl protons on the isopropyl substituent (6 protons) shown in the alkyl region. Again, in the alkyl region, another signal appearing at 2.34, 2.32, 2.34 and 2.34 ppm (3protons) for TM 8A, 8B, 8C and 8G respectively shows protons on the aromatic methyl group of the respective compounds. Two other signals were seen at 5.55 ppm (2protons) and 5.23 ppm (2protons) for TM 8A, 5.56 ppm (2protons) and 5.22 ppm (2protons) for TM 8B, 5.57 ppm (2protons) and 5.22 ppm (2protons) for TM 8C, as well as 5.99 ppm (2protons) and 5.29 ppm (2protons) for TM 8G, in the alkyl region which represents the two methylene protons respectively in the respective compounds. Seven signals were observed in the aromatic region for TM 8A, TM 8B and TM 8G whilst eight signals were seen in the aromatic region for TM 8C which has no substitution on the second aromatic ring either than the thymol moiety. A triplet signal at 7.33 ppm, two singlet signals at 7.35 ppm and 7.40 ppm respectively and four doublet signals at 6.80, 7.13, 7.20, and 7.30 ppm respectively for TM 8A, a triplet signal at 7.30 ppm, two singlet signals at 6.98 ppm and 7.33 ppm respectively and four doublet signals at 6.78, 6.91, 7.11, and 7.14 ppm respectively for TM 8B, two triplet signals at 7.58 and 7.63 ppm respectively, a singlet signal at 7.64 ppm, and four doublet signals at 6.80, 7.08, 7.12 and 7.29 ppm respectively for TM 8G. Three triplet signals at 7.31, 7.40 and 7.41 ppm respectively, a singlet signal at 6.79 ppm and four doublet signals at 6.80, 7.11, 7.12 and 7.29 ppm respectively for TM 8C. (Table 14; Appendices A-1, A-2, A-3 & A-7).

Analysis of the ^{13}C-NMR revealed twenty carbon environments for all the derivatives TM 8A, 8B, 8C and 8G. The alkyl region showed the isopropyl methyl carbons at 21.36, 21.32, 22.76 and 21.26(21.36) ppm for TM 8A, 8B, 8C and 8G respectively. The methyl carbon attached to the aromatic nucleus at were showed at 22.81, 22.77, 22.80 and 22.79 ppm for TM 8A, 8B, 8C and 8G respectively as well as the methyne carbon of the isopropyl substituent at 26.57, 26.63, 26.54 and 26.62 ppm for TM 8A, 8B, 8C and 8G respectively. Again in the alkyl region, the methylene carbon closer to the oxygen of the ether linkage was observed further downfield at 62.52, 64.38, 62.58, and 62.48 ppm for TM 8A, 8B, 8C and 8G respectively. Also the other methylene carbon were observed at 53.61, 53.59, 54.21 and 50.97 ppm for TM 8A, 8B, 8C and 8G respectively due to the fact that, it is further away from the ether linkage oxygen atom. The carbon in the triazole ring were observed at 136.59, 136.98, 145.34 and 136.49 ppm for TM 8A, 8B, 8C and 8G respectively. They were observed further downfield due to their attachment closer to the oxygen atom of the ether linkage as well as the triazole nitrogen atoms. The other triazole carbon was observed at 121.95, 121.94, 127.80 and 121.95 ppm for TM 8A, 8B, 8C and 8G respectively due to their proximity of attachement from the oxygen atom of the ether functional group linkage. The aromatic carbon of the thymol moiety which is involved in the ether functional group unit was observed a little further downfield at 155.26, 155.22, 155.34 and 155.21 ppm for TM 8A, 8B, 8C and 8G respectively compared to the triazole carbons because of its direct attachment to the oxygen of the ether functional group linkage. On the same thymol aromatic nucleus was observed two quaternary carbons appearing at 136.50 and 134.32 ppm for TM 8A, 136.50 and 134.29

ppm for TM 8B, 136.47 and 134.65 ppm for TM 8C, as well as 134.43 and 130.71 ppm for TM 8G respectively. The other thymol aromatic nucleus carbons appeared at 130.44, 116.49 and 112.94 ppm for TM 8A, 129.96, 123.51 and 115.89 ppm for TM 8B, 126.03, 121.88 and 112.95 ppm for TM 8C as well as 129.70, 125.43 and 112.88 ppm for TM 8G respectively. The other aromatic nucleus either than the thymol nucleus, with an aromatic carbon substituted with chlorine was observed further downfield at 135.01 ppm for TM 8A due to the attachment of the chlorine atom. The substituted carbon with fluorine atom on TM 8B resulted in a further downfield signal of the carbon at 163.99 ppm due tom its attachment to the fluorine atom with a strong shielding effect. The carbon atom with the nitro group was observed further downfield at a signal at 147.40 ppm due to its attachment to the nitro group with a strong shielding effect. This aromatic nucleus had one quaternary carbon also appearing at 135.01 ppm for TM 8A, 136.92 ppm for TM 8B, and 130.40 ppm for TM 8G. The remaining four aromatic carbons appeared at 128.99, 128.04, 126.06 and 126.05 ppm for TM 8A, 130.78, 126.05 116.48 and 114.85 for TM 8B, 134.32, 130.25, 126.09 and 123.51 ppm for TM 8G respectively. The non-substituted aromatic nucleus of TM 8C revealed only one quaternary aromatic carbon atom with a signal at 134.33 ppm. The remaining five aromatic carbons appeared at 129.14, 129.04, 128.78, 128.01 and 122.34 ppm for TM 8C (Table 12; Appendices A-1, A-2, A-3 & A-7).

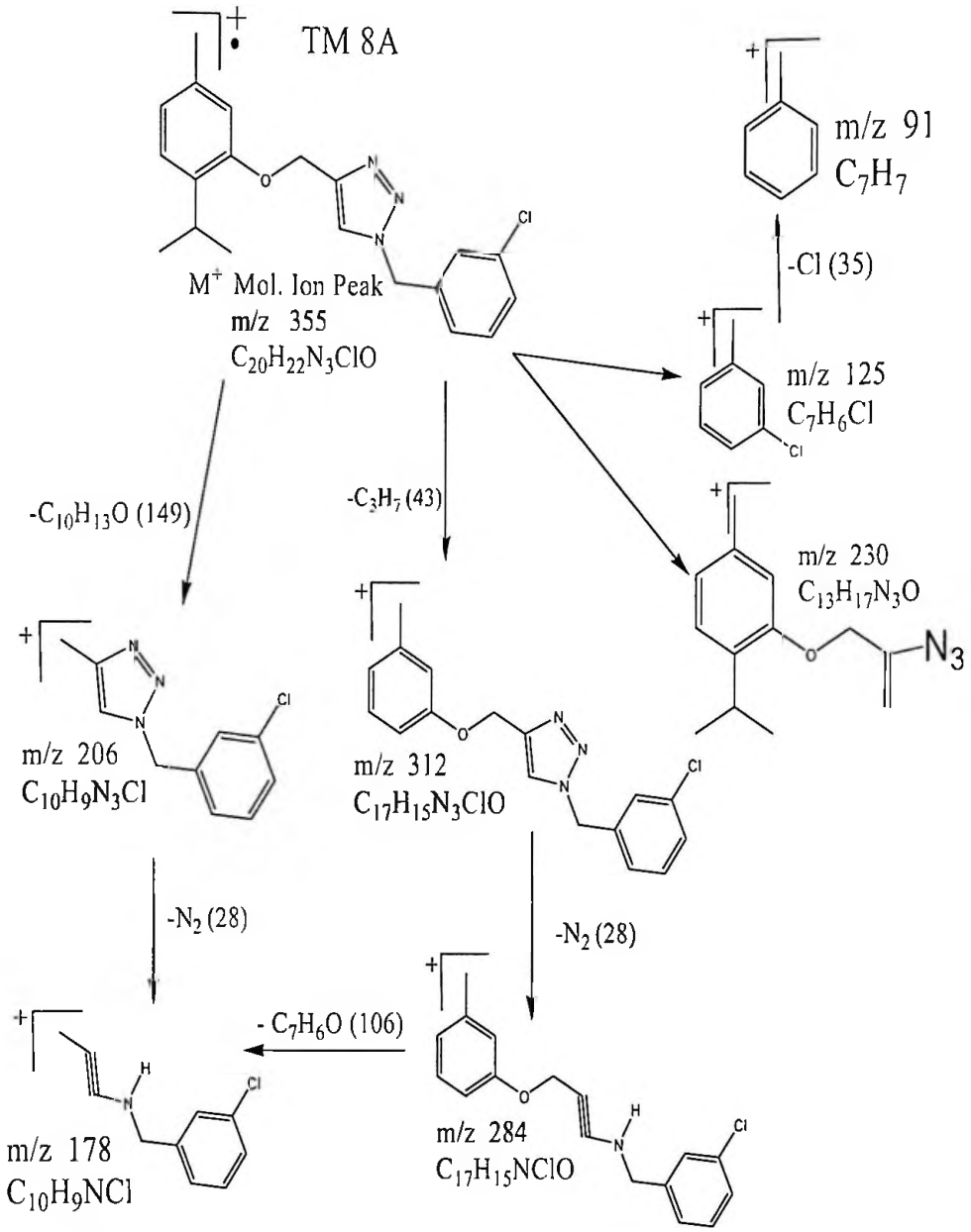
The infra-red spectrum of the compounds was very revealing as it indicated all the major functional groups expected in the target molecules (TM 8A, 8B, 8C, and 8G) (Table 11). A weak absorption at 3158.2 and 3085.4 cm^{-1} for TM 8A, 3156.4 and 3080.8 cm^{-1} for TM 8B, 3150.2, 3087.5, and

3031.3 cm^{-1} for TM 8C as well as 3132.4 and 3088.8 cm^{-1} for TM 8G signifies aromatic proton (Ar-H). The C - H stretching in alkyl region was characterised by absorption peaks in 2957.7 and 2927.7 cm^{-1} for TM 8A, 2963.4 and 2938.2 cm^{-1} for TM 8B, 2961.6 and 2928.5 cm^{-1} for TM 8C as well as 2981.0 , 2951.3 and 2921.7 cm^{-1} for TM 8G with their corresponding aliphatic methylene (-CH_2-) and methyl (-CH_3) groups being observed at 2885.5 and 2867.0 cm^{-1} for TM 8A, 2872.9 cm^{-1} for TM 8B, 2868.8 cm^{-1} for TM 8C as well as 2886.6 and 2867.2 cm^{-1} for TM 8G . An azo group –N=N- observed at 1885.6 cm^{-1} for TM 8A, 1793.0 and 1730.7 cm^{-1} for TM 8B, 1973.1 and 1733.2 cm^{-1} for TM 8C, as well as 1733.3 cm^{-1} for TM 8G confirms the formation of the triazole moiety. The ether functional group linkage –C–O (aromatic) was characterised by an absorption in the region 1241.9 cm^{-1} for TM 8A, 1249.1cm^{-1} for TM 8B, 1256.8 cm^{-1} for TM 8C and 1251.9 cm^{-1} for TM 8G. Again, the absence of the representative absorption peak for azido group at 2100.89 cm^{-1} confirms the formation of the triazole moiety in all the compounds (Appendices B-7, B-8 & B-10)

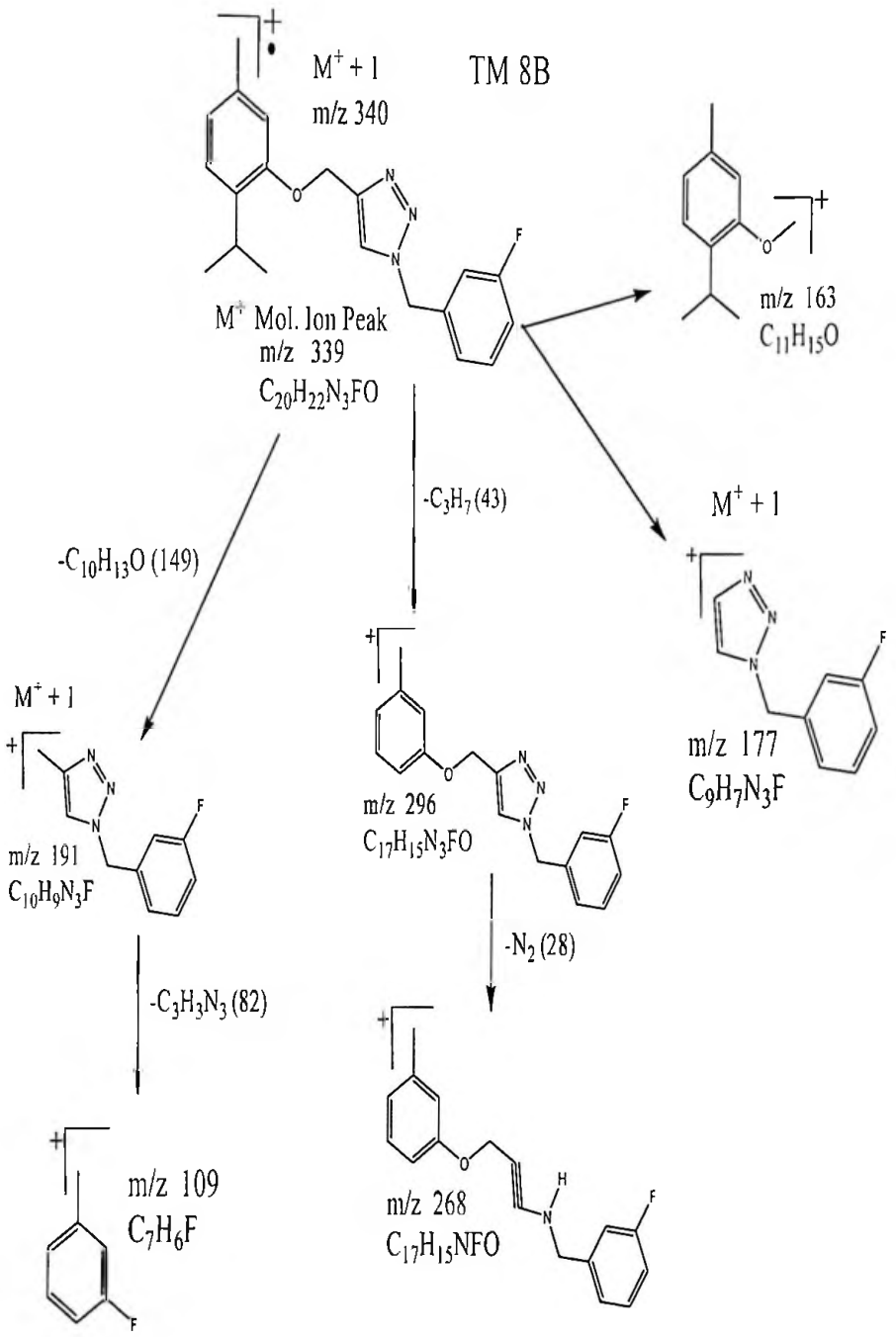
The mass spectrum (EI) of the compounds(TM 8A, 8B, 8C and 8G) which led to a confirmation of their structure gave the molecular ion $[M]^+$ peak at m/z 355 and a corresponding base peak was observed at m/z 125 for TM 8A, molecular ion $[M]^+$ peak at m/z 339 and a corresponding base peak was observed at m/z 109 for TM 8B, molecular ion $[M]^+$ peak at m/z 321 and a corresponding base peak was observed at m/z 91 for TM 8C, molecular ion $[M]^+$ peak at m/z 366 and a corresponding base peak was observed at m/z 135 for TM 8G. A characteristic tropelium ion peak was observed at 91 for TM 8A and TM 8C. The other prominent mass fragments for the compound are 312,

284, and 178 for TM 8A, 296, 268,191 and 162 for TM 8B, 278, 250, 173 and 144 for TM 8C as well as 218, 189, 105 and 78 for TM 8G as accounted for in the fragmentation pattern of the compound (Schemes 22-25 & Appendices C-1, C-2, C-3 & C-7).

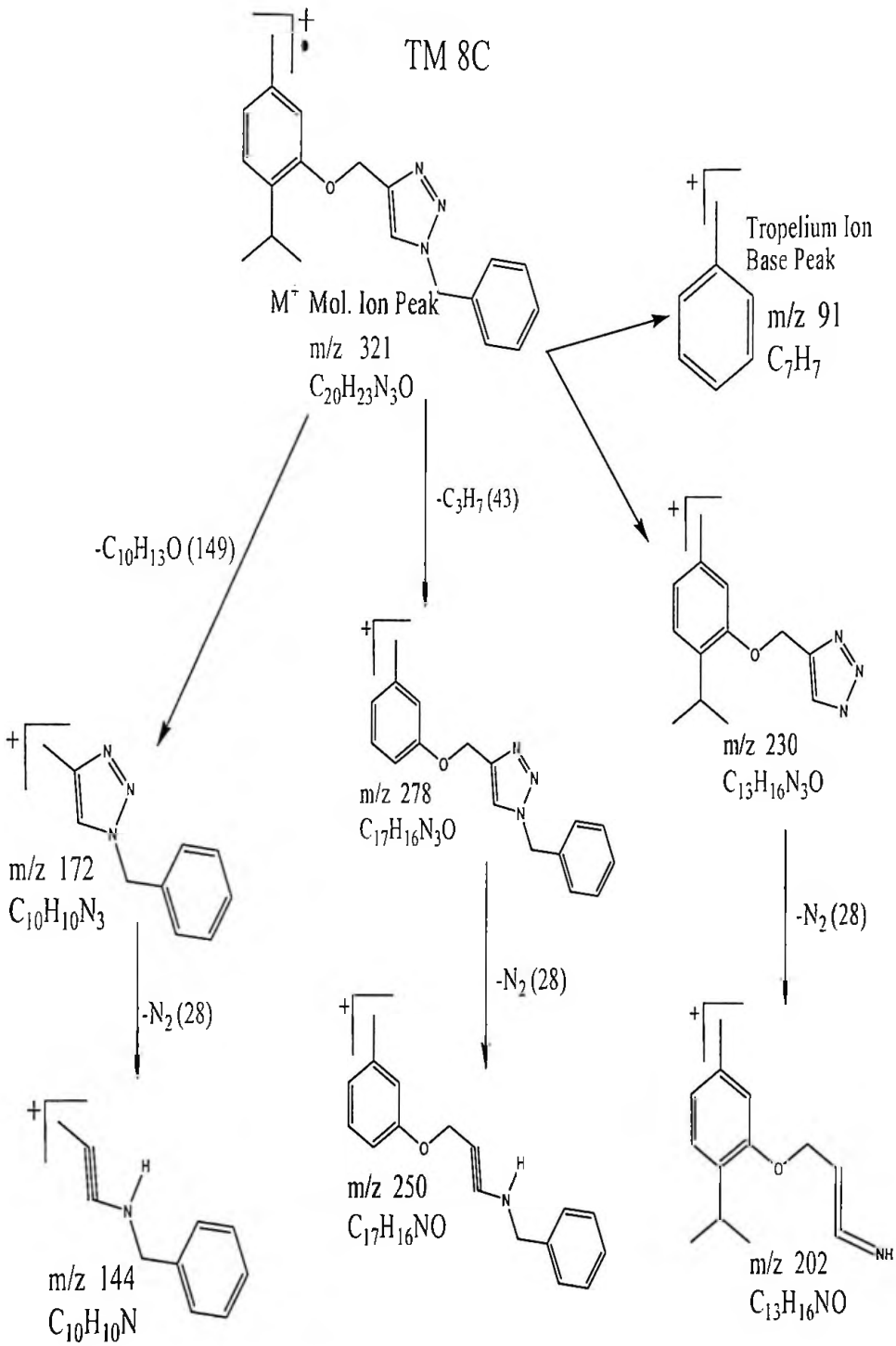
The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 356 and 384 respectively for TM 8A (Appendix F-1), 340 and 368 respectively for TM 8B (Appendix F-1), 322 and 350 respectively for TM 8C (Appendix F-2) as well as 367 and 395 respectively for TM 8G (Appendix F-4). The TOF MS (ES+) gave the *m/z* $[M+H]^+$ and $[M+K]^+$ as 356 and 394 respectively for TM 8A (Appendix I-1) , 340 and 378 respectively for TM 8B (Appendix I-2). 322 and 360 respectively for TM 8C (Appendix I-3), as well as 367 and 405 respectively for TM 8G (Appendix I-7). There is a characteristic dimeric peak of the $[M+K]^+$ ion at 788 for TM 8A (Appendix I-1).



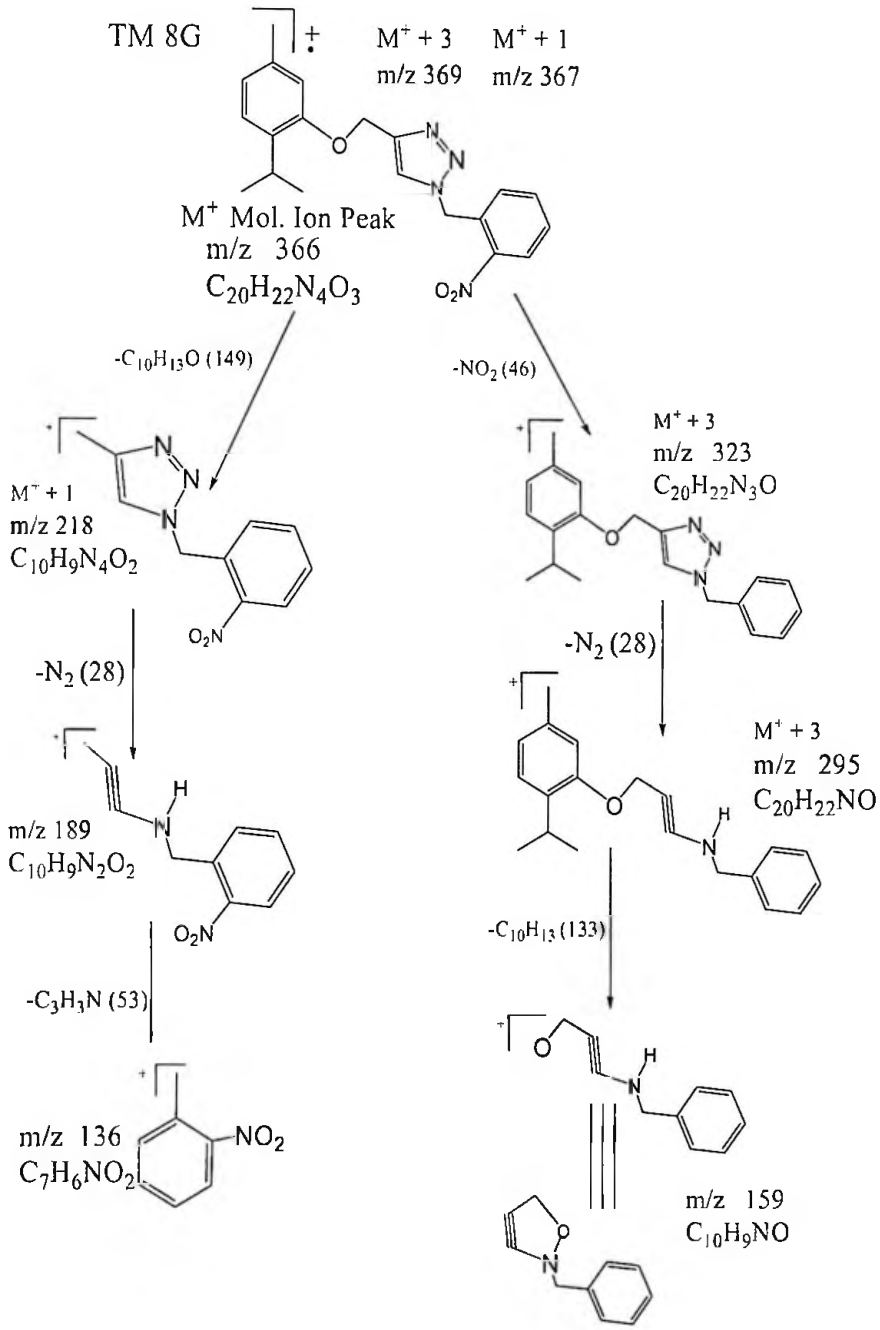
Scheme 22: Mass spectral fragmentation details of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-chloro-methylbenzene (TM 8A).



Scheme 23: Mass spectral fragmentation details of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-fluoro-methylbenzene (TM 8B).



Scheme 24: Mass spectral fragmentation details of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene (TM 8C).



Scheme 25: Mass spectral fragmentation details of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro-methylbenzene (TM 8G).

Triazoles with chlorothymol moiety (TM 8D, TM8E, TM 8F & TM 8H)

Analysis of the ^{1}H-NMR indicated the presence of thirteen major signals for all the derivatives (TM 8D, 8E and 8H), although the proton signal labelled-18(18-H) was not seen and thus, resulted in a total of twelve signals being observed in the ^{1}H-NMR of TM 8D. Again, TM 8F showed fourteen major signals because the other aromatic nucleus other than the chlorothymol nucleus is non-substituted. The signal at 7.63, 7.63, 7.56 and 7.73 ppm (1proton) is due to the triazolyl proton in the triazole ring which is found between the two aromatic rings for TM 8D, 8E, 8F and 8H respectively. The signal at 3.23, 3.26, 3.21 and 3.14 ppm (1proton) for TM 8D, 8E, 8F and 8H respectively is a multiplet which is an indicative of the proton on a methyne carbon of the isopropyl group with two adjacent methyl protons in these compounds. The multiplet is a combination of two doublets (doublets of doublets) at 1.15 and 1.17 ppm for TM 8D, 1.17 and 1.17 ppm for TM 8E, 1.13 and 1.15 ppm for TM 8F, and 1.07 and 1.08 ppm for TM 8H respectively of the two methyl protons on the isopropyl substituent (6 protons) shown in the alkyl region. Again, in the alkyl region, another signal appeared at 2.33, 2.35, 2.31 and 2.25 ppm (3protons) for TM 8D, 8E, 8F and 8H respectively represent protons on the methyl group attached to the aromatic nucleus of the respective compounds. Two other signals were seen at 5.55 ppm (2protons) and 5.20 ppm (2protons) for TM 8D, 5.67 ppm (2protons) and 5.63 ppm (2protons) for TM 8E, 5.54 ppm (2protons) and 5.16 ppm (2protons) for TM 8F as well as 5.89 ppm (2protons) and 5.12 ppm (2protons) for TM 8H, in the alkyl region which represents the two methylene protons respectively in the respective compounds. Six signals were observed in the aromatic region for

TM 8E and TM 8H with five signals been observed for TM 8D as the expected sixth signal could not be seen. Seven signals were seen in the aromatic region for TM 8F which has no substitution on the second aromatic ring either than the chlorothymol moiety. A triplet signal at 7.33 ppm, two doublet signals at 7.16 ppm and 7.28 ppm respectively and two singlet signals at 6.84 and 7.31 ppm respectively for TM 8D as the third doublet signal could not be seen, a triplet signal at 7.06 ppm, two singlet signals at 6.95 ppm and 7.29 ppm respectively and three doublet signals at 7.00, 7.34, and 7.38 ppm respectively for TM 8E, two triplet signals at 7.49 and 7.56 ppm respectively, a doublet signal at 6.96 ppm, and three singlet signals at 6.80, 7.04, and 7.29 ppm respectively for TM 8H. Three triplet signals at 7.28, 7.37 and 7.39 ppm respectively, two singlet signal at 6.84 and 7.13 ppm and two doublet signals at 7.26 and 7.35ppm respectively for TM 8F (Table 15; Appendices A-4, A-5, A-6 & A-8).

Analysis of the ^{13}C-NMR revealed twenty carbon environments for all the derivatives TM 8D, 8F and 8H, except TM 8E which showed nineteen carbon environments with the signal at carcon-14, one of the methylene carbons not seen in the alkyl region. The alkyl region showed the isopropyl methyl carbons at 22.61, 22.63(22.72), 22.59 and 20.91 ppm for TM 8D, 8E, 8F and 8H respectively. The aromatic methyl carbon at 20.06, 20.33, 20.02 and 14.07 ppm for TM 8D, 8E, 8F and 8H respectively as well as the methyne carbon of the isopropyl substituent at 26.61, 26.60, 26.58 and 26.53 ppm for TM 8D, 8E, 8F and 8H respectively. Again in the alkyl region, the methylene carbon closer to the oxygen of the ether linkage was observed further downfield at 62.72, 64.60, 62.72, and 62.48 ppm for TM 8D, 8E, 8F and 8H

respectively compared to the other methylene carbon observed at 53.62, 54.16 and 50.87 ppm for TM 8D, 8F and 8H respectively which is further away from the ether linkage oxygen atom. The signal of the methylene carbon labelled C-14 for TM 8E was not seen. The triazole ring carbons were observed at 136.69, 136.69, 136.73 and 144.53 ppm for TM 8D, 8E, 8F and 8H respectively further downfield due to its attachment closer to the oxygen atom of the ether linkage as well as the triazole nitrogen atoms and the other observed at 118.03, 122.22, 116.55 and 125.29 ppm for TM 8D, 8E, 8F and 8H respectively due to its distant proximity from the oxygen atom of the ether functional group linkage to which it is attached. The aromatic carbon of the thymol moiety which is involved in the ether functional group unit was observed a little further downfield at 153.80, 153.68, 153.90 and 153.82 ppm for TM 8D, 8E, 8F and 8H respectively compared to the triazole carbons because of its direct attachment to the oxygen of the ether functional group linkage. On the same chlorothymol aromatic nucleus was observed three quaternary carbons appearing at 135.03, 133.80 and 126.75 ppm for TM 8D, 130.84, 130.77 and 126.49 ppm for TM 8E, 133.74, 133.74 and 126.24 ppm for TM 8F, as well as 134.35, 133.64 and 126.56 ppm for TM 8H respectively. The other thymol aromatic nucleus carbons appeared at 126.75 and 114.66 ppm for TM 8D, 126.75 and 114.78 ppm for TM 8E, 126.68, and 114.74 ppm for TM 8F as well as 129.67 and 114.72 ppm for TM 8H respectively. The other aromatic nucleus either than the chlorothymol nucleus, with an aromatic carbon substituted with chlorine was observed further downfield at 133.80 ppm for TM 8D due to the attachment of the chlorine atom on that carbon. The substituted carbon with fluorine atom on TM 8E resulted in a further

downfield signal of the carbon at 163.93 ppm due to its attachment to the fluorine atom with a much stronger shielding effect. The substituted carbon atom with nitro group was observed further downfield at a signal at 147.42 ppm due to its attachment to the nitro group with a strong shielding effect.This aromatic nucleus had second quaternary carbon also appearing at 136.50 ppm for TM 8D, 133.85 ppm for TM 8E, and 130.71 ppm for TM 8H. The remaining four aromatic carbons appeared at 130.46, 129.03, 128.03 and 126.04 ppm for TM 8D, 130.10, 123.73, 116.12 and 113.78 for TM 8E, 136.69, 130.62, 130.14 and 126.06 ppm for TM 8H respectively. The non-substituted aromatic nucleus of TM 8F revealed only one quaternary aromatic carbon atom with a signal at 134.65 ppm. The remaining five aromatic carbons appearing at 129.12, 129.12, 128.77, 128.00 and 122.67 ppm for TM 8F (Table 13; Appendices A-4, A- 5, A-6 & A-8)

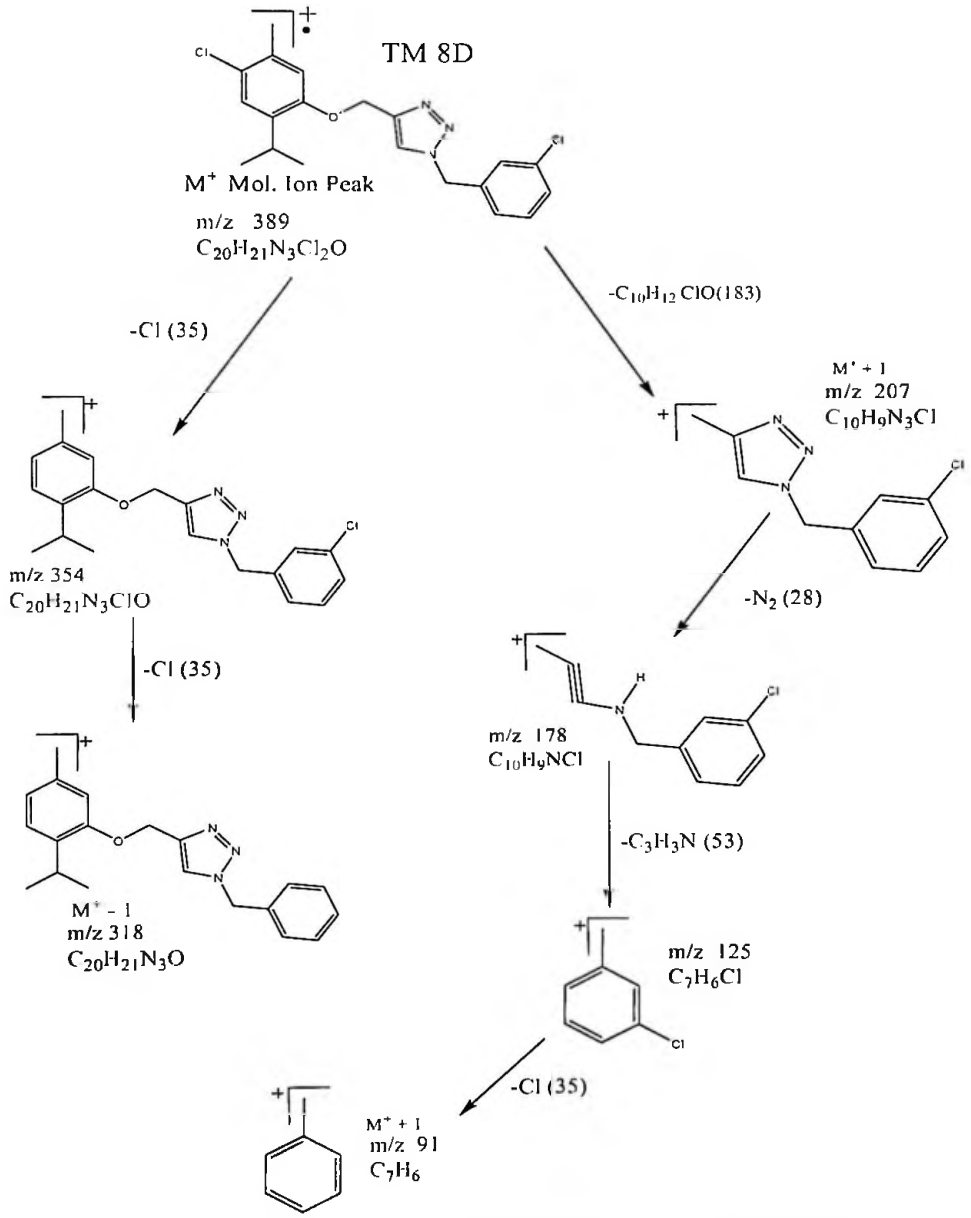
The infra-red spectra of the compounds were very revealing as they indicated all the major functional groups expected in the target molecules (TM 8D, 8E, 8F and 8H) (Table 11). A weak absorption at 3155.7 and 3073.7 cm^{-1} for TM 8D, 3117.1 and 3075.5 cm^{-1} for TM 8E, 3124.4, 3076.5, and 3041.6 cm^{-1} for TM 8F as well as 3152.8,3068.7,3037.4 and 3011.8 cm^{-1} for TM 8G signifies aromatic proton (Ar-H). The C - H stretching in alkyl region was characterised by absorption peaks in 2982.5, 2959.5 and 2927.8 cm^{-1} for TM 8D, 2967.7 and 2924.9 cm^{-1} for TM 8E, 2962.2 and 2926.6 cm^{-1} for TM 8F as well as 2980.9 , 2960.2 and 2918.5 cm^{-1} for TM 8H with their corresponding aliphatic methylene ($-CH_2-$) and methyl ($-CH_3$) groups being observed at 2868.3 cm^{-1} for TM 8D, 2872.2 cm^{-1} for TM 8E, 2875.0 cm^{-1} for TM 8F as well as 2864.2 cm^{-1} for TM 8H . An azo group –N=N- observed at

1738.8 cm^{-1} for TM 8D, 1729.3 cm^{-1} for TM 8E, 1762.8 cm^{-1} for TM 8F, as well as 1745.5 cm^{-1} for TM 8H confirms the formation of the triazolyl ring moiety. The ether functional group linkage –C– O(aromatic) was characterised by an absorption in the region 1248.2 cm^{-1} for TM 8D, 1245.4cm^{-1} for TM 8E, 1243.9 cm^{-1} for TM 8F and 1248.7 cm^{-1} for TM 8H. Again, the absence of the representative absorption peak for azido group at 2100.89 cm^{-1} confirms the formation of the triazole moiety in all the compounds (Appendices B-8, B-9 & B-10).

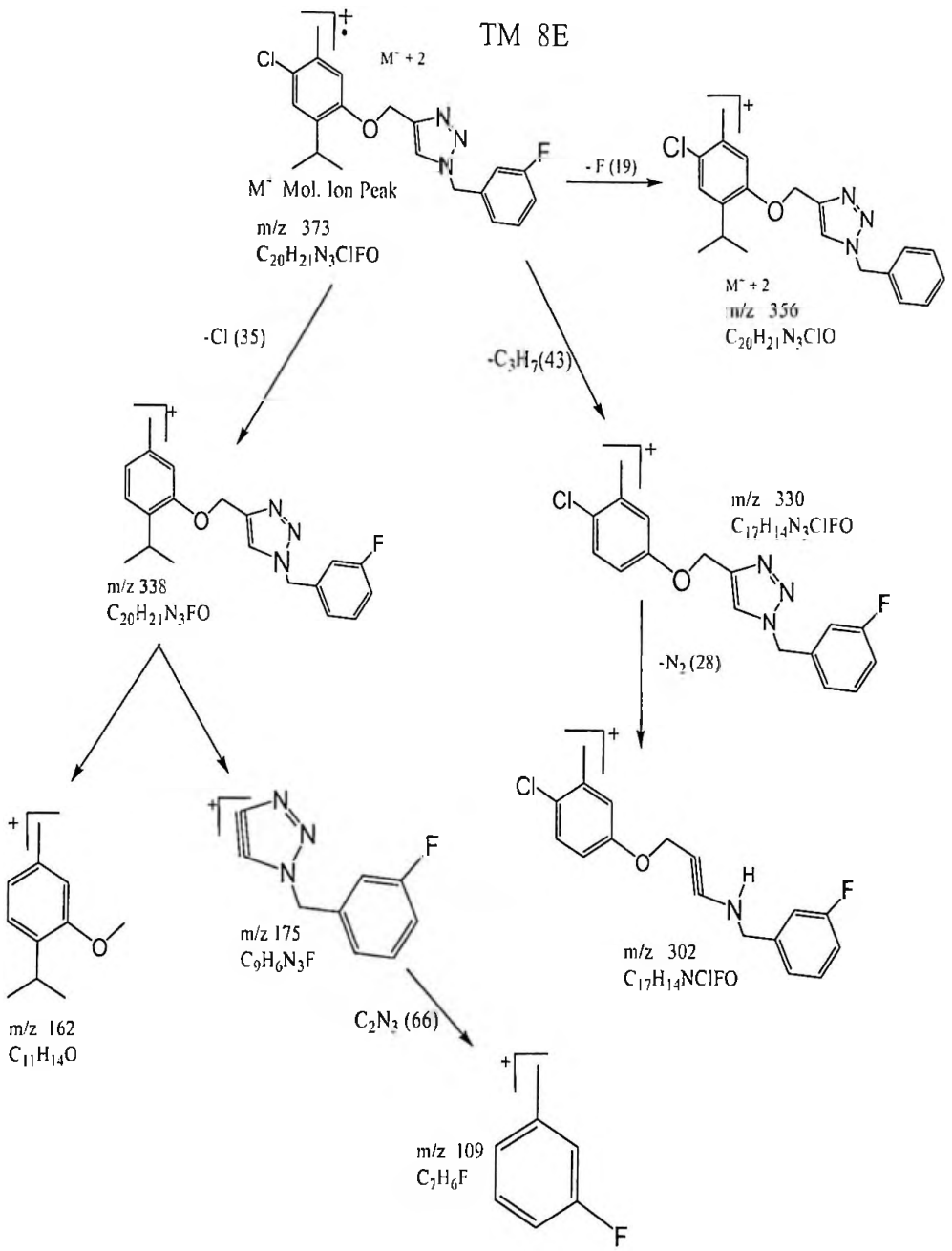
The mass spectrum (EI) of the compounds (TM 8D, 8E, 8F and 8H) which led to a confirmation of their structure gave the molecular ion $[M]^+$ peak at m/z 389 and a corresponding base peak was observed at m/z 125 for TM 8D, molecular ion $[M]^+$ peak at m/z 373 and a corresponding base peak was observed at m/z 109 for TM 8E, molecular ion $[M]^+$ peak at m/z 355 and a corresponding base peak was observed at m/z 91 for TM 8F, molecular ion $[M]^+$ peak at m/z 400 and a corresponding base peak was observed at m/z 189 for TM 8H. A characteristic tropelium ion peak was observed at 91 for TM 8D and TM 8F. The other prominent mass fragments for the compound are 354, 207, and 178 for TM 8D; 356, 302 and 162 for TM 8E; 284, 169 and 144 for TM 8F as well as 357, 218 and 136 for TM 8H as accounted for in the fragmentation pattern of the compounds (Schemes 26-29; Appendices C-4, C-5, C-6 & C-8).

The mass spectrum (Cl) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 390 and 418 respectively for TM 8D (Appendix F-2), 374 and 402 respectively for TM 8E (Appendix F-3), 356 and 384 respectively for TM 8F (Appendix F-3) as well as 401 and 429 respectively for TM 8H (Appendix F-4). The TOF MS

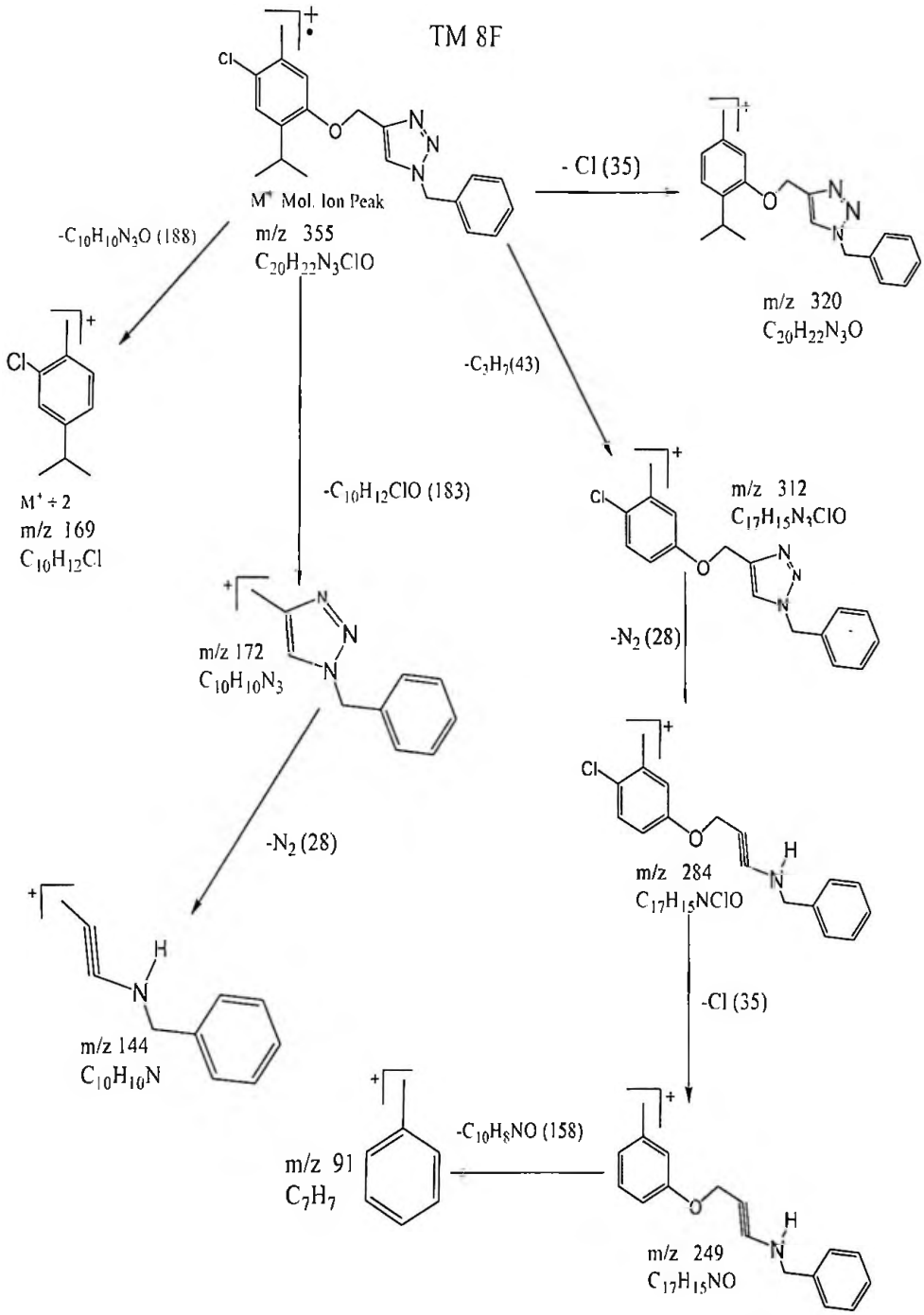
(ES+) gave the *m/z* $[M+H]^+$ and $[M+K]^+$ as 390 and 428 respectively for TM 8D (Appendix I-4), 374 and 412 respectively for TM 8E (Appendix I-5), 356 and 394 respectively for TM 8F (Appendix I-6), as well as 401 and 439 respectively for TM 8H (Appendix I-8). There was a characteristic dimeric peak of the $[M+K]^+$ ion at 788 for TM 8F (Appendix I-6).



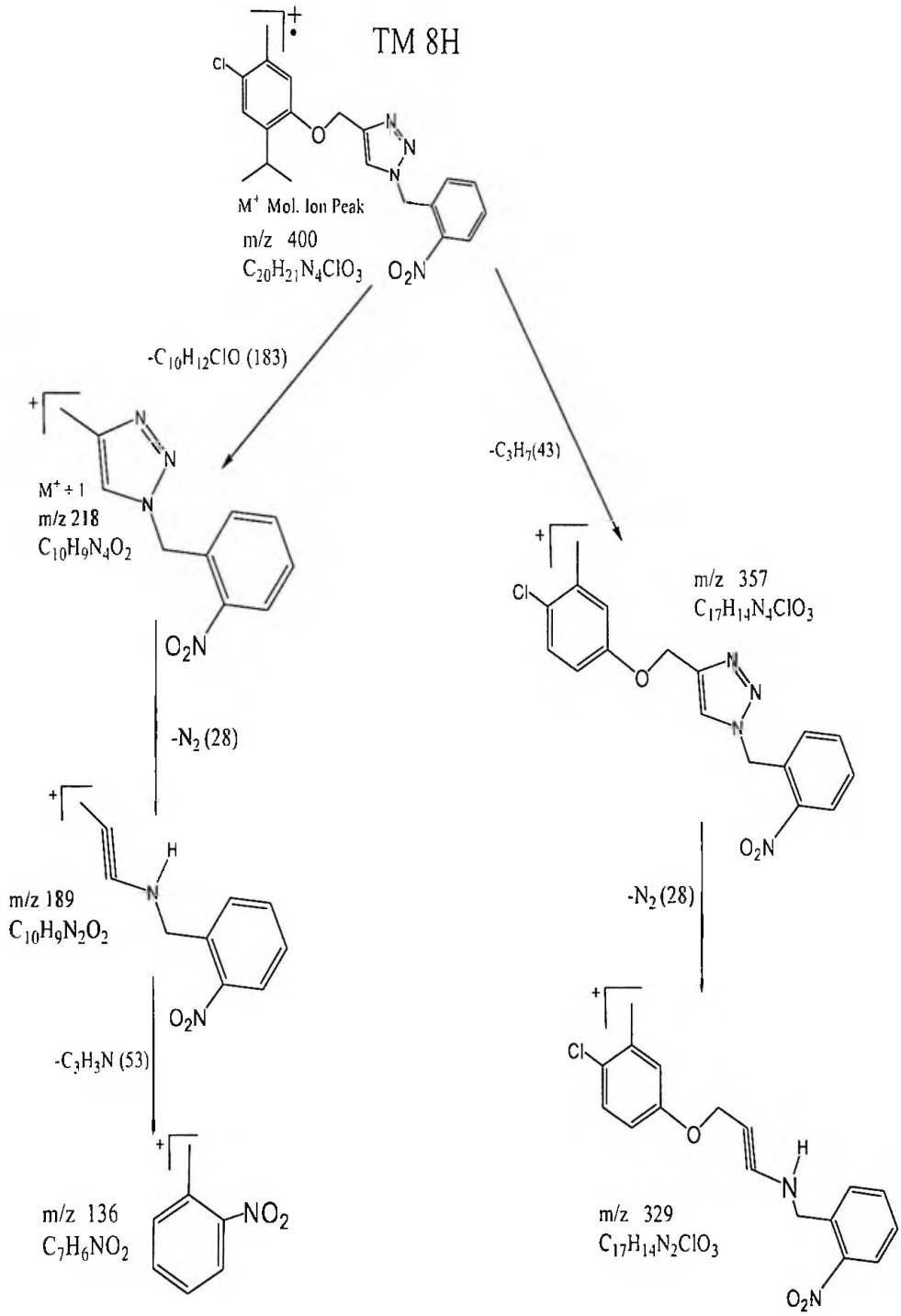
Scheme 26: Mass spectral fragmentation details of 1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-chloro-methylbenzene (TM 8D).



Scheme 27: Mass spectral fragmentation details of 1-[4-(4-chloro-2-isopropyl-5- methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-3-fluoro-methylbenzene (TM 8E).



Scheme 28: Mass spectral fragmentation details of 1-[4-(4-chloro-2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-methylbenzene (TM 8F).



Scheme 29: Mass spectral fragmentation details of 1-[4-(4-chloro-2-isopropyl-5- methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-nitro-methylbenzene (TM 8H).

Triazoles with two thymol groups (TM 8I AND TM 8J)

Analysis of the ^{1}H-NMR indicated the presence of fifteen major signals for the derivative TM 8I and fourteen major signals for TM 8J , the reason being the chlorine atom on TM 8J which is absent in TM 8I. A characteristic triazolyl proton signal was observed at 7.80 ppm (1proton) each for TM 8I and TM 8J. The signal at 3.30 and 3.20 ppm (1proton) each for TM 8I and TM 8J are multiplet, which is an indicative of the two methyne protons of the isopropyl groups with two adjacent methyl protons on each of these compounds (TM 8I and TM 8J). The observed multiplet signal is a combination of two doublets (doublets of doublets) at 1.20 and 1.20 ppm for TM 8I and 1.20 and 1.20 ppm for TM 8J respectively of the two methyl protons on the isopropyl substituent (6 protons) on each isopropyl unit, making a total of twelve(12) protons as shown in the alkyl region. Again, in the alkyl region, two signals appeared at 2.33 and 2.34 ppm (3protons) respectively each for TM 8I and TM 8J show protons on the methyl group attached to the aromatic nucleus of the respective compounds. Three other signals were seen at 5.54 ppm (2protons), 5.32ppm (2protons) and 5.23 ppm (2protons) for TM 8I, as well as 5.21ppm (2protons), 4.84ppm (2protons) and 4.40ppm (2protons) were also recorded for TM 8J in the alkyl region which represent the three methylene protons respectively in the respective compounds. Six signals were observed in the aromatic region for TM 8I whilst five signals was observed in the aromatic region for TM 8J. The five aromatic signals observed in TM 8J is as a result of the substituted chlorine group on one of the thymol moiety. Four doublet signals at 6.81ppm, 7.11ppm, 7.11ppm and 7.13 ppm respectively and two singlet signals at 6.63ppm and 7.29 ppm

respectively for TM 8I, as well three singlet signals at 5.32 ppm, 6.63 ppm and 7.29 ppm respectively were observed for TM 8J (Tables 14 & 15; Appendices A-9 & A-11).

Analysis of the ^{13}C-NMR revealed twenty five carbon environments for both dimeric thymol derivatives TM 8I and TM 8J. The alkyl region showed the isopropyl methyl carbons at 21.29 and 21.36 ppm for TM 8I as well as 22.62 and 22.74 ppm for TM 8J. The methyl carbons bonded to the aromatic ring were observed at 22.74 ppm and 22.81ppm for TM 8I as well as 20.06 ppm and 21.29 ppm for TM 8J. The methyne carbons of the two isopropyl substituents at 26.48 ppm and 26.52 ppm for TM 8I as well as 26.48 ppm and 26.58 ppm for TM 8J were seen. Again in the alkyl region, the methylene carbons closer to the oxygen of the ether linkage were both observed further downfield at 62.48 ppm and 66.49 ppm with the third methylene carbon further away from the oxygen of the ether linkage also observed at 53.47 ppm for TM 8I. That of TM 8J were also observed at 62.71 ppm and 66.47 ppm for the two methylene carbons very close to the oxygen atom of the ether linkage and the third methylene carbon appearing at 53.45 ppm because of its further away distance from the ether linkage oxygen atom. The triazole ring carbons were observed at 144.97 ppm and 123.45 ppm for TM 8I as well as 136.62 ppm and 123.55 ppm for TM 8J. The high downfield signals observed for the triazole carbons at position 12 (C-12) for both TM 8I and TM 8J is due to its attachment to the oxygen atom of the ether linkage and the triazole nitrogen atoms. The other triazole carbons observed much lower downfield were due to their distance from the oxygen atom of the ether functional group linkage to which it is attached. The two aromatic carbons of the thymol moiety which are

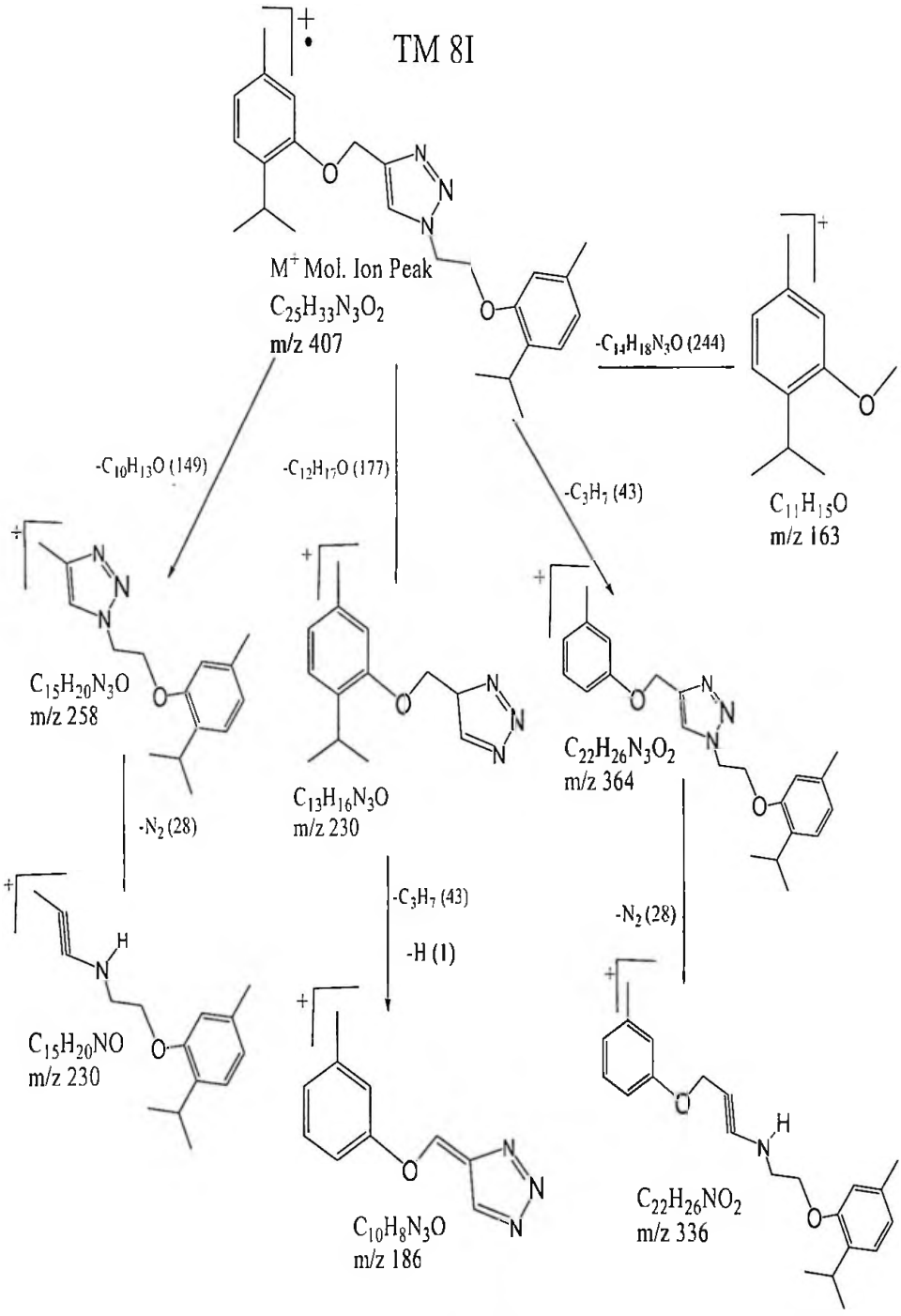
involved in the ether functional group unit were observed a little further downfield at 155.33 ppm and 154.18 ppm respectively for TM 8I as well as 154.77 ppm and 153.89 ppm respectively for TM 8J which are quaternary carbons compared to the triazole carbons because of their direct attachment to the oxygen of the ether functional group linkage. The two aromatic nuclei of TM 8I observed additional four quaternary carbons appearing at 133.98 ppm, 134.24 ppm, 136.50 ppm and 136.59 ppm whilst that of TM 8J showed signal for five additional quaternary carbons appearing at 126.73 ppm, 133.79 ppm, 133.79 ppm, 133.94 ppm and 133.94 ppm. The other six aromatic carbons for TM 8I appeared at 126.12 ppm, 126.01ppm, 122.22 ppm, 121.82 ppm, 112.71ppm and 112.30 ppm as well as five other aromatic carbons for TM 8J also appeared at 126.27 ppm, 126.15 ppm, 122.27 ppm, 114.44 ppm and 112.30 ppm (Tables 12 & 13; Appendices A-10 & A-12).

The infra-red spectrum of the compounds was very revealing as it indicated all the major functional groups expected in the target molecules (TM 8I and 8J) (Table 11). A weak absorption at 3159.2 and 3031.5 cm^{-1} for TM 8I as well as 3160.3 and 3033.7 cm^{-1} for TM 8J, signifies aromatic proton (Ar-H). The C - H stretching in alkyl region was characterised by absorption peaks in 2958.6 and 2923.6 cm^{-1} for TM 8I as well as 2959.3 and 2924.1 cm^{-1} for TM 8J with their corresponding aliphatic methylene ($-CH_2-$) and methyl ($-CH_3$) groups being observed at 2868.9 cm^{-1} for TM 8I and 2868.3 cm^{-1} for TM 8J. An azo group –N=N- observed at 1880.4 and 1698.9 cm^{-1} for TM 8I as well as 1742.7 and 1704.1 cm^{-1} for TM 8J confirms the formation of the triazolyl ring moiety. The ether functional group linkage –C–O (aromatic) was characterised by an absorption in the region 1252.6 cm^{-1} for TM 8I and

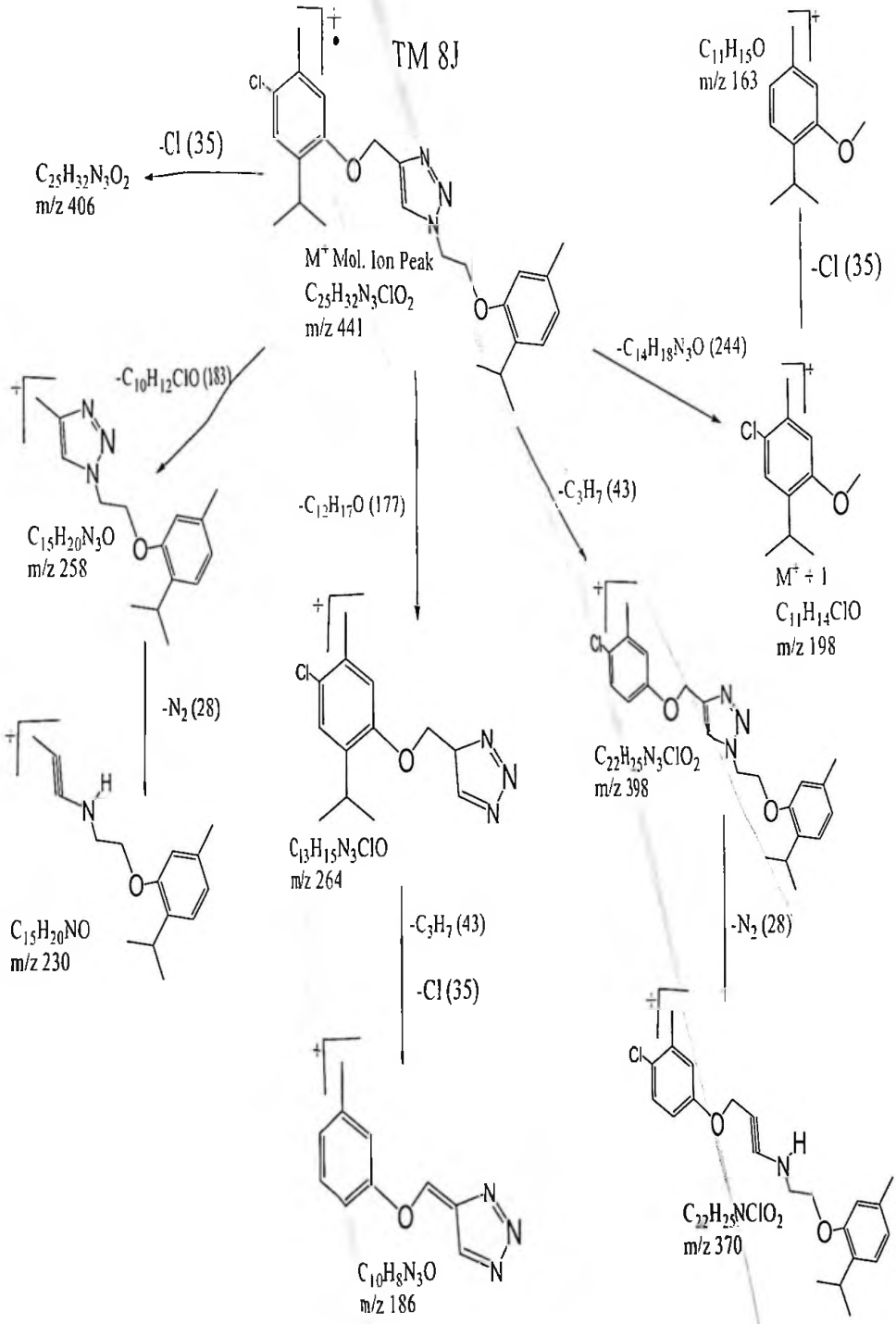
1245.1cm^{-1} for TM 8J. Again, the absence of the representative absorption peak for azido group at 2100.0 cm^{-1} confirms the formation of the triazole moiety in all the compounds (Table 11; Appendix B-11).

The mass spectrum (EI) of the compounds(TM 8I and 8J) which led to a confirmation of their structure gave the molecular ion $[M]^+$ peak at m/z 407 and a corresponding base peak was observed at m/z 135 for TM 8I. Also, a molecular ion $[M]^+$ peak at m/z 441 and a corresponding base peak was observed at m/z 230 for TM 8J. The other prominent mass fragments for the compound are 364, 336, 258, 230 and 163 for TM 8I as well as 406, 370, 258, 230 and 186 for TM 8J as accounted for in the fragmentation pattern of the compounds (Schemes 30 & 31; Appendices C-9 & C-10) respectively.

.The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 408 and 436 respectively for TM 8I (Appendix F-5) as well as 442 and 470 for $[M+H]^+$ and $[M+C_2H_5]^+$respectively for TM 8J (Appendix F-5). The TOF MS (ES+) gave the *m/z* $[M+H]^+$ and $[M+K]^+$ as 408 and 446 respectively for TM 8I (Appendix I-9) as well as 440, 442 and 456 for $[M-H]^+$, $[M+H]^+$ and $[M+CH_3]^+$ respectively for TM 8J (Appendix I-10).



Scheme 30: Mass spectral fragmentation details of 1-[4-(2-isopropyl-5-methylphenoxy) methyl-1, 2, 3-triazol-1-yl]-2-isopropyl-5-methyl-phenoxyethane (TM 8I).



Scheme 31: Mass spectral fragmentation details of 1-[4-(4-chloro-2-isopropyl-5-methyl phenoxy) methyl-1, 2, 3-triazol-1-yl]-2-isopropyl-5-methyl- phenoxyethane (TM 8J).

Ester derivatives of thymol

Formation of the ester derivatives of thymol were also confirmed by the absence of –OH stretching absorption of the thymol at 3310 - 3510 cm^{-1} and the presence of the a strong characteristic carbonyl -C=O group at 1731.2 cm^{-1} in the IR spectra. The C - H stretching in alkyl region was characterised by absorption peaks with a shoulder at 2961.9, 2870.1 and 2836.0 cm^{-1} for the esters which is indicative of the aliphatic methylene ($-CH_2-$) and methyl($-CH_3$) groups (Appendix B-2)

The mass spectra (EI & CI) of the ester derivatives (TM 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1I , 1N, 1K, 1L, 1M, 1P, 1Q, 1R and 1U) which led to confirmation of their structures are indicated as follows:

TM 1A: 2-Isopropyl-5-methylphenyl ethanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 192 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150,121, 105, 77 and 43 as accounted for in the fragmentation pattern of the compound (Scheme 32; Appendix D-1). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 193 and 221 respectively (Appendix G-1).

TM 1B: 2-Isopropyl-5-methylphenyl propanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 206 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 121, 105 and 57 as accounted for in the fragmentation pattern of the compound (Scheme 33; Appendix D-2).

The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 207 and 235 respectively (Appendix G-1).

TM 1C: 2-Isopropyl-5-methylphenyl 2-methylpropanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 220 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 105, 71 and 43 as accounted for in the fragmentation pattern of the compound (Scheme 34; Appendix D-3). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 221 and 249 respectively (Appendix G-2).

TM 1D: 2-Isopropyl-5-methylphenyl butanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 220 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 121, 105, 71 and 43 as accounted for in the fragmentation pattern of the compound (Scheme 35; Appendix D-4). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 221 and 249 respectively (Appendix G-2).

TM 1E: 2-Isopropyl-5-methylphenyl-2-methyl butanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 234 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 115, 105, 77 and 57 as accounted for in the fragmentation pattern of the compound (Scheme 36; Appendix D-5).

The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 235 and 263 respectively (Appendix G-3).

TM 1F: 2-Isopropyl-5-methylphenyl pentanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 234 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 121, 105, 77 and 57 as accounted for in the fragmentation pattern of the compound (Scheme 37; Appendix D-6). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 235 and 263 respectively (Appendix G-3).

TM 1G: 2-Isopropyl-5-methylphenyl hexanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 248 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 121, 105, 71 and 43 as accounted for in the fragmentation pattern of the compound (Scheme 38; Appendix D-7). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 249 and 277 respectively (Appendix G-4).

TM 1I: 2-Isopropyl-5-methylphenyl 2-phenylethanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 268 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 119, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 39; Appendix D-8). The

mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 269 and 297 respectively (Appendix G-4).

TM 1K: 2-Isopropyl-5-methylphenyl benzoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 254 and a corresponding base peak at m/z 105. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 149, 135, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 40; Appendix D-9). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 255 and 283 respectively (Appendix G-5).

TM 1L: 2-Isopropyl-5-methylphenyl 3-bromo-4-methylbenzoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 346 and a corresponding base peak at m/z 197. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 169, 149 and 118 as accounted for in the fragmentation pattern of the compound (Scheme 41; Appendix D-10). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 347 and 375 respectively (Appendix G-5).

TM 1M: 2-Isopropyl-5-methylphenyl 2-hydroxylbenzoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 270 and a corresponding base peak at m/z 121. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 252, 150, 135 and 105 as accounted for in the fragmentation pattern of the compound (Scheme 42; Appendix D-11). The

mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 271 and 299 respectively (Appendix G-6).

TM 1N: 2-Isopropyl-5-methylphenyl 2, 2-dichloroethanoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 260 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 245,177, 150, 121, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 43; Appendix D-12). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 261 and 289 respectively (Appendix G-6).

TM 1P: 2-Isopropyl-5-methylphenyl 4-ethylbenzoate

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 282 and a corresponding base peak at m/z 133. The other prominent mass fragments for the compound are m/z 149, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 44; Appendix D-13). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 283 and 311 respectively (Appendix G-8).

TM 1Q: 2-Isopropyl-5-methylphenyl 3-chlorobenzoate

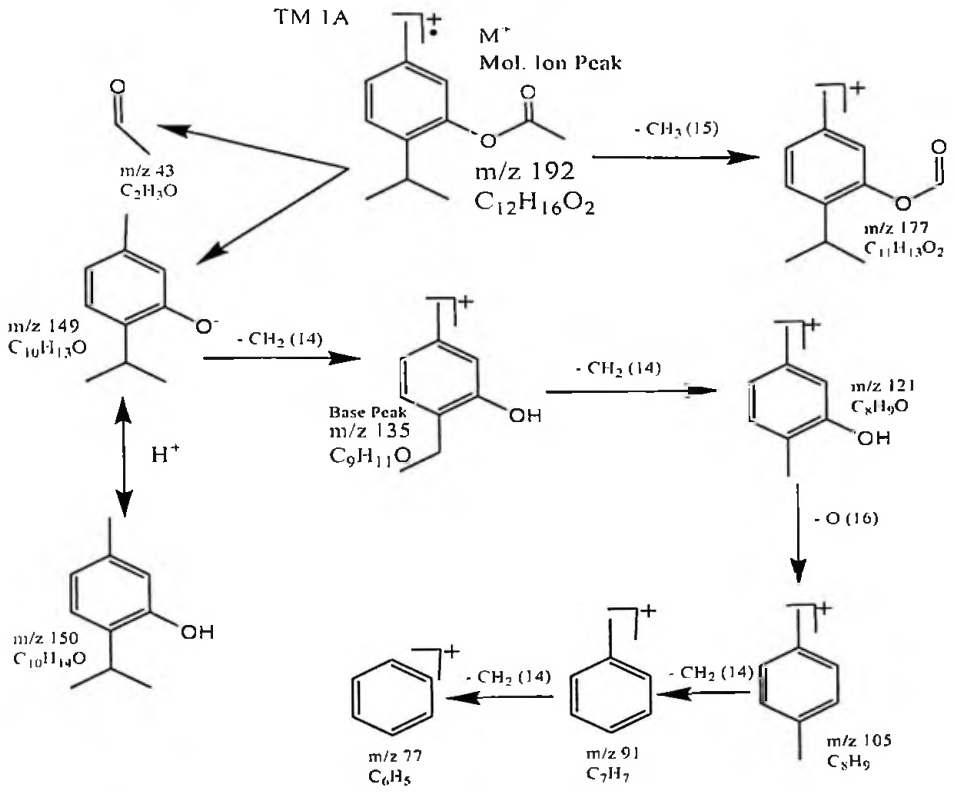
The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 288 and a corresponding base peak at m/z 139. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 111 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 45; Appendix D-14). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 289 and 317 respectively (Appendix G-7).

TM 1R: 2-Isopropyl-5-methylphenyl 3-methoxybenzoate

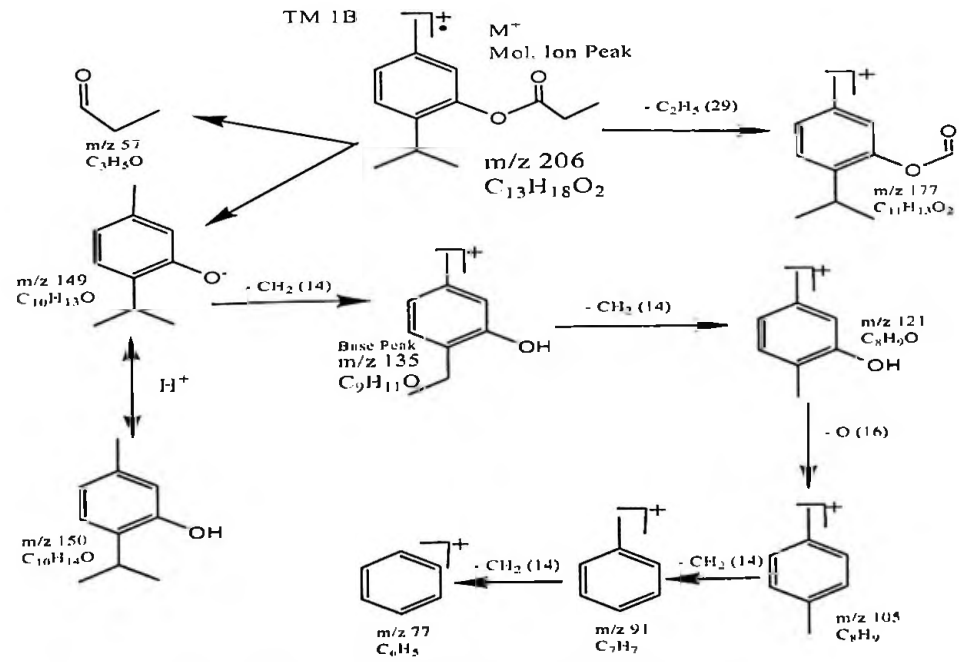
The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 284 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 107 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 46; Appendix D-15). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 285 and 313 respectively (Appendix G-7).

TM 1U: Di-(2-Isopropyl-5-methylphenyl) hexanedioate

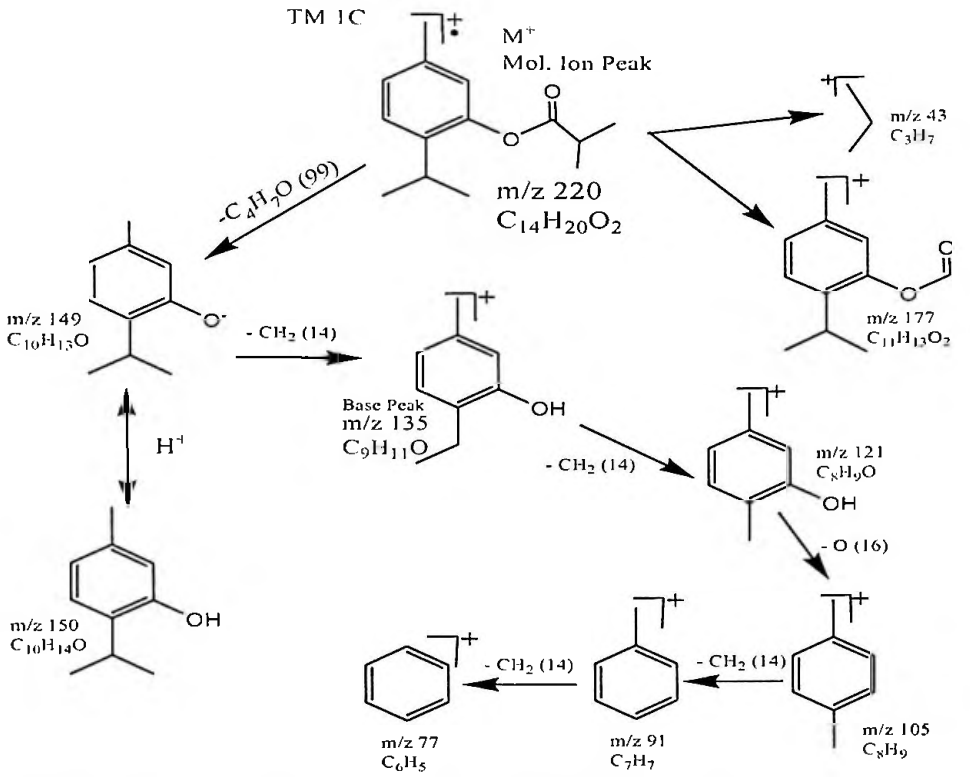
The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 410 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 261, 177, 150, 112 and 55 as accounted for in the fragmentation pattern of the compound (Scheme 47; Appendix D-16). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 411 and 439 respectively (Appendix G-8).



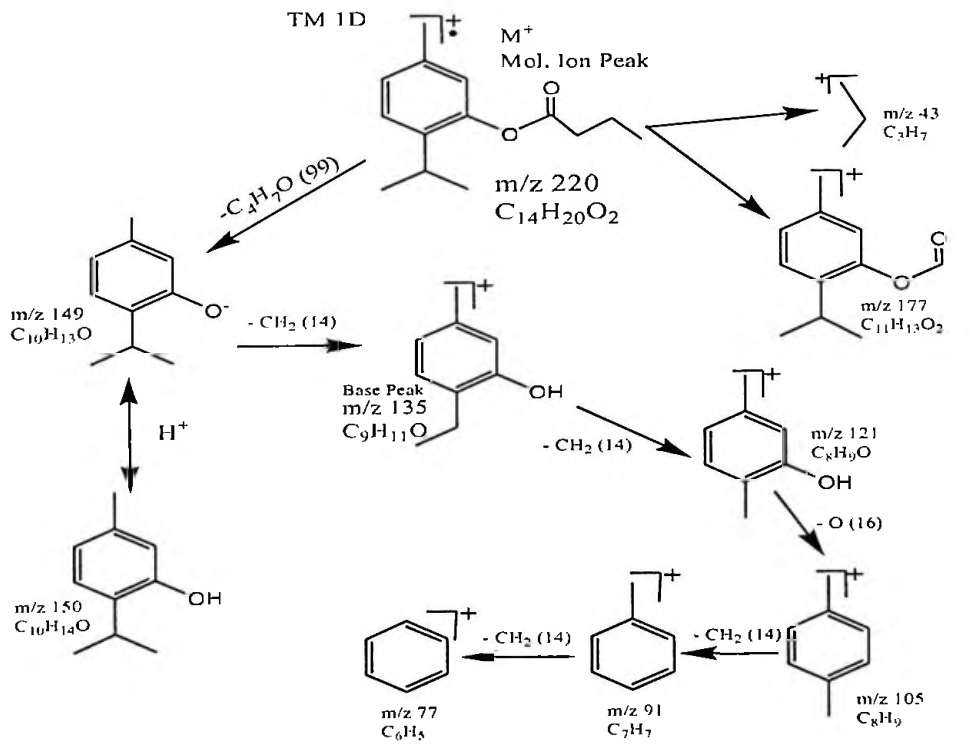
Scheme 32: Mass spectral fragmentation details of TM 1A.



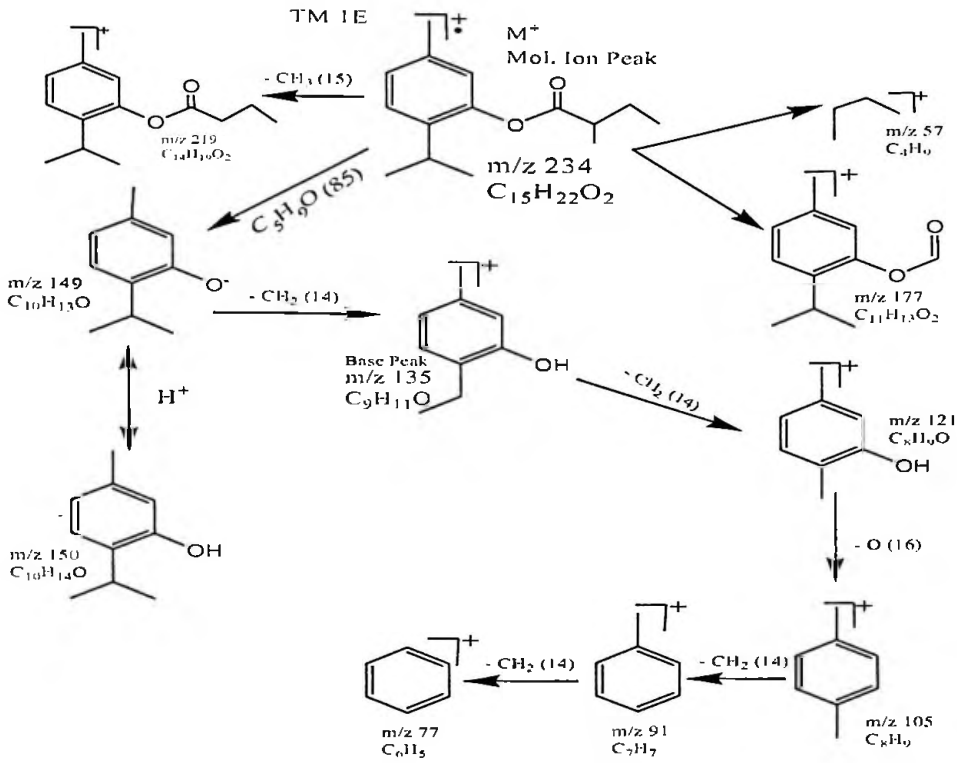
Scheme 33: Mass spectral fragmentation details of TM 1B.



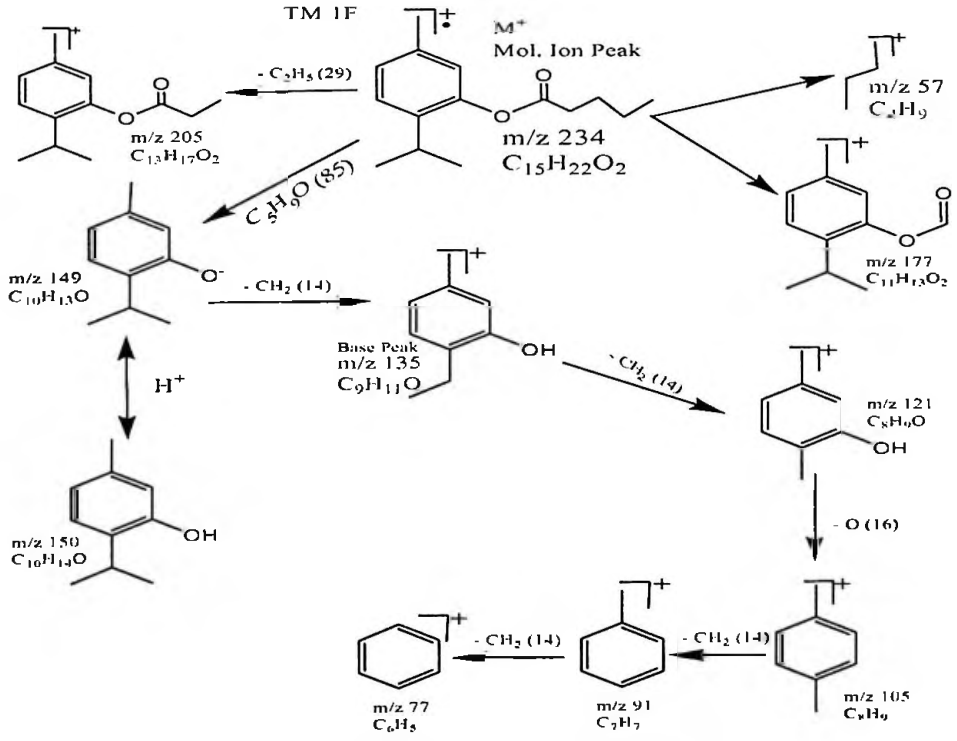
Scheme 34: Mass spectral fragmentation details of TM 1C.



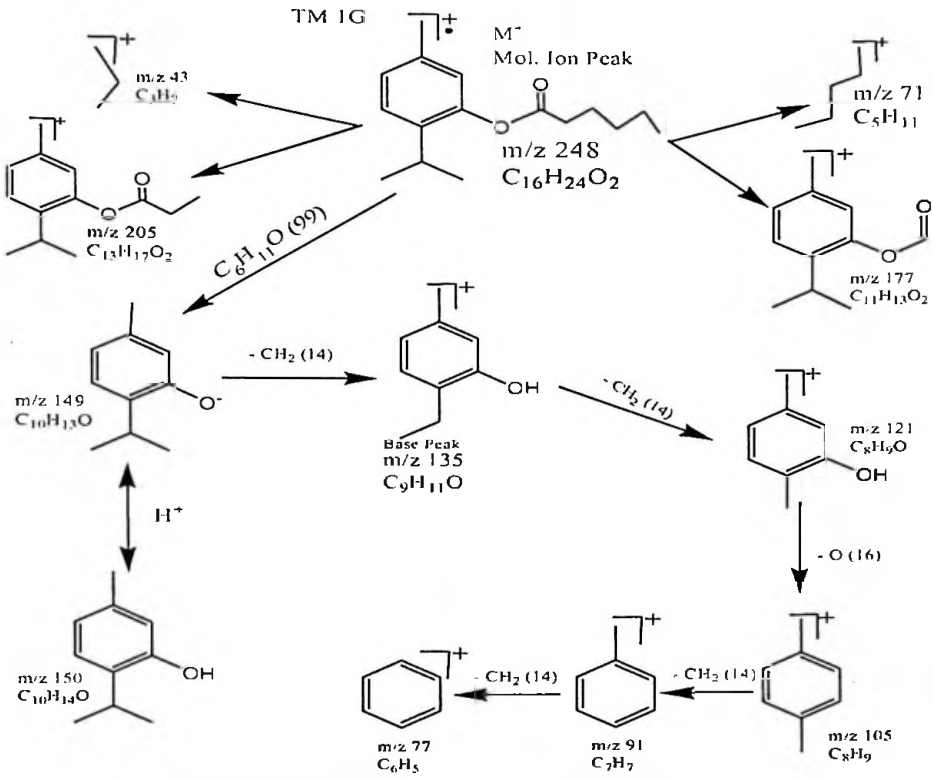
Scheme 35: Mass spectral fragmentation details of TM 1D.



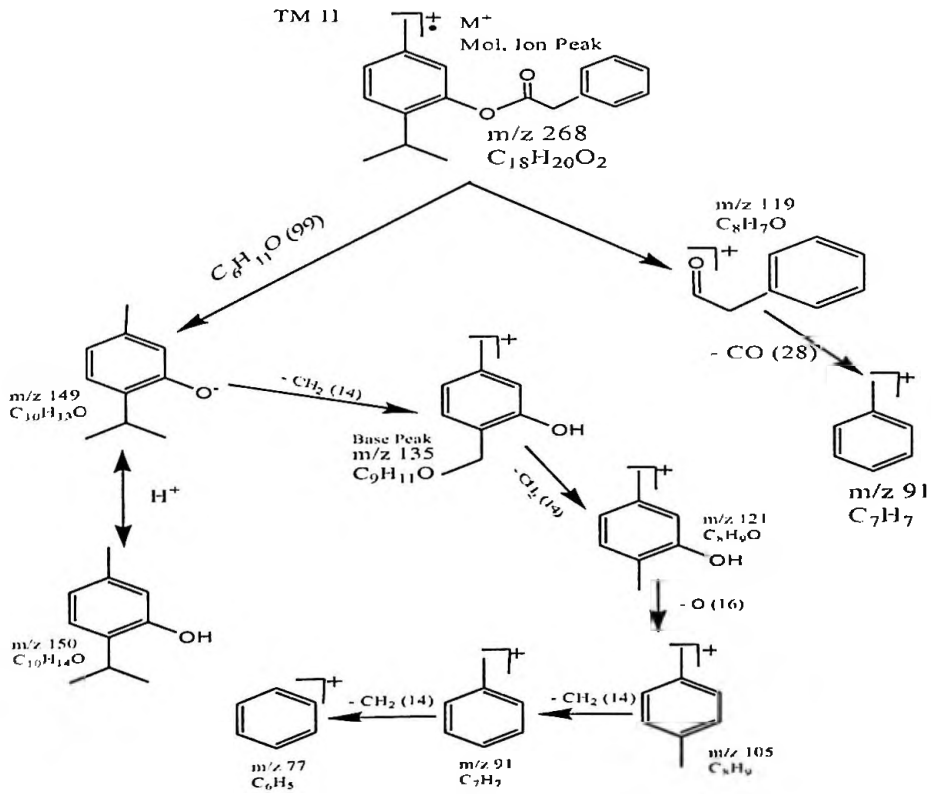
Scheme 36: Mass spectral fragmentation details of TM 1E.



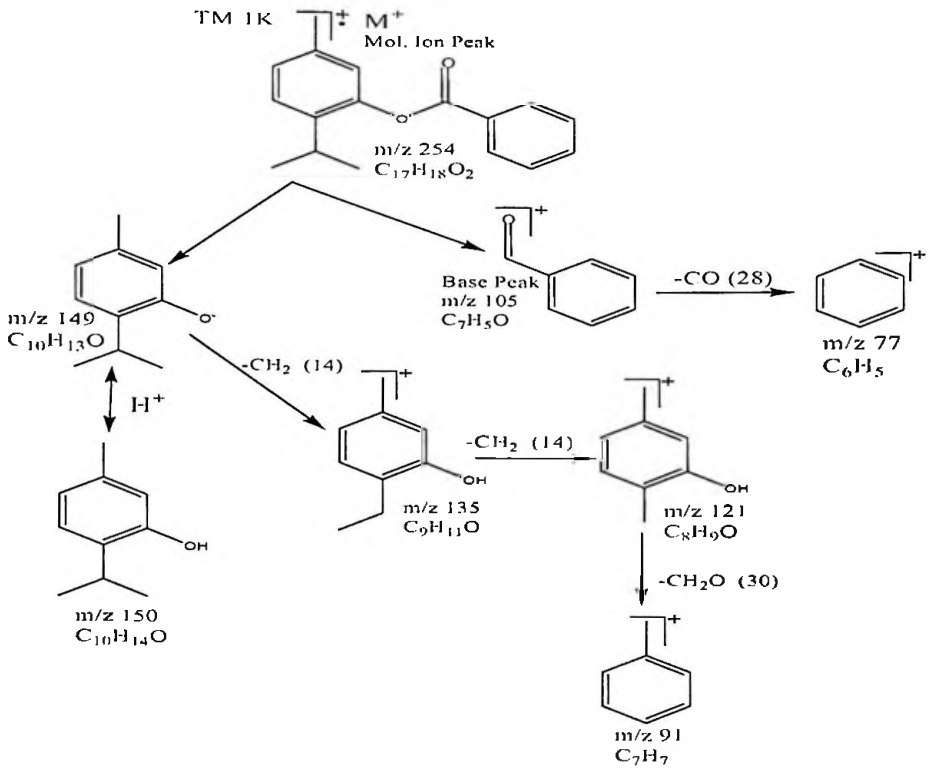
Scheme 37: Mass spectral fragmentation details of TM 1F.



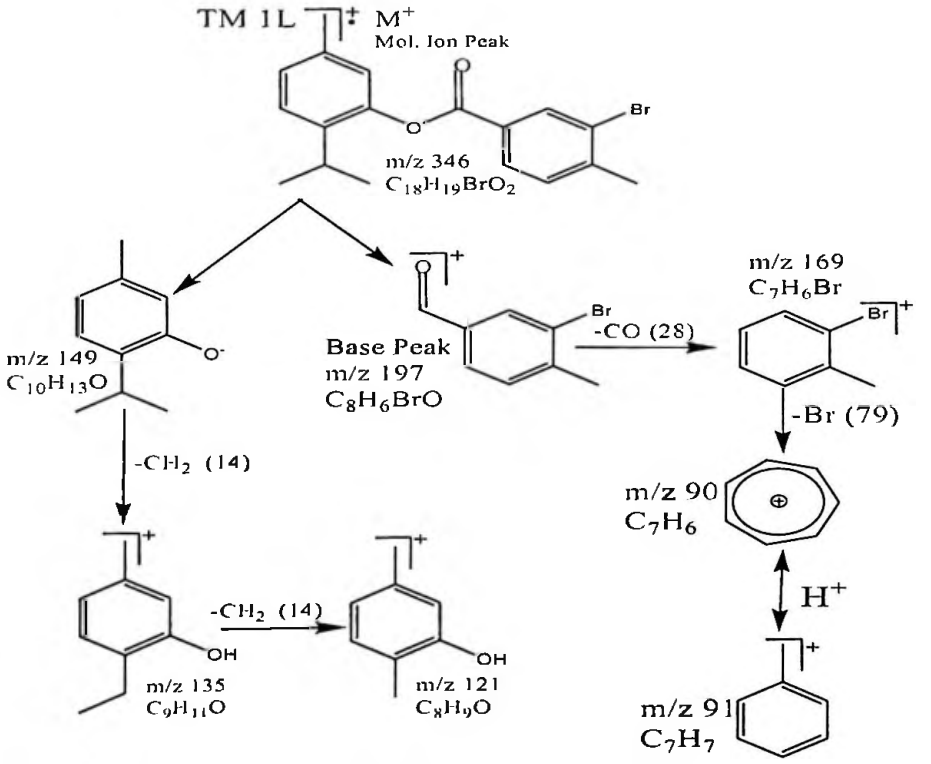
Scheme 38: Mass spectral fragmentation details of TM 1G.



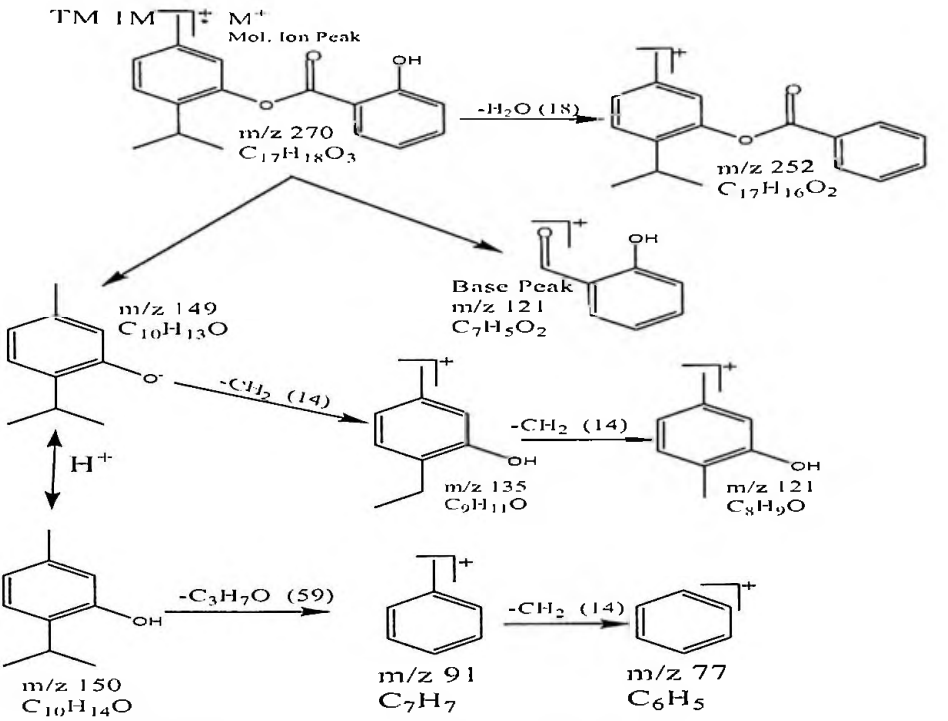
Scheme 39: Mass spectral fragmentation details of TM 11.



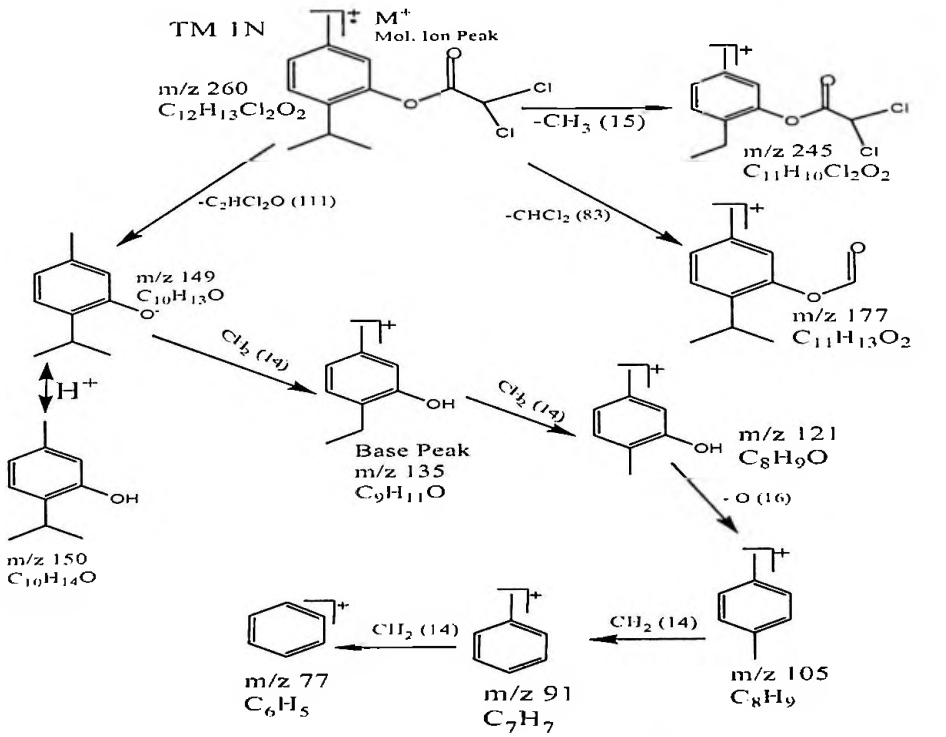
Scheme 40: Mass spectral fragmentation details of TM 1K.



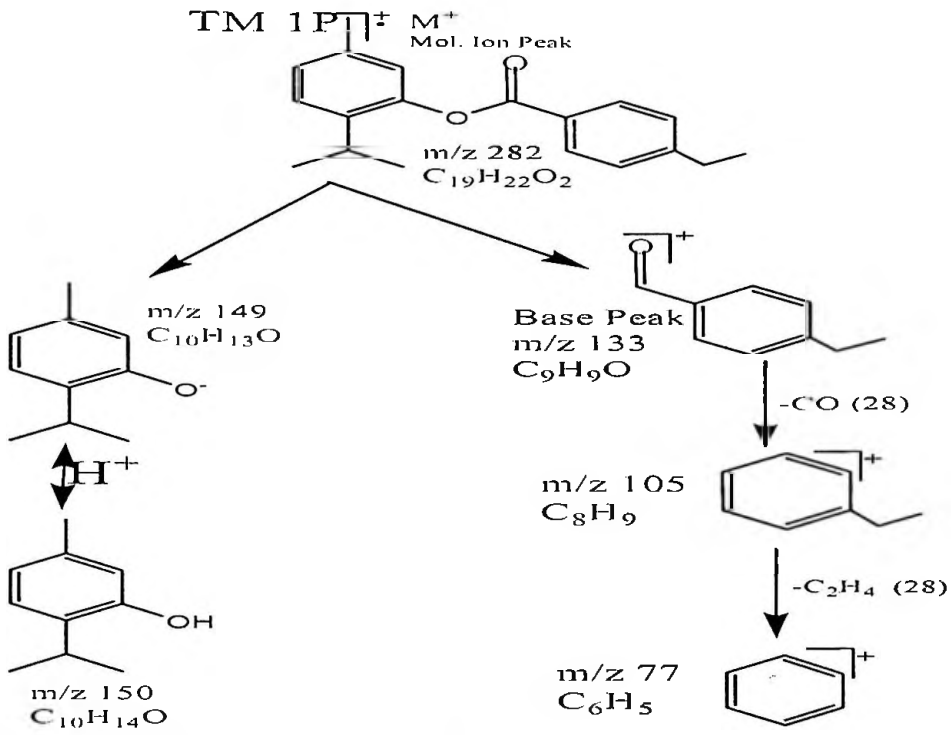
Scheme 41: Mass spectral fragmentation details of TM 1L.



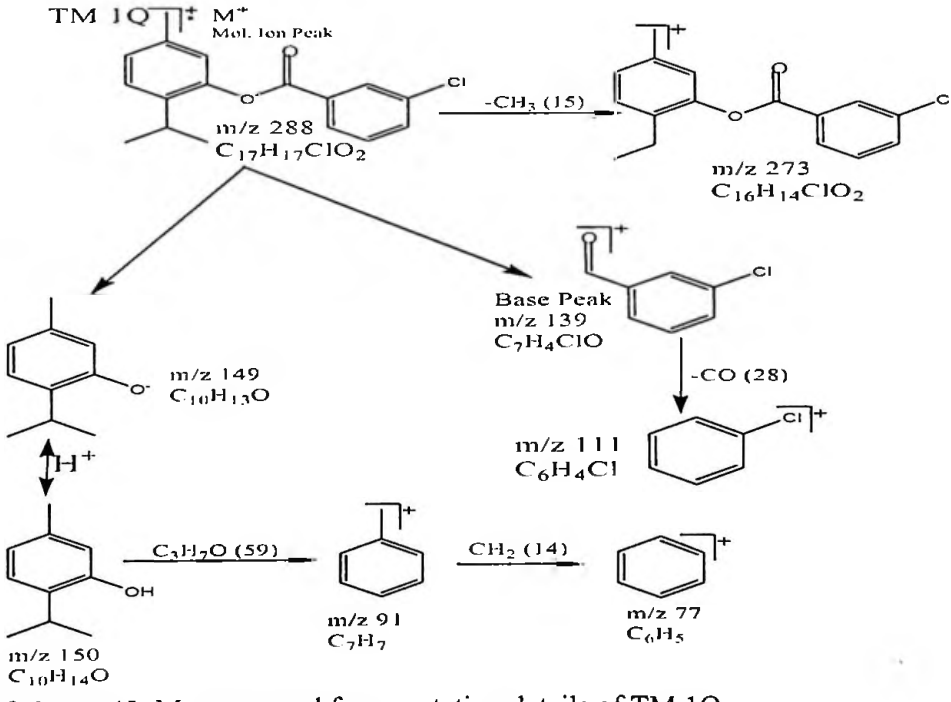
Scheme 42: Mass spectral fragmentation details of TM 1M.



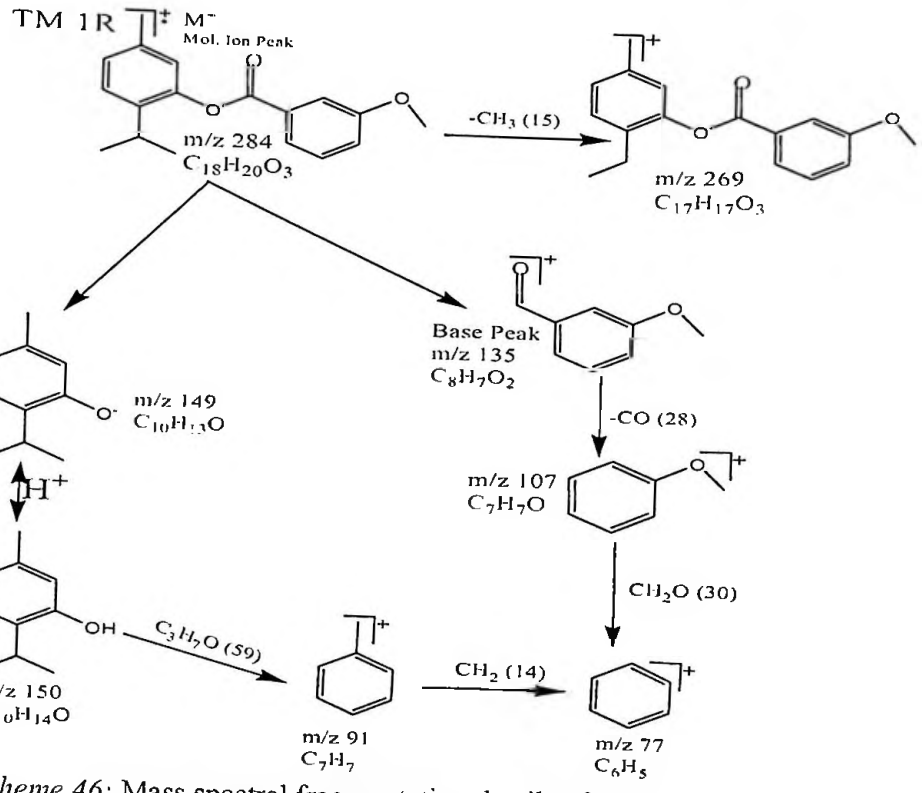
Scheme 43: Mass spectral fragmentation details of TM 1N.



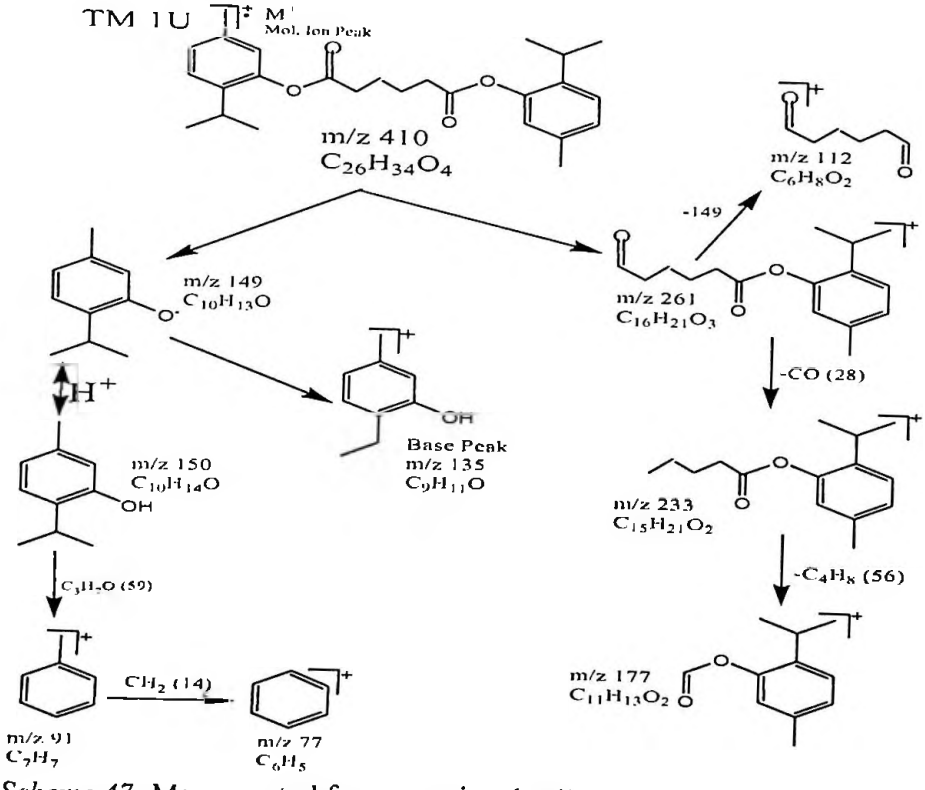
Scheme 44: Mass spectral fragmentation details of TM 1P.



Scheme 45: Mass spectral fragmentation details of TM 1Q.



Scheme 46: Mass spectral fragmentation details of TM 1R.



Scheme 47: Mass spectral fragmentation details of TM 1U.

Ether derivatives of thymol

Formation of the ether derivatives of thymol was confirmed by the absence of –OH stretching absorption of the thymol at 3310 - 3510 cm^{-1} and the presence of C-O group at 1255.3 cm^{-1} in the IR spectra. The C - H stretching in alkyl region was characterised by a strong absorption peaks with a shoulder at 2967.5, 2938.7 and 2879.9 cm^{-1} for the ethers which is indicative of the aliphatic methylene ($-CH_2-$) and methyl ($-CH_3$) groups (Appendix B-1).

The mass spectra (EI & CI) of the ether derivatives (TM 2C, 2D, 2E, 2F, 2I, 2K, 2N, 2O and 2P) which led to confirmation of their structures are indicated as follows:

TM 2C: 2-Isopropyl-5-methylphenoxy propane

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 192 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 177, 150, 121, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 48; Appendix E-1). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 193 and 221 respectively (Appendix H-1).

TM 2D: 2-Isopropyl-5-methylphenoxy methylethane

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 192 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 177, 150, 121, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 49; Appendix E-2). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ as 193 (Appendix H-2).

TM 2E: 2-Isopropyl-5-methylphenoxy 1-methylpropane

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 206 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 191, 150, 121, 105, 77and 57 as accounted for in the fragmentation pattern of the compound (Scheme 50; Appendix E-3). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 207 and 235 respectively (Appendix H-2).

TM 2F: 2-Isopropyl-5-methylphenoxy butane

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 206 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 150, 121, 105, 77 and 57 as accounted for in the fragmentation pattern of the compound (Scheme 51; Appendix E-4). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ at 207 (Appendix H-3).

TM 2I: 2-Isopropyl-5-methylphenoxy hexane

The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 234 and a corresponding base peak at m/z 135. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 219, 150, 121, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 52; Appendix E-5). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 235 and 263 respectively (Appendix H-3).

TM 2K: 2-Isopropyl-5-methylphenoxy 2-chloroethane

The mass spectrum (EI) gave a molecular ion $[M]^{+}$ peak of m/z 212 and a corresponding base peak at m/z 197. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 163, 135,121, 105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 53; Appendix E-6). The mass spectrum (CI) gave the *m/z* $[M+H]^{+}$ and $[M+C_2H_5]^{+}$ as 255 and 283 respectively (Appendix H-4).

TM 2N: 2-Isopropyl-5-methylphenoxy methylbenzene

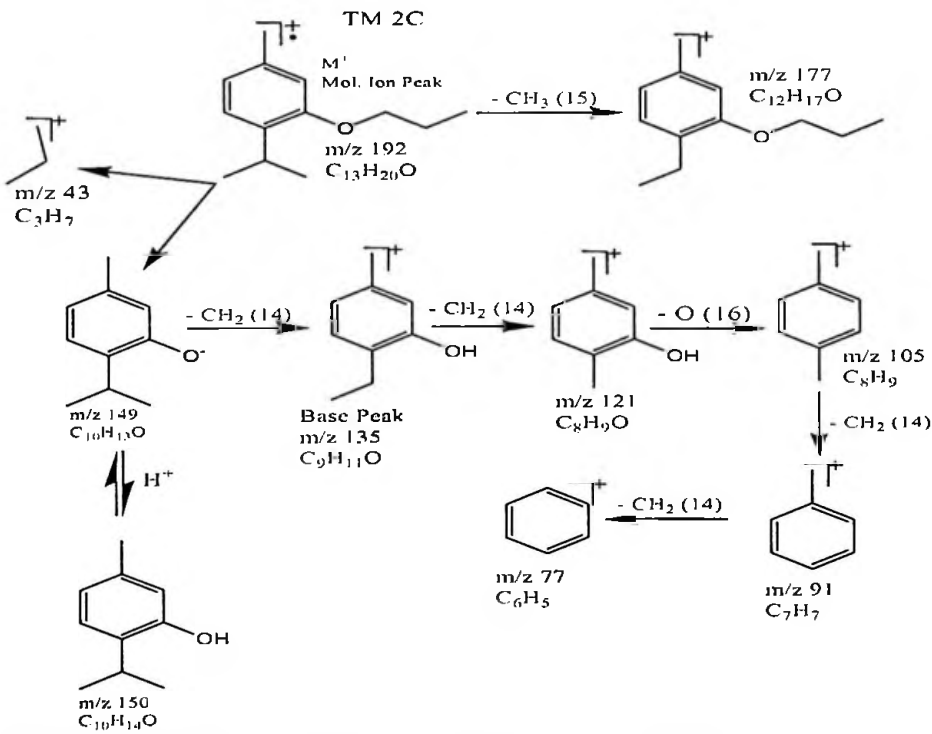
The mass spectrum (EI) gave a molecular ion $[M]^{+}$ peak of m/z 240 and a corresponding base peak at m/z 91 which is the characteristic tropelium ion peak. The other prominent mass fragments for the compound are m/z 225, 197, 149, 135, 121,105 and 77 as accounted for in the fragmentation pattern of the compound (Scheme 54; Appendix E-7). The mass spectrum (CI) gave the *m/z* $[M+H]^{+}$ and $[M+C_2H_5]^{+}$ as 241 and 269 respectively (Appendix H-4).

TM 2O: 2-Isopropyl-5-methylphenoxy 3-chloromethylbenzene

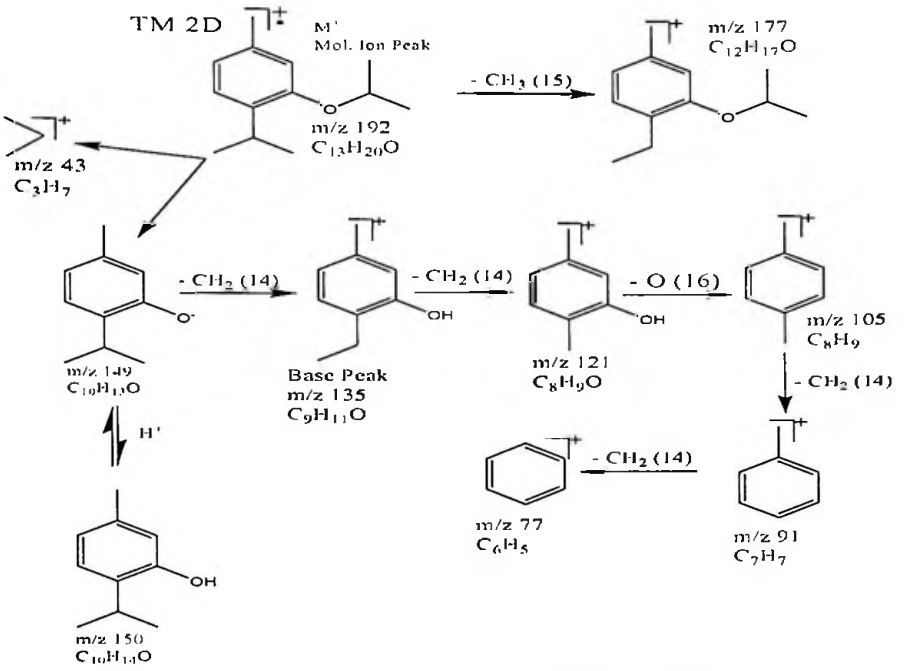
The mass spectrum (EI) gave a molecular ion $[M]^{+}$ peak of m/z 274 and a corresponding base peak at m/z 125. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 259, 231, 149, 121, and 105 as accounted for in the fragmentation pattern of the compound (Scheme 55; Appendix E-8). The mass spectrum (CI) gave the *m/z* $[M+H]^{+}$ and $[M+C_2H_5]^{+}$ as 275 and 303 respectively (Appendix H-5).

TM 2P: 2-Isopropyl-5-methylphenoxy 3-fluoromethylbenzene

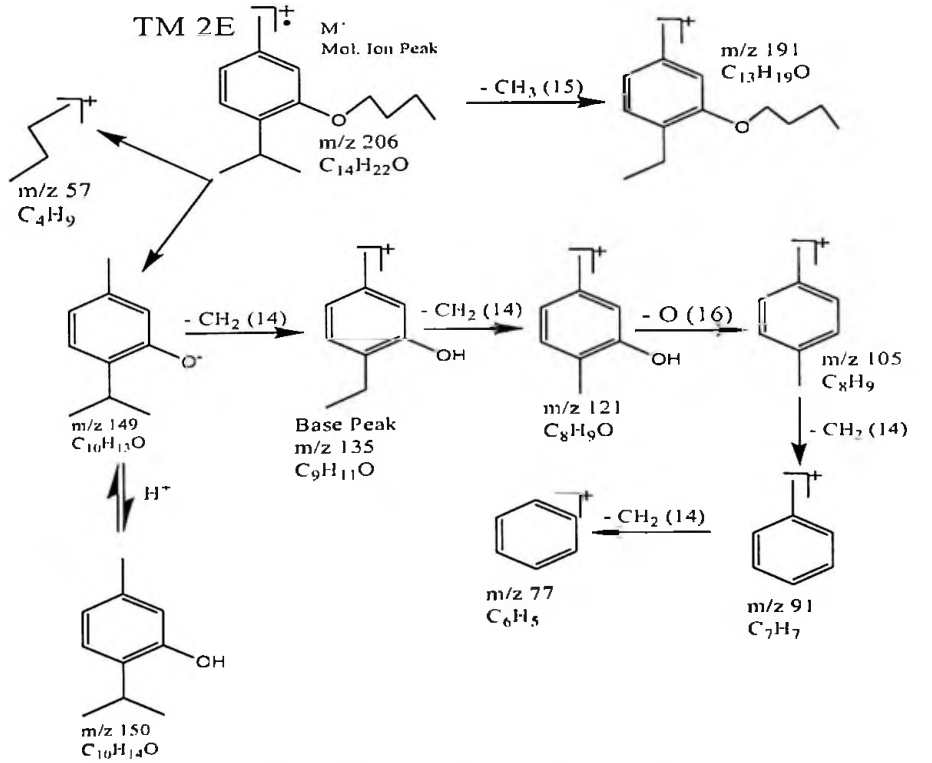
The mass spectrum (EI) gave a molecular ion $[M]^+$ peak of m/z 258 and a corresponding base peak was at m/z 109. A characteristic tropelium ion peak at m/z 91 was also observed in the spectrum. The other prominent mass fragments for the compound are m/z 243, 215, 149 and 135 as accounted for in the fragmentation pattern of the compound (Scheme 56; Appendix E-9). The mass spectrum (CI) gave the *m/z* $[M+H]^+$ and $[M+C_2H_5]^+$ as 259 and 287 respectively (Appendix H-5).



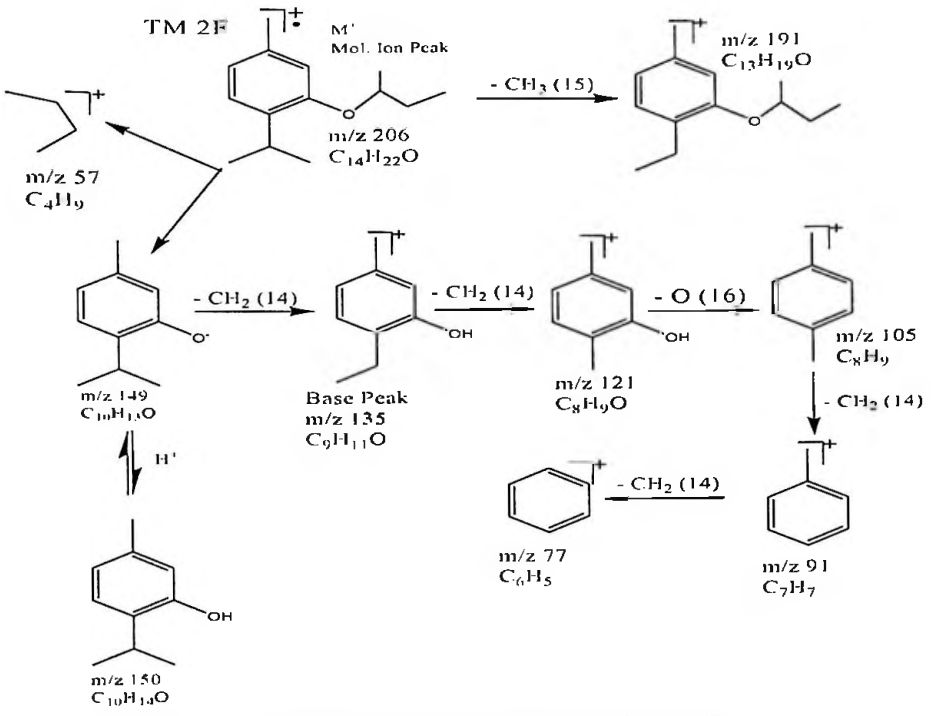
Scheme 48: Mass spectral fragmentation details of TM 2C.



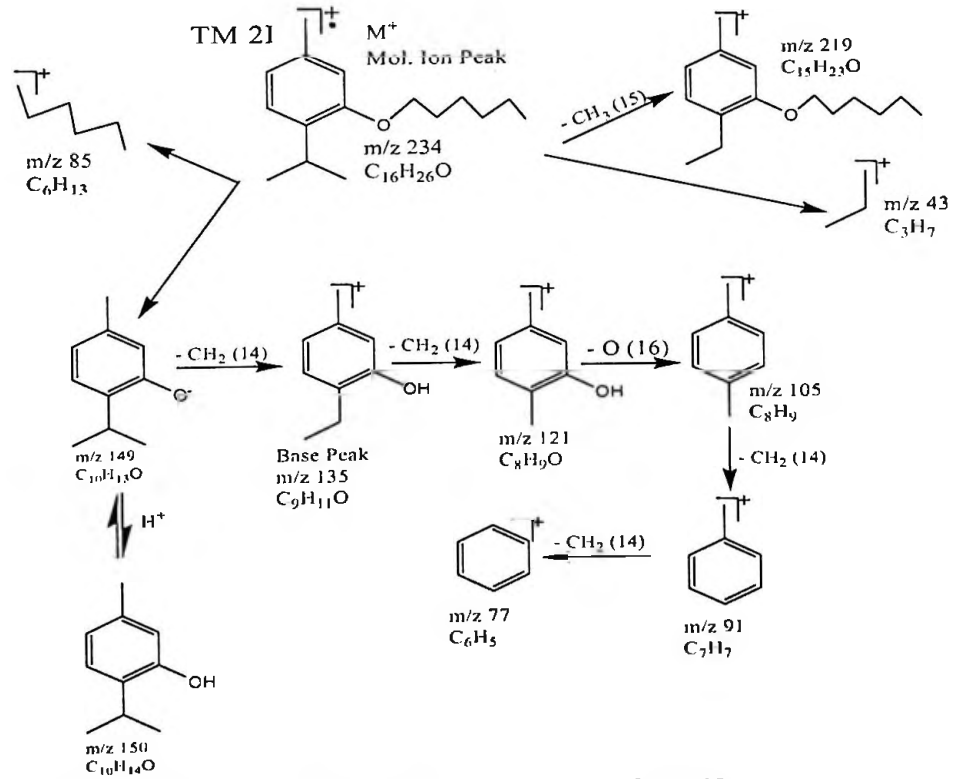
Scheme 49: Mass spectral fragmentation details of TM 2D.



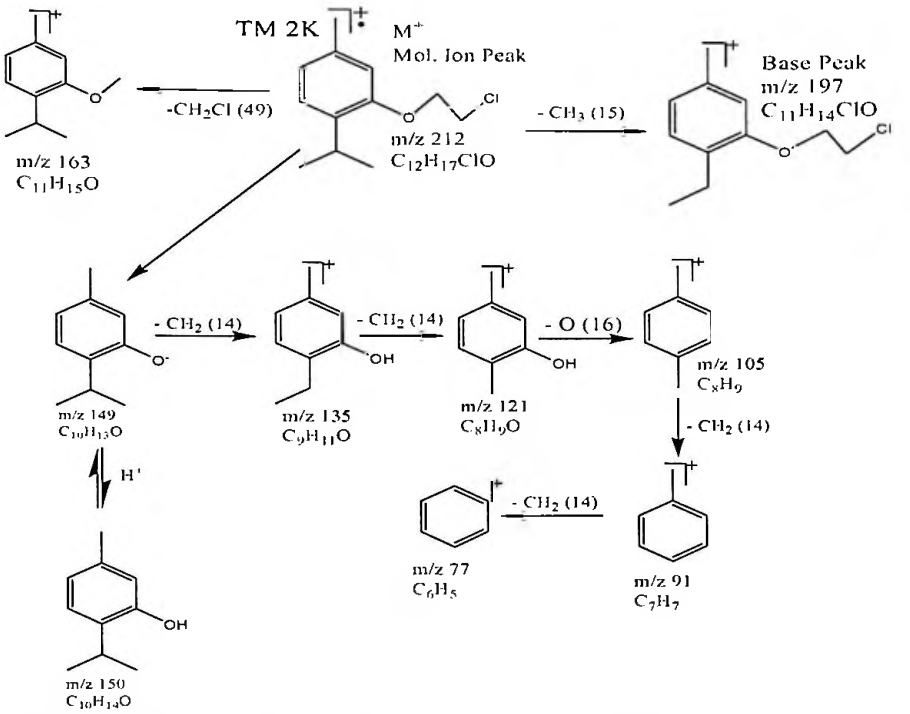
Scheme 50: Mass spectral fragmentation details of TM 2E.



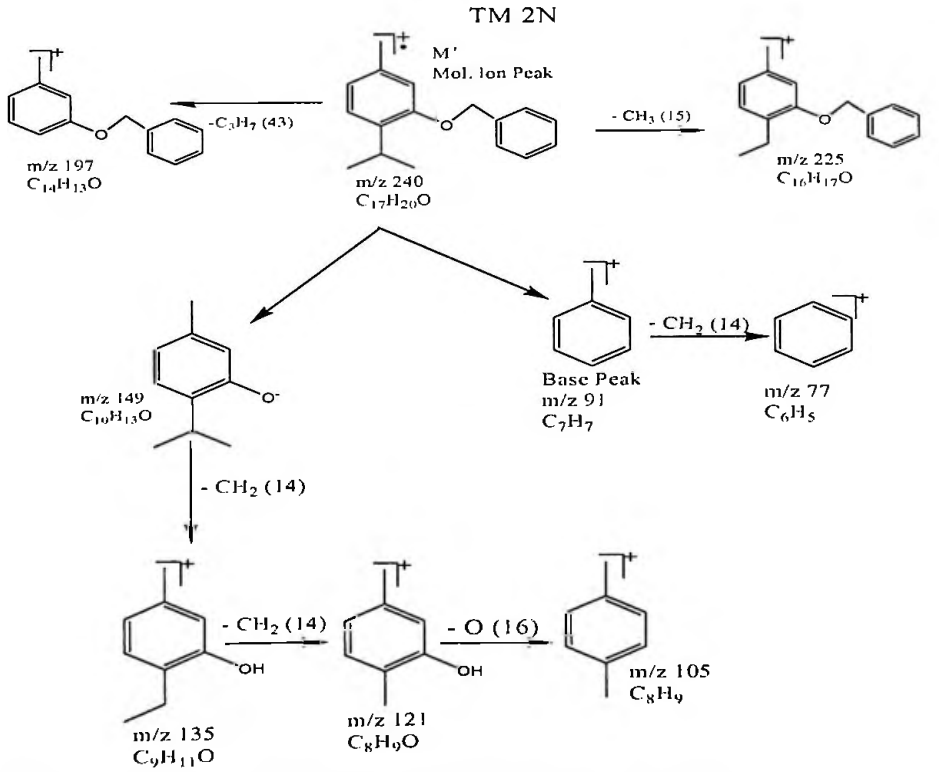
Scheme 51: Mass spectral fragmentation details of TM 2F.



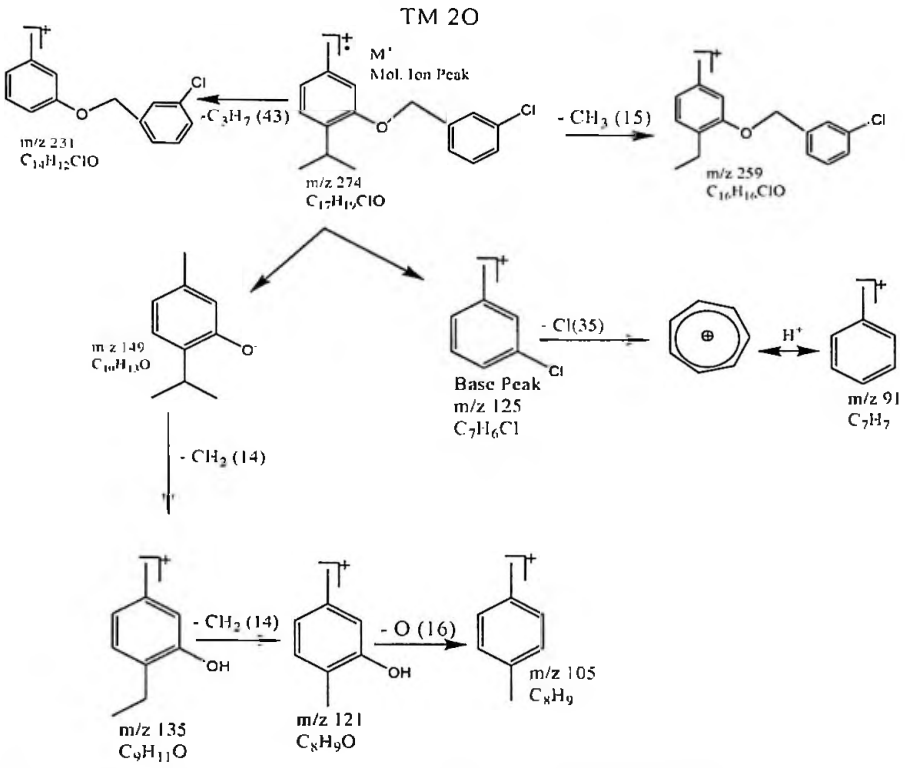
Scheme 52: Mass spectral fragmentation details of TM 2I.



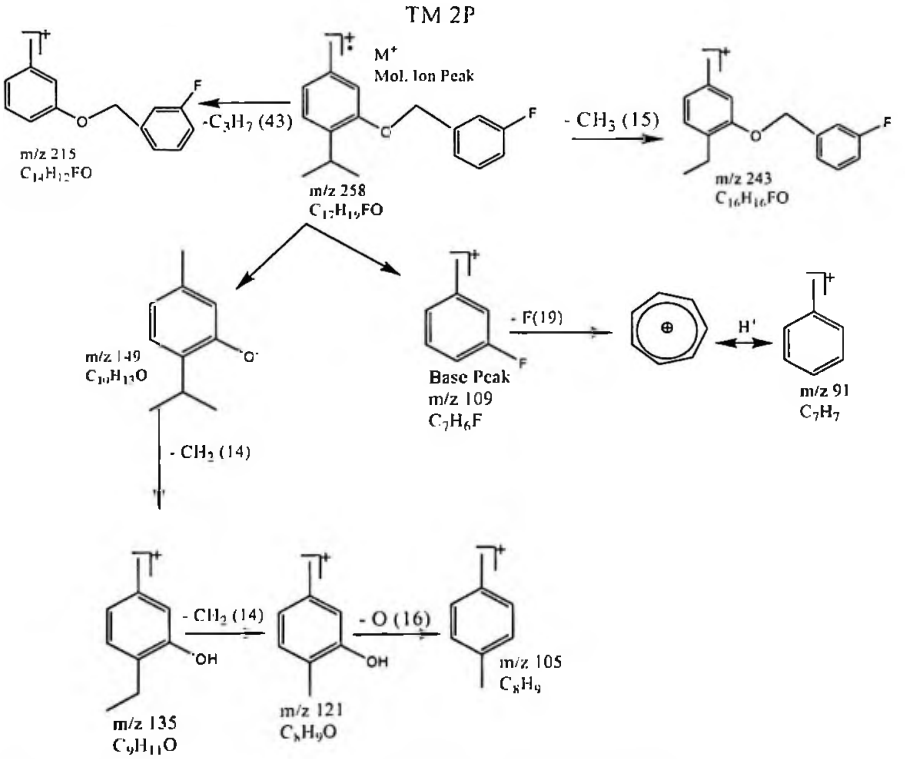
Scheme 53: Mass spectral fragmentation details of TM 2K.



Scheme 54: Mass spectral fragmentation details of TM 2N.



Scheme 55: Mass spectral fragmentation details of TM 2O.



Scheme 56: Mass spectral fragmentation details of TM 2P.

Larvicidal assay of alkyl and alkyl substituted ether derivatives

Generally, the synthetic alkyl and alkyl substituted ether derivatives of thymol showed significantly improved larvicidal activity over the tested alkyl and substituted alkyl ester derivatives, with the exception of TM 1I, where the larvicidal activity was comparable to the ether derivatives. The reason could be attributed to the substituted aromatic ring on the alkyl side chain of the ester functional group, which confirms the importance of the aromatic nucleus to the enhanced larvicidal activity of the thymol derivatives. The higher potency among the ether derivatives, was as a result of the introduction of aromatic ring in the alkyl chain as in TM 2O, TM 2P and TM 2N. Again, the number of straight chain carbons in the alkyl group of the ether derivative showed the highest activity in TM 2C with three carbons, and a decreased in the activity from TM 2E with four carbons and TM 2I with six carbons. The increase in the straight chain carbon length resulted in a corresponding decrease in larvicidal activity of the ether derivative. The degree of branching in the aliphatic side chain of the ethers was also seen to decrease the larvicidal activity considerably as in TM 2D and TM 2F. The higher the degree of branching in the aliphatic side chain, the lower the activity. This observation might be due to the poor solubility of the test compound as a result of the reduced surface area arising from the branching of the side carbon chain.

It is also observed that, halogens in the aromatic nucleus of the ether carbon chain confer a much more potency as in TM 20 and TM 2P, than when the halogen is in the alkyl chain of the ether derivative as in TM 2K.

Larvicidal assay of alkyl and alkyl substituted ester derivatives

Among the alkyl and alkyl substituted ester derivatives, activity was enhanced by the kind of substituent on the second carbon to the ester functional group. The most potent ester derivative, TM 1I had an aromatic ring as substituent on the α-carbon of the ester functional group, followed by TM 1N with two chlorine atom substituents on the α-carbon. It is also observed that, the nature of the aliphatic alkyl chain, whether branched or straight chain in the ester functional group affected the activity. The activity of TM 1A, TM 1B, TM 1F, and TM 1G, suggests that, the side chain enhanced the larvicidal activity up to three carbons atoms in a straight alkyl chain as in TM 1A, TM 1B and TM 1C respectively. The activity decreases from four to five carbon atoms in a straight alkyl chain as in TM 1F and TM 1G respectively. On the contrary to the ether derivatives, the degree of branching in the alky chain (-R) to the ester functional group contributes significantly and played an effective role in the enhanced larvicidal activity of the ester derivatives of thymol. For example TM 1C recorded the highest larvicidal potency, followed by TM 1E among the aliphatic alkyl chain, either straight or branched. The parent compound, thymol exhibited a higher larvicidal activity than some of the ester derivatives except those with aromatic ring, halogens and branched alkyl carbon chains.

Larvicidal assay of aromatic and substituted aromatic ester derivatives

Among the aromatic ester derivatives, activity was enhanced by the presence of an aromatic ring attached to the ester functional group. This was confirmed by the significant larvicidal activity possessed by all the aromatic ester derivatives of thymol as compared to thymol itself. Again, the kind of

substituent on the aromatic ring also played a vital role in conferring an enhanced activity, as it was realized that, the most potent derivatives were those with an alkyl residue substituent on the aromatic ring. The most potent aromatic ester derivative, TM 1P had an aromatic ring and an alkyl substituent of two carbons on the ring. This was followed by TM 1L with an alkyl substituent of one carbon on the aromatic ring as well as a bromine atom substituent at different position on the ring. The lower activity of TM 1K compared to that of TM 1P and TM 1L signifies that, the presence of alkyl substituents on the aromatic ring of the esters or substituents with an alkyl moiety as in the case of TM 1R and TM 1U contribute significantly to the activity of the ester derivatives. Although substituents like methoxy (-OCH_3), Halogens (Cl or Br) and the hydroxyl group (-OH) might have contributed in the activity of the aromatic ester derivatives, their significance is not comparable to those with alkyl residues as substituents on the aromatic ring. A hydroxyl group as a substituent on TM 1M has a minimal contribution to its activity as TM 1M had the least activity compared to the derivatives with substituents Cl, Br, and OCH_3 as well as aromatic esters without substituent(s) and the dimeric ester . This confirm that, the hydroxyl group is not essential for the larvicidal activity of thymol and its aromatic ester derivatives, compared to the alkyl and halogen substituents.

The zero proportion of dead larvae recorded by the 1% DMSO solution and the tap water is an indication that, the mortality of the larvae recorded was as a result of the toxicity of the test compounds and not the DMSO and tap water as shown in (Figures 11, 13 & 15).

Adulticidal assay of alkyl and alkyl substituted ether derivatives

Thymol demonstrated a better adulticidal activity than most of its alkyl and alkyl substituted ether derivatives with an LC_{50} value of 27.60 mg/L.This is an indication that, the hydroxyl functional group (-OH) is very critical and essential in determining the adulticidal activity on the *An. gambiae s.s.*

From the ether derivatives screened for adulticidal activity, it was realized that only TM 2O and TM 2P exhibited a greater activity than thymol, with TM 2O showing the strongest activity with an LC_{50} value of 19.30 mg/L, which was followed closely by TM 2P with an LC_{50} value of 23.20 mg/L. This can be attributed to the presence of an aromatic ring as well as the halogens (chlorine and fluorine) on the substituted aromatic ring on the alkyl side chain of the ether functional group. Although the aromatic substituted group contributed significantly to the activity of the TM 2O and TM 2P which is also seen in TM 2N with an LC_{50} value of 31.30 mg/L, the contributions of the substituted halogens, Cl and F are enormous. The significant difference in activity among TM 2O and TM 2P over TM 2N is the presence of Chlorine (Cl) and Fluorine (F) on the aromatic ring on the alkyl chain of the ether functional group of TM 2O and TM 2P which conferred on them higher potency as compared to the non-substituted aromatic ring on the alkyl carbon chain of the ether functional group of TM 2N. The contribution of the activity of the halogens (Cl and F) on the derivatives was more significant when in aromatic ring on the alkyl chain than being in the alkyl chain of the ether as in TM 2K with an LC_{50} value of 46.70 mg/L. TM 2N recorded an activity less by two folds compared to TM 2O and TM 2P.

It is also observed that, the number of straight chain carbons in the ether side chain showed an increased in the activity with a corresponding increase in the number of carbon atoms attached to the ether functional group. TM 2C and TM 2D with three carbons, showed the least activity with an LC_{50} values of 73.00 mg/L and 98.00 mg/L respectively. There was a slight increase in activity in TM 2E and TM 2F with four carbons with LC_{50} values of 54.20 mg/L and 69.10 mg/L respectively. Among the alkyl chain ethers, TM 2I with six carbons recorded the highest activity with an LC_{50} value of 35.00 mg/L. The branching in the aliphatic chain of the ethers contributed to a decrease in the adulticidal activity considerably as seen in TM 2C and TM 2D as well as TM 2E and TM 2F, where each pair possess the same number of carbon atoms in the aliphatic chain but branching in TM 2D and TM 2F respectively caused a reduction in the observed activity. This observation might as well be due to the poor solubility of the test compound as a result of the reduced surface area resulting from the branching of the carbon chain.

Adulticidal assay of alkyl and alkyl substituted ester derivatives

Thymol (LC_{50} value of 27.60 mg/L) again showed a significant adulticidal activity over most of its alkyl and alkyl substituted derivatives with the exception of TM 1C and TM 1D. Thus, the presence of the hydroxyl functional group (-OH) is essential in determining the adulticidal activity of thymol and its ester derivatives on the adult mosquito of *An. gambiae s.s.*

TM 1C was the most potent derivative with an LC_{50} value of 16.02 mg/L, which was followed by TM 1D with an LC_{50} value of 23.10 mg/L. The enhanced activity is linked to the number of carbons in the alkyl group of the esters, either branch or straight chain. Activity was greatest for up to three

carbons and also branching contributed significantly to the activity. Although both TM 1C and TM 1D had three carbons in the ester side carbon chain, TM 1C has alkyl residue branched whilst the TM 1D has the alkyl residue as a straight chain. This behavior was also seen between TM 1E (LC_{50} value of 59.60 mg/L) and TM 1F (LC_{50} value of 121.05 mg/L), where although both derivatives have four carbons in the ester side chain, the branching in TM 1E resulted in an activity which is two folds that in TM 1F, which is characterised by a straight carbon chain in the ester side alkyl chain. The LC_{50} value of 57.10 mg/L recorded for TM 1A shows that, the adulticidal activity increased from derivatives with ester alkyl side chain from one carbon up to three carbons where maximum activity was recorded. Activity of the alkyl ester derivatives was seen diminishing with a corresponding increasing in the number of carbon atoms in the ester side alkyl chain beyond three carbons up to five carbons as in TM IE, TM 1F and TM 1G with TM 1G recording the least activity of an LC_{50} value of 195.00 mg/L, which is seven folds less the activity of the parent compound, thymol. Substitution on the alpha carbon to the ester functional group with a halogen (Chlorine, Cl) and an aromatic ring also contributed to the activity of the derivatives. The substituted halogen, Chlorine contributed significantly in the activity of TM 1N with an LC_{50} value of 45.50 mg/L which is about two folds more active than when the substituted group was an aromatic ring as in TM 1I with an LC_{50} value of 82.10 mg/L.

Structure-Activity Relationship (SAR)

Larvicidal

It can be seen from the estimated LC_{50} values of both the ether and ester derivatives of thymol, that although the hydroxyl group in thymol is important

for its activity, it is not essential. This is because the modification of the hydroxyl group into ether and ester functional groups in the thymol derivatives resulted in significantly enhanced larvicidal potency of the synthesised ether and ester derivatives, except in the case of TM 1A, TM 1B, TM 1D, TM 1F and TM 1G. It can be established that the high larvicidal potency of the alkyl and alkyl substituted ether and ester derivatives of thymol on the larvae of *Anopheles gambiae s.s* is most important related to the presence of certain functional groups. The functional groups such as aromatic ring attached to the side alkyl chains of both ether and ester functional groups, substituted halogens like chlorine and fluorine on the aromatic ring as was seen in the case of the ether derivatives, the number of carbon atoms up to a certain limit in the side chain of both the ether and ester functional groups.

Again, the nature of the aliphatic side chain, as branching of the alkyl chain resulted in a decrease in activity in ether functional group derivative, branching of the alkyl side chain contributed to a corresponding increase in activity of the ester functional group derivatives.

The larvicidal potency of the aromatic and substituted aromatic esters is seen to have been contributed largely by the presence of an aromatic nucleus. This is because with the introduction of an aromatic nucleus attached to the ester functional group, resulted in all the aromatic ester derivatives of thymol showing an enhanced activity compared to the parent compound, thymol. Again, the substituted groups on the aromatic ester derivatives also conferred varying degree of activity with the alkyl substituted residual groups possessing the highest activity. The weakly activating alkyl residues on the aromatic nucleus contributed a corresponding significant increase in the larvicidal

activity of the aromatic ester derivatives as compared to the moderately and strongly activating methoxy (-OCH_3) and hydroxyl (-OH) groups respectively. This is also confirmed by the higher activity exhibited by the non-substituted aromatic ester derivative compared to the substitution of moderately and strongly activating groups. The contribution of the weakly deactivating groups of halogens like Br and Cl to the activity of the aromatic ester derivatives were very minimal.

Adulticidal

From the estimated LC_{50} value of thymol throughout the entire study period and in comparison to the different ether and ester derivatives of thymol, the hydroxyl group in thymol is important for its adulticidal activity. This contradicts the contribution of the hydroxyl group in the assessment of the larvicidal activity. Thus, thymol exhibited significant adulticidal activity over most of its alkyl and alkyl substituted ester derivatives with the exception of TM 1C and TM 1D for the alkyl esters. The same adulticidal superiority of thymol was seen compared to its alkyl and alkyl substituted ether derivatives, as it was realized that only TM 2O and TM 2P exhibited a greater activity than thymol. Chemical modification of the hydroxyl group into ether and ester functional groups in the thymol derivatives resulted in significantly decreased in adulticidal potency of the most of the synthesised ether and ester derivatives, except in the case of TM 1C, TM 1D, TM 2O and TM 2P.

It can also be established that the adulticidal potency of the alkyl and alkyl substituted ether and the alkyl and alkyl substituted esters derivatives of thymol on the adult mosquitoes of the *Anopheles gambiae s.s* is most important related to the presence of certain functional groups, such as aromatic

ring attached to the side alkyl chains of ether functional group, as an aromatic ring attached to the ester functional group contributed very little to its activity. Substituents like chlorine and fluorine on the aromatic ring as in the case of the ether derivatives, and halogens (Chlorine) on the alkyl chain of both the ether and ester functional groups also confer on such derivatives an enhanced adulticidal activity. The number of carbon in the chain of both the ether and ester functional groups, as there is an increase in activity of the ethers from carbon chain of three to carbon chain of four. In the esters, increasing the carbon chain increases the activity up to three carbons where maximum activity was recorded and activity starts to decreases beyond carbon three up to carbon five. Again, the nature of the aliphatic chain, such as branching of the alkyl chain resulted in a decrease in activity in ether functional group derivatives. Branching of the alkyl chain also contributed to a corresponding increase in activity of the ester functional group derivatives.

Triazoles

Triazoles have attracted considerable attention in the fields of medicine and agrochemical research as well as in materials science, due to their unique structures and properties. Triazole and its derivatives belong to a class of exceptionally active compounds possessing many pharmacological properties. Modern day research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The success of imidazole as an important moiety of a number of medicinal agents led to introduction of the triazoles. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen. Triazoles nucleus have been incorporated into a wide variety of therapeutically

interesting drug candidates including anticonvulsant, antineoplastic, antimalarial, antiviral, antiproliferative, anticancer, analgesic, anti-inflammatory, CNS stimulants, sedatives, antianxiety, antimicrobial, antifungal, antioxidant activities, etc. They are used as optical brightening agents, corrosion inhibitors and additives with a variety of other functions. Many dye stuffs and pigments have heterocyclic rings. The importance of triazole derivatives lies in the field that these have good position in heterocyclic chemistry, due to its various biological activities (Khatak & Verma 2014; Didwagh & Piste, 2013; Saini & Dwivedi, 2013; Guzeldemirci & Kucukbasmaci, 2010; Isloor, Kalluraya & Shetty, 2009; Amir, Kumar & Javed, 2008; Asif, 2015). Thus triazole acts as a promising medicinal agent for the scientists working over this field to develop new candidates of drugs to help address the issue of resistance by plant and animal pathogens.

Summary

All the synthesised derivatives of thymol were fully characterised and their structures elucidated with the following spectral analysis: proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR), liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS), Electron ionization/ chemical ionization gas chromatography mass spectroscopy (GC-MS-EI/CI) and infra-red spectroscopy (IR).

Most of the ester and ether derivatives showed high larvicidal and adultiicdal potency against the larvae and adult mosquito of the *Anopheles gambiae s.s* in comparison to the parent compound, thymol.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

In summary, the ever increasing demand for new effective drugs as well as the discovery and optimisation of existing drugs have resulted in the continuous modification of existing biological molecules by the introduction of new functional groups of interest. There are numerous active compounds and biological active molecules of plant origin that had been isolated, characterised and screened for their biological activity. Most of such active compounds in plants are either alkaloids, tannins, saponins, terpenoids etc. which exhibit various or diverse activity in the plant. Some of these active compounds exist in the salt form in the plant and are associated to perform specific task. Extraction and isolation of these compounds could alter their biological activity compared to their primary use in the plant. There is therefore the need to chemically modify these existing biological molecules isolated from plants by the introduction of certain functional groups and to study their structure activity relationship, in order to enhance the potency exhibited by these active compounds as well as maintaining their eco-friendly properties.

Triazole is a unique moiety that is responsible for various biological activities. This study was able to synthesize ten novel 1, 2, 3-triazole derivatives of thymol successfully in moderate to excellent yields using the azide-alkyne "click" reaction. All the prepared compounds, of thymol derivatives with triazole moiety were characterised by the following spectral data; proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear

magnetic resonance (^{13}C-NMR), liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS) , Electron ionization / chemical ionization gas chromatography mass spectroscopy (GC-MS-EI/CI) and infra-red spectroscopy (IR). Many heterocycles containing embedded triazole moiety have been shown to possess a wide range of biological activity (Asif, 2015). It is also established that, the 1, 2, 3-triazole moiety is stable against acidic and basic hydrolysis as well as against oxidative and reductive conditions, reflecting a high aromatic stabilisation and relative resistance to metabolic degradation (Ferreira *et al.,* 2010). At the same time, it has a high dipole moment, of about 5 D, (Bourne, Kolb, Radić, Sharpless, Taylor & Marchot, 2004) and participates actively in hydrogen bond formation as well as in dipole–dipole interactions (Whiting *et al.,* 2006). It is expected that, further investigations are carried out to evaluate the activities of these synthesised triazole derivatives of thymol for the many diseases whose treatment are difficult in the medical sciences. This is because, it has been noticed that chemical modifications involving the introduction of triazole moiety results in the formation of compounds with valuable biological activities. It will be interesting to observe that these modifications can be utilised as potent therapeutic agents in future. Thus many more modifications of thymol derivatives with the triazole moiety can be possible and thus need to be explored for the use of mankind.

The numerous biological activities of thymol and the enormous biological potentials that the triazole moiety possesses, present a new series of thymol derivatives with the triazole moiety with excellent biological activity.

This would help the medicinal chemist to assemble a large number of potentially active compounds which are derivatives of thymol. Again, this class of compounds of outstanding biological properties and medicinal importance would lead to discovery of new drugs as well as giving enormous energy to modern drug discovery.

The successful extraction and isolation of parthenin from *Parthenium hysterophorus* and the coupling of thymol and chlorothymol to it, accorded two novel compounds. This implies that, other derivatives of thymol with biological activity can be coupled to parthenin by the introduction of the triazole moiety. These will provide us with another class of medicinally potent drugs with thymol, a monoterpene and parthenin, a sesquiterpene moieties. To the best of our knowledge, this is the first time, through this study that thymol and chlorothymol had been coupled to parthenin with a triazole moiety such as in TM 10A and TM 10B respectively. These two compounds were characterised on the basis of liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS) and infra-red spectroscopy (IR).

Conclusions

A series of sixteen ester derivatives of thymol were successfully synthesised in excellent yields and of high purity using esterification reaction as described by Mathela *et al.,* 2010 and Kumbhar & Dewang, 2001 with minor modifications. A total of nine of these ester derivatives are classified as alkyl and alkyl substituted ester derivatives and seven of them aromatic and aromatic substituted ester derivatives. These synthesised compounds were characterised by the following spectral data; Electron ionization gas chromatography mass spectroscopy (GC-MS-EI), Chemical ionization gas

chromatography mass spectroscopy (GC-MS-CI) and infra-red spectroscopy (IR). The synthesis, characterisation and *in vitro* antibacterial activity of TM 1A, TM 1B, TM IC, TM ID, TM 1I, TM IK, TM 1N and TM IU had been reported earlier by Mathela *et al* whilst the synthesis and antifungal efficacy and larvicidal activity of TM 1A and IK was reported by Kumbhar and Dewang; Jack, Okorosaye-Orubite and Bobmanuel respectively. The synthesis, characterization, larvicidal and adulticidal activity of the following ester derivatives; TM 1E, TM 1F, TM 1G, TM 1L, TM 1M, TM 1P, TM 1Q and TM 1R are being reported for the first time in this study to the best of our knowledge.

A series of nine ether derivatives of thymol were synthesised successfully in excellent yield and of high purity using an etherification reaction as described by Mathela *et al* and also Kumbhar and Dewang with minor modifications. Six of these ether derivatives are classified as alkyl ether derivatives with the remaining three as alkyl substituted derivatives of thymol. Like some of the esters, the synthesis and antifungal efficacy of TM 2C, TM 2D, TM 2F and TM 2N had been reported earlier by Kumbhar and Dewang. The synthesis, characterisation, larvicidal and adulticidal activity of the remaining ether derivatives; TM 2E, TM 2I, TM 2K, TM 2O and TM 2P are being reported for the first time in this study to the best of our knowledge. In addition, with the exception of TM 1A and TM 1K whose larvicidal activity had been reported by Jack *et al*, the larvicidal and adulticidal activity of the other known ester and ether derivatives of thymol are being reported for the first time.

Thymol and carvacrol's antimicrobial activity is comparable to that of 2-amino-p-cymene, which indicates that the hydroxyl group although is important, but not essential for their activity (Veldhuizen *et al.*, 2006). The antimicrobial activity of essential oils can often be correlated to their content of phenolic constituents (Aligiannis *et al.*, 2001; Kalemba & Kunicka, 2003; Rhayour *et al.*, 2003). The antifungal potency of thymol derivatives is enhanced marginally with the introduction of an aromatic ring, additional olefinic bond and side chain up to three carbons (Kumbhar & Dewang, 2001). The synthesis of thymyl ester derivatives showed significant activity against gram-positive bacterial strains. These thymyl ester derivatives showed moderate activity against *B. subtilis* and *S. epidermidis (*Mathela *et al.*, 2010). The most notable enhancement in the activity noticed in the thymyl ester derivatives against gram-positive bacterial strains were as a result of the introduction of alkyl groups up to three carbons and an aromatic nucleus. Thymyl acetate and thymyl isobutyrate were found to be more effective than thymol (Mathela *et al.*, 2010).

The modification of the hydroxyl functional group of thymol into ester and ether functional groups resulted in enhanced antifungal and antibacterial potentials of some of the derivatives, which confirms that, although the hydroxyl (-OH) group is important for the activity of thymol, it is not essential (Veldhuizen *et al.*, 2006). This study has also established that, the larvicidal activity of the ester derivatives of thymol is greatly due to the presence of certain functional groups. The introduction of an aromatic nucleus to the side chain of the ester functional group was very essential for the enhancement of the larvicidal activity of the synthesised compounds compared to the parent

compound, thymol with the hydroxyl(-OH) group attached. The number of carbon atoms up to a certain limit in the side chain of the ester functional group, as a side chain of three carbons resulted in the highest larvicidal activity. Again, the nature of the aliphatic side chain played a critical role in the activity, the activity was much pronounced in the branched side chain, as a straight side chain confer a corresponding decrease in larvicidal activity of the same number of carbon in the side chain. The weakly activating group ($-CH_2CH_3$) on the aromatic nucleus attached to the ester functional group contributed significantly to the larvicidal activity compared to the methoxy ($-OCH_3$) and hydroxyl (-OH) activating groups which are moderate and strong activating groups respectively.The contribution of the weakly deactivating groups, halogens like Br and Cl to the activity of the aromatic ester derivatives were very minimal.

The larvicidal activity of the ether derivatives is due to an aromatic nucleus attached to the side chain of the ether functional group, substituted halogens like chlorine and fluorine on the aromatic ring and the nature of the aliphatic side chain, as activity was enhanced in a straight side chain compared to a branch chain.

Generally, the synthetic alkyl and alkyl substituted ether derivatives of thymol showed significantly improved larvicidal activity over the tested alkyl and substituted alkyl ester derivatives, with the exception of TM 1I, where the larvicidal activity was comparable to the ether derivatives. The most potent larvicidal derivative, TM 2O recorded an LC_{50} value of 1.90 mg/L after 12 hours of exposure time. This is about 8 folds higher in potency compared to the parent compound, thymol with an LC_{50} value of 15.01 mg/L after 72 hours

of exposure time. This was followed by TM 2P also with an LC_{50} value of 4.61 mg/L after 12 hours of exposure time which is also about 4 folds higher in larvicidal potency than thymol. Contrary to the larvicidal activity, the adulticidal activity of the synthesised ester and ether derivatives were very much influenced by the presence of the hydroxyl (-OH) group. The parent compound, thymol demonstrated a better adulticidal activity on the Anopheles mosquito compared to the synthesised derivatives with an LC_{50} value of 27.60 mg/L after seven days of exposure time, with the exception of TM 1C, TM 2O, TM 1D and TM 2P with LC_{50} values of 16.02 mg/L, 19.30 mg/L, 23.11mg/L and 23.20 mg/L respectively. The most potent adulticidal derivative been TM 1C.

The derivatives of thymol that were subjected to the virtual screening studies of larvicidal and adulticidal assays revealed that the compounds under study possess moderate to excellent drug-like characteristics. The modifications resulted in change in the larvicidal and adulticidal activity against the larvae and adult female anopheles mosquitoes. Based on these findings, compounds TM 2N, TM 2O, TM 2P, TM 1C, TM 1D, TM 1I, TM 1M and TM 1Q possess a potential to be developed as larvicidal agents (larvicides) and adulticidal agents (adulticides) and can as well be useful candidate insecticides against the larvae and adult female *Anopheles gambiae s.s* since they showed the highest bioactivity.

Recommendations

Since the structures of the synthesised derivatives of thymol are fully characterised and elucidated, there is the need to screen for their biological activities against several human and plant pathogens.

With the enormous medicinal potentials of the triazole moiety in the search of potent drugs for antifungal, anticancer, antibacterial, antitubercular, antiviral, anti-inflammatory, analgesic, anticonvulsant, antiparasitic, antidiabetic, anti obesitic, antihistaminic, anti-neuropathic, antihypertensive and so on, it is expected that, the ten novel 1, 2, 3-triazole derivatives of thymol synthesised in this study would be screened for their potency in all the above listed biological assays.

The successful coupling of thymol and its derivative chlorothymol on parthenin should create a backbone for several of the synthesised thymol derivatives to be coupled to parthenin in a similar way. Modifications around the aromatic nucleus of thymol by the introduction of electronegative and highly polar groups like the amino ($-NH_2$), hydroxyl (-OH), halogenated (X= Br, I, F) groups etc. should be prepared and coupled to parthenin. The thymol-parthenin coupled products, TM 10A and TM 10B should also be screened for their biological activities as anti-cancer, anti-tumor and anti-parasitic agents. The proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) data of TM 10A and TM 10B is needed to establish fully their structural elucidation.

There is also the need to confirm fully the chemical structures of the newly synthesised ester and ether derivatives of thymol using the following spectral data; proton nuclear magnetic resonance (^{1}H-NMR), carbon-13

nuclear magnetic resonance (^{13}C-NMR) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS).The synthesised ester and ether derivatives should be screened for their antifungal, antibacterial, leishmanial, antioxidant and anti-inflammatory activity in the continuous search for potent and lead drugs.

Since the parthenin was isolated from *Parthenium hysterophorus*, natural plant source, it is expected that thymol may as well be extracted and isolated from certain plant sources such as *Thymus vulgaris L* (Thyme) and *Carum copticum* (Ajwan) to be employed in further syntheses instead of using synthetic thymol. This will help us to access these biological compounds with their semi-synthetic derivatives as lead drugs.

Since thymol is a skin irritant, future investigations are expected to be carried out on these synthesised thymol derivatives; the triazole derivatives of thymol, the ester derivatives as well as the ether derivatives to ascertain and evaluate their respective toxicity. This would serve as a guide to help in further studies on the biological and pharmaceutical activities of these derivatives, thereby reducing their potential risk to human, animals, plants and the environment in general. This study can be helpful in synthesizing new compounds possessing thymol moiety that could be better in terms of efficacy and lesser toxicity. From the discussions of the larvicidal and adulticidal biological assay results, it may be concluded that the modifications of thymol into its ester and ether derivatives displayed valuable biological activities and these modifications can be utilised to develop potentially active agents for future investigations.

REFERENCES

Adams, R., & Levine, I. J. (1923). *Journal of the American Chemical Society, 45,* 2373.

Afanador-Kafuri, L., Minz, D., Maymon, M., & Freeman, S. (2003). Characterization of colletotrichum isolates from tamarillo, passiflora, and mango in Colombia and identification of a unique species from the genus. *Phytopathology, 93*(5), 579-587.

Aftab, K., Atta-Ur-Rahman, & Usmanghani, K. (1995). Blood pressure lowering action of active principle from trachyspermum ammi (L.) sprague. *Phytomedicine, 2*(1), 35-40.

Ahmad, A., Khan, A., Akhtar, F., Yousuf, S., Xess, I., Khan, L. A., & Manzoor, N. (2011). Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against candida. *European Journal of Clinical Microbiology and Infectious Diseases, 30*(1), 41-50.

Ahmad, A., Khan, L. A., Khan, A., Yousuf, S., & Manzoor, N. (2010). Proton translocating ATPase mediated fungicidal activity of eugenol and thymol. *Fitoterapia, 81*(8), 1157-1162.

Ahmadifar, E., Falahatkar, B., & Akrami, R. (2011). Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, oncorhynchus mykiss. *Journal of Applied Ichthyology, 27*(4), 1057-1060.

Ahvenainen, R. (2003). Novel food Packaging Techniques. Cambridge: CRC Press, Woodhead Publishing Limited.

Akhtar, Y., & Isman, M. B. (2004). Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. *Journal of Applied Entomology, 128*(1), 32-38.

Ali, A. A., & Gaikar, V. G. (2011). Microwave-assisted process intensification of synthesis of thymol using carbonized sulfonic acidic resin (CSA) catalyst. *Industrial and Engineering Chemistry Research, 50*(11), 6543-6555.

Aligiannis, N., Kalpoutzakis, E., Mitaku, S., & Chinou, I. B. (2001). Composition and antimicrobial activity of the essential oils of two origanum species. *Journal of Agricultural and Food Chemistry, 49*(9), 4168-4170.

Amandi, R., Hyde, J. R., Ross, S. K., Lotz, T. J., & Poliakoff, M. (2005). Continuous reactions in supercritical fluids; a cleaner, more selective synthesis of thymol in supercritical CO 2. *Green Chemistry, 7*(5), 288-293.

Amslinger, S. (2010). The tunable functionality of alpha, beta-unsaturated carbonyl compounds enables their differential application in biological systems. *Chemmedchem, 5*(3), 351-356.

Ambikadevi, D., & Samarjit, R. (1997) Chemical control of red spider mite Tetranychus cinnabarinus (Boisduval) on okra. *Journal of Tropical Agricultural Science*, 35: 38–40.

Amir, M., Kumar, H., & Javed, S. A. (2008). Condensed bridgehead nitrogen heterocyclic system: Synthesis and pharmacological activities of 1, 2, 4-triazolo-[3, 4-b]-1, 3, 4-thiadiazole derivatives of ibuprofen and

biphenyl-4-yloxy acetic acid. *European Journal of Medicinal Chemistry, 43*(10), 2056-2066.

Andersen, A. (2006). Final report on the safety assessment of sodium p-chloro-m-cresol, p-Chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol. *International Journal of Toxicology, 25*(1), 29-127.

Arnason, J. T., MacKinnon, S., Durst, A., Philogene, B. J. R., Hasbun, C., Sanchez, P., Poveda, L., San Roman, L., Isman, M. B. & Satasook, C. (1993). Insecticides in tropical plants with non-neurotoxic modes of action. In Downum, K. R., Romeo, J. T. & Stafford, H. A. (Eds.), *Phytochemical Potential of Tropical Plants.* New York: Plenum.

Arrigoni M. E. (1977). *Inflammation and anti-inflammatory.* New York: Spectrum Publication.

Asif, M. (2015). Antiviral and antiparasitic activities of various substituted triazole derivatives: A mini. *Chemistry International, 1*(2), 71-80.

Astani, A., Reichling, J., & Schnitzler, P. (2011). Screening for antiviral activities of isolated compounds from essential oils. *Evidence-Based Complementary and Alternative Medicine, 2011,* 1-8.

Azuma, Y., Ozasa, N., Ueda, Y. & Takagi, N. J. (1986). *Dental Research, 65,* 53.

Bagamboula, C. F., Uyttendaele, M., & Debevere, J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards shigella sonnei and S. flexneri. *Food Microbiology, 21*(1), 33-42.

Bai, X., Zhou, C. H., & Mi, J. L. (2007). Research and application of triazoles. *Chemical Research and Application 19*, 721-729.

Barnard, C., Padgitt, M., & Uri, N. D. (1997). Pesticide use and its measurement. *International Pest Control, 39*(5), 161-164.

Bay, H. A., Quaddouri, B., Guaadaoui, A., Touzani, R., Benchat, N. E., Hamal, A., & Kadiri, S. E. (2010). Synthesis and biological activity of new triazole compounds. *Letters in Drug Design and Discovery, 7*(1), 41-45.

Braga, P. C., Culici, M., Alfieri, M., & Dal Sasso, M. (2008). Thymol inhibits Candida albicans biofilm formation and mature biofilm. *International Journal of Antimicrobial Agents, 31*(5), 472-477.

Braga, P. C., Dal Sasso, M., Culici, M., Bianchi, T., Bordoni, L., & Marabini, L. (2006). Anti-inflammatory activity of thymol: Inhibitory effect on the release of human neutrophil elastase. *Pharmacology, 77*(3), 130-136.

Bassolé, I. H. N., Lamien-Meda, A., Bayala, B., Tirogo, S., Franz, C., Novak, J., & Dicko, M. H. (2010). Composition and antimicrobial activities of lippia multiflora moldenke, mentha x piperita L. and ocimum basilicum L. essential oils and their major monoterpene alcohols alone and in combination. *Molecules, 15*(11), 7825-7839.

Bell, F., & Henry, T. A. (1928). CCLXXXVIII.—By-products of the gattermann aldehyde reaction. *Journal of American Chemical Society,* 2215-2227.

Ben, Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N., & Chalier, P. (2006). Antimicrobial activity of carvacrol related to its chemical structure. *Letters in Applied Microbiology, 43*(2), 149-154.

Benjamin, Y. K., Nogbou, E. A., Ado, G., Azzaro-Pantel, C., & Davin, A. (2007). Modeling and optimization of M-cresol isopropylation for obtaining N-thymol: combining a hybrid artificial neural network with a genetic algorithm. *International Journal of Chemical Reactor Engineering, 5*(1).

Beović, B. (2006). The issue of antimicrobial resistance in human medicine. *International Journal of Food Microbiology, 112*(3), 280-287.

Bennis, S., Chami, F., Chami, N., Bouchikhi, T., & Remmal, A. (2004). Surface alteration of saccharomyces cerevisiae induced by thymol and eugenol. *Letters in Applied Microbiology, 38*(6), 454-458.

Bhat, U. G., & Nagasampagi, B. A. (1988). Alumina-catalyzed reactions of Parthenin and Its Derivatives. *Indian Journal Of Chemistry Section B-Organic Chemistry Including Medicinal Chemistry, 27*(11), 989-993.

Bischof, L. J., & Enan, E. E. (2004). Cloning, expression and functional analysis of an octopamine receptor from periplaneta americana. *Insect Biochemistry and Molecular Biology, 34*(6), 511-521.

Bohlmann, F., Mahanta, P. K., Jakupovic, J., Rastogi, R. C., & Natu, A. A. (1978). New sesquiterpene lactones from inula species. *Phytochemistry, 17*(7), 1165-1172.

Bohlmann, F., & Zdero, C. (1977). Neue sesquiterpenlactone und thymol-derivate aus inula-arten. *Phytochemistry, 16*(8), 1243-1245.

Bokadia, M. M., Macleod, A. J., Mehta, B. K., Mehta, S. C., & Patel, H. (1986). The essential oil of Inula racemosa. *Phytochemistry, 25*(12), 2887-2888.

Bourne, Y., Kolb, H. C., Radić, Z., Sharpless, K. B., Taylor, P., & Marchot, P. (2004). Freeze-frame inhibitor captures acetylcholinesterase in a unique conformation. *Proceedings of the National Academy of Sciences of the United States of America, 101*(6), 1449-1454.

Brenes, A., & Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. *Animal Feed Science and Technology, 158*(1), 1-14.

Buckingham, J. (1998). *Dictionary of natural products*. London: Chapman and Hall.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiol*ogy, *94,* 223–253.

Burt, S. A., Ruurd van der Zee, Koets, A. P., Anko M. de Graaff, Knapen, F. v., Gaastra, W., . . . Edwin J. A. Veldhuizen. (2007). Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in escherichia coli O157:H7. *Applied and Environmental Microbiology, 73*(14), 4484-4490.

Caballero, B., Trugo, L., & Finglas, P. (2003). In *Encyclopedia of food sciences and nutrition,* (pp. 1-10). Elsevier Science BV.

Cava-Roda, R. M., Taboada-Rodríguez, A., Valverde-Franco, M. T., & Marín-Iniesta, F. (2012; 2010). Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling listeria

dihydroxy-β- himachalene by botrytis cinerea. *Journal of Agricultural and Food Chemistry, 53*(17), 6673-6677.

Davis, E., & Croteau, R. (2000). Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpenes. *Biosynthesis*, 53-95.

Davidson, P., & Parish, M. (1989). *Methods for testing the efficacy of food antimicrobials*. Chicago: Institute of Food Technologists.

Deans, S. G., & Ritchie, G. (1987). Antibacterial properties of plant essential oils. *International Journal of Food Microbiology, 5*(2), 165-180.

Delaquis, P. J., Stanich, K., Girard, B., & Mazza, G. (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology, 74*(1), 101-109.

Degenhardt, J., Köllner, T. G., & Gershenzon, J. (2009). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry*, *70*(15), 1621-1637.

Demirbas, N., Karaoglu, S. A., Demirbas, A., & Sancak, K. (2004). Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1, 3, 4] thiadiazol-2-yl) methyl-5-oxo-[1, 2, 4] triazole and 1-(4-phenyl-5-thioxo-[1, 2, 4] triazol-3-yl) methyl-5-oxo-[1, 2, 4] triazole derivatives. *European Journal of Medicinal Chemistry*, *39*(9), 793-804.

Dev, S., & Koul, O. (1997). *Insecticides of natural origin.* Amsterdam: Harwood Academic Publishers.

Di Pasqua, R., Betts, G., Hoskins, N., Edwards, M., Ercolini, D., & Mauriello, G. (2007). Membrane toxicity of antimicrobial compounds from

essential oils. *Journal of Agricultural and Food Chemistry, 55*(12), 4863-4870.

Di Pasqua, R., Mamone, G., Ferranti, P., Ercolini, D., & Mauriello, G. (2010). Changes in the proteome of salmonella enterica serovar thompson as stress adaptation to sublethal concentrations of thymol. *Proteomics, 10*(5), 1040-1049.

Didry, N., Didry, N., Dubreuil, L., Dubreuil, L., Pinkas, M., & Pinkas, M. (1994). Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharmaceutica Acta Helvetiae, 69*(1), 25-28.

Dirsch, V., Stuppner, H., & Vollmar, A. (2001). Helenalin triggers a CD95 death receptor-independent apoptosis that is not affected by overexpression of bcl-X-L or bcl-2. *Cancer Research, 61*(15), 5817-5823.

Desai, J. M. & Shah, V. H. (2003). Synthesis and biological activity of cyanopyridine, isoxazole and pyrazoline derivatives having thymol moeity. *Indian Journal of Chemistry, 42B,* 382-385.

Donsì, F., Annunziata, M., Sessa, M., & Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT - Food Science and Technology, 44*(9), 1908-1914.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*(2), 308-316.

Dwivedi, S. K., & Dubey, N. K. (1993). Potential use of the essential oil of Trachyspermum ammi against seed-borne fungi of Guar (Cyamopsis tetragonoloba L. (Taub.)). *Mycopathologia, 121*(2), 101-104.

Dwivedi, A. K., Shukla, V. K., Bhandari, K., Setty, B. S., Kamboj, V. P., & Khanna, N. M. (1991). 1 aryloxy-3- substituted aminopropan- 2-ols aminomethyl substituted phenols and 1, 3- benzoxazines as potential spermicides. *Indian Journal of Chemistry Section B Organic Chemistry Including Medicinal Chemistry, 30(*2), 281-287.

Eliopoulos, G. M., Moellering, R. C. J., & Pillai, S. K. (1996). *Antimicrobial combinations in antibiotics in laboratory medicine.* Philadelphia: Williams & Wilkins.

Espina, L., Somolinos, M., Lorán, S., Conchello, P., García, D., & Pagán, R. (2011). Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control, 22*(6), 896-902.

Enan, E. (2001). Insecticidal activity of essential oils: octopaminergic sites of action. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 130*(3), 325-337.

Falcone, P., Speranza, B., Del Nobile, M. A., Corbo, M. R., & Sinigaglia, M. (2005). A study on the antimicrobial activity of thymol intended as a natural preservative. *Journal of Food Protection, 68*(8), 1664-1670.

FAO (1999). The use of spices and medicinals as bioactive protectants for grains. *Agriculture Service Bulletin*, 137, 201–213.

Ferreira, S. B., Sodero, A. C., Cardoso, M. F., Lima, E. S., Kaiser, C. R., Silva Jr, F. P., & Ferreira, V. F. (2010). Synthesis, biological activity, and molecular modeling studies of 1 h-1, 2, 3-triazole derivatives of carbohydrates as α-glucosidases inhibitors. *Journal of Medicinal Chemistry, 53*(6), 2364-2375.

Filoche, S. K., Soma, K., & Sissons, C. H. (2005). Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. *Molecular Oral Microbiology*, *20*(4), 221-225.

Fitzgerald, D. J., Stratford, M., & Narbad, A. (2003). Analysis of the inhibition of food spoilage yeasts by vanillin. *International Journal of Food Microbiology*, *86*(1), 113-122.

Fletcher, J., Bender, C., Budowle, B., Cobb, W. T., Gold, S. E., Ishimaru, C. A., & Seem, R. C. (2006). Plant pathogen forensics: capabilities, needs, and recommendations. *Microbiology and Molecular Biology Reviews*, *70*(2), 450-471.

Floris, I., Satta, A., Cabras, P., Garau, V. L., & Angioni, A. (2004). Comparison between two thymol formulations in the control of varroa destructor: effectiveness, persistence, and residues. *Journal of Economic Entomology*, *97*(2), 187-191.

Fraga, B. M. (2006). Natural sesquiterpenoids. *Natural Product Reports*, *23*(6), 943-972.

Fraenkel, G. S. (1959). The raison d'etre of secondary plant substances. *Science*, *129*(3361), 1466-1470.

Furka, A., & Szell, T. J. (1960). Compounds derived from acetic acid. *Chemical Society, 459,* 2312.

Gabel, C. V., & Berg, H. C. (2003). The speed of the flagellar rotary motor of Escherichia coli varies linearly with protonmotive force. *Proceedings of the National Academy of Sciences*, *100*(15), 8748-8751.

Gallucci, N., Casero, C., Oliva, M., Zygadlo, J., & Demo, M. (2006). Interaction between terpenes and penicillin on bacterial strains resistant

to beta-lactam antibiotics. *Molecular Medicinal Chemistry*, *10*(1), 30-2.

Gaonkar, A., & Kirtany, J. K. (1991). *Indian Journal of Chemistry, 30,* 800.

García-García, R., López-Malo, A., & Palou, E. (2011). Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against Listeria innocua. *Journal of Food Science*, *76*(2).

Gershenzon, J., Maffei, M., & Croteau, R. (1989). Biochemical and histochemical localization of monoterpene biosynthesis in the glandular trichomes of spearmint (Mentha spicata). *Plant Physiology*, *89*(4), 1351-1357.

Gershenzon, J., & Kreis, W. (1999). Biochemistry of terpenoids: Monoterpenes, sesquiterpenes, diterpenes, sterols, cardiac glycosides and steroid saponins. *Biochemistry of Plant Secondary Metabolism*, *2*, 222-299.

Gershenzon, J., & Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology*, *3*(7), 408-414.

George, D. R., Smith, T. J., Shiel, R. S., Sparagano, O. A. E., & Guy, J. H. (2009). Mode of action and variability in efficacy of plant essential oils showing toxicity against the poultry red mite, dermanyssus gallinae. *Veterinary Parasitology, 161*(3), 276-282.

Ghannoum, M. A., & Rice, L. B. (1999). Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clinical Microbiology Reviews*, *12*(4), 501-517.

Ghosheh, O. A., Houdi, A. A., & Crooks, P. A. (1999). High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (nigella

sativa L.). *Journal of Pharmaceutical and Biomedical Analysis, 19*(5), 757-762.

Gilani, A. H., Jabeen, Q., Ghayur, M. N., Janbaz, K. H., & Akhtar, M. S. (2005). Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the carum copticum seed extract. *Journal of Ethnopharmacology, 98*(1), 127-135.

Gill, A. O., Delaquis, P., Russo, P., & Holley, R. A. (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. International *Journal of Food Microbiology, 73*(1), 83-92.

Govindarajan, M., Jebanesan, A., & Pushpanathan, T. (2008). Larvicidal and ovicidal activity of cassia fistula linn leaf extract against filarial and malarial vector mosquitoes. *Parasitology Research*, *102*(2), 289-292.

Govindarajan, M. (2009). Bioefficacy of cassia fistula linn. (leguminosae) leaf extract against chikungunya vector, aedes aegypti (diptera: Culicidae). *European Review for Medical and Pharmacological Sciences, 13*(2), 99.

Govindarajan, M., & Sivakumar, R. (2011). Mosquito adulticidal and repellent activities of botanical extracts against malarial vector, anopheles stephensi liston (diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine, 4*(12), 941-947.

Grabowska, H., & Wrzyszcz, J. (2001). C-alkylation of m-cresol with n-and iso-propanol over iron catalyst. *Research on Chemical Intermediates*, *27*(3), 281-285.

Grabowska, H., Miśta, W., Trawczyński, J., Wrzyszcz, J., & Zawadzki, M. (2001). A method for obtaining thymol by gas phase catalytic

alkylation of m-cresol over zinc aluminate spinel. *Applied Catalysis A, General, 220*(1), 207-213.

Grodnitzky, J. A., & Coats, J. R. (2002). QSAR evaluation of monoterpenoids' insecticidal activity. *Journal of Agricultural and Food Chemistry, 50*(16), 4576-4580.

Guo, L., Wei, C., Jia, J., Zhao, L., & Quan, Z. (2009). Design and synthesis of 5-alkoxy-[1,2,4]triazolo[4,3-a]quinoline derivativeswith anticonvulsant activity. *European Journal of Medicinal Chemistry, 44*(3), 954-958.

Güzeldemirci, N. U., & Küçükbasmacı, Ö. (2010). Synthesis and antimicrobial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazo[2,1- b]thiazole moiety. *European Journal of Medicinal Chemistry, 45*(1), 63-68.

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology, 124*(1), 91-97.

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2009). Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiology, 26*(2), 142-150.

Gupta, V. K., Fatima, A., Faridi, U., Negi, A. S., Shanker, K., Kumar, J. K., & Darokar, M. P. (2008). Antimicrobial potential of Glycyrrhiza glabra roots. *Journal of Ethnopharmacology, 116*(2), 377-380.

Guo, N., Liu, J., Wu, X., Xingming, B., Rizeng Meng, Wang, X., Xiang, H., Deng, X., & Yu, L. (2009). Antifungal activity of the ajwain ethanol

extract, of various fungi; A. flavus, A. ochraceus, A. niger, A. orzya, fusarium moniliforme, penicillium sp . *Journal Medical Microbiology*, *58*(90), 1074.

Habon, T., Szabados, E., Kesmarky, G., Halmosi, R., Past, T., Sumegi, B., & Toth, K. (2001). The effect of carvedilol on enhanced ADP-ribosylation and red blood cell membrane damage caused by free radicals. *Cardiovascular Research*, *52*(1), 153-160.

Hadad, M., Zygadlo, J. A., Lima, B., Derita, M., Egly Feresin, G. A. B. R. I. E. L. A., Zacchino, S. A., & Tapia, A. (2007). Chemical composition and antimicrobial activity of essential oil from baccharis grisebachii hieron (asteraceae). *Journal of the Chilean Chemical Society*, *52*(2), 1186-1189.

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, *86*(6), 985-990.

Hartmann, T. (2007). From waste products to ecochemicals: Fifty years research of plant secondary metabolism. *Phytochemistry*, *68*(22), 2831-2846.

Hartl, F. U., Bracher, A., & Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature*, *475*(7356), 324-332.

Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., . . . Wright, v., A. (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. *Journal of Agricultural and Food Chemistry, 46*(9), 3590-3595.

He, L., Mo, H., Hadisusilo, S., Qureshi, A. A., & Elson, C. E. (1997). Isoprenoids suppress the growth of murine B16 melanomas in vitro and in vivo. *The Journal of Nutrition*, *127*(5), 668-674.

Heilmann, J., Wasescha, M. R., & Schmidt, T. J. (2001). The influence of glutathione and cysteine levels on the cytotoxicity of helenanolide type sesquiterpene lactones against KB cells. *Bioorganic and Medicinal Chemistry*, *9*(8), 2189-2194.

Hernández, Y. S., Sánchez, L. B., Bedia, M. M. G., Gómez, L. T., Rodríguez, E. J., San Miguel, H. M. G., & Pieters, L. (2011). Determination of parthenin in Parthenium hysterophorus L. by means of HPLC-UV: Method development and validation. *Phytochemistry Letters*, *4*(2), 134-137.

Horváth, G., Kovács, K., Kocsis, B., & Kustos, I. (2009). Effect of thyme (*Thymus vulgaris L.*) essential oil and its main constituents on the outer membrane protein composition of Erwinia strains studied with microfluid chip technology. *Chromatographia*, *70*(11), 1645-1650.

Hsieh, P. (2000). Antimicrobial effect of cinnamon extract. *Taiwanese Journal of Agricultural Chemistry and Food Science*, *38*(2), 184-193.

Hu, D., & Coats, J. (2008). Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory. *Pest Management Science*, *64*(7), 775-779.

Hummelbrunner, L. A., & Isman, M. B. (2001). Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, Spodoptera litura (Lep., Noctuidae). *Journal of Agricultural and Food Chemistry*, *49*(2), 715-720.

Huang, M., Abel, C., Sohrabi, R., Petri, J., Haupt, I., Cosimano, J., & Tholl, D. (2010). Variation of herbivore-induced volatile terpenes among Arabidopsis ecotypes depends on allelic differences and subcellular targeting of two terpene synthases, TPS02 and TPS03. *Plant Physiology, 153*(3), 1293-1310.

Hunashal, R. D., & Satyanarayana, D. (2012). One pot synthesis of 3-(substituted phenoxymethyl)-6-phenyl/substituted phenoxymethyl-1, 2, 4-triazolo [3, 4-b][1, 3, 4] thiadiazole derivatives as antimicrobial agents. *International Journal of Pharmacy and Biological Sciences, 3*, 183-192.

Huisgen, R. (1961). Centenary lecture-1, 3-dipolar cycloadditions. *Proceedings of the Chemical Society*, 357-369.

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology, 3,* 12.

Isman, M. (1999). Pesticides based on plant essential oils. *Journal of Pesticide Outlook, 10* (2), 68–72.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection, 19*(8), 603-608.

Isman, M. B. (2004). Plant essential oils as green pesticides for pest and disease management. In Nelson, W. M. (Eds.), *Agricultural applications in green chemistry* (pp. 41–51). Washington, DC: American Chemical Society.

Isloor, A. M., Kalluraya, B., & Shetty, P. (2009). Regioselective reaction: synthesis, characterization and pharmacological studies of some new

Mannich bases derived from 1, 2, 4-triazoles. *European Journal of Medicinal Chemistry*, *44*(9), 3784-3787.

Iwalokun, B. A., Gbenle, G. O., Smith, S. I., Ogunledun, A., Akinsinde, K. A., & Omonigbehin, E. A. (2001). Epidemiology of shigellosis in Lagos, Nigeria: trends in antimicrobial resistance. *Journal of Health, Population and Nutrition*, 183-190.

Jabbar, A., Iqbal, Z., & Khan, M. N. (2006). In vitro anthelmintic activity of trachyspermum ammi seeds. *Pharmacognosy Magazine*, *2*(6), 126.

Jack, I. R., Okorosaye-Orubite, K., & Bobmanuel, R. B. (2006). Assessment of the larvicidal potentials of thymol derivatives on anopheles mosquitoes. *Journal of Applied Sciences and Environmental Management*, *10*(1), 63-65.

Jensen, K., & Møller, B. L. (2010). Plant NADPH-cytochrome P450 oxidoreductases. *Phytochemistry*, *71*(2), 132-141.

Jordão, A. K., Ferreira, V. F., Souza, T. M., de Souza Faria, G. G., Machado, V., Abrantes, J. L., & Cunha, A. C. (2011). Synthesis and anti-HSV-1 activity of new 1, 2, 3-triazole derivatives. *Bioorganic & Medicinal Chemistry*, *19*(6), 1860-1865.

Juglal, S., Govinden, R., & Odhav, B. (2002). Spice oils for the control of co-occurring mycotoxin-producing fungi. *Journal of Food Protection*, *65*(4), 683-687.

Juven, B. J., Kanner, J., Schved, F., & Weisslowicz, H. (1994). Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *Journal of Applied Microbiology*, *76*(6), 626-631.

Kalemba, D. A. A. K., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, *10*(10), 813-829.

Kamat, S. P., & Paknikar, S. K. (1981). *Indian Journal of Chemistry, 20,* 244.

Kank, H. H., Rho, H. S., Hwang, J. S., & O. H, S.G. (2003). *Chemical and Pharmaceutical Bulletin,* 51(9), 1085.

Khatak, M., & Verma, P. K. (2014). Microwave synthesis and pharmacological importance of 1, 2, 4-triazole derivatives-a review. *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*(3), 388-409.

Kharb, R., Sharma, P. C., & Yar, M. S. (2011). Pharmacological significance of triazole scaffold. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *26*(1), 1-21.

Kim, S. H., Oh, S. M., & Kim, T. S. (2005). Induction of human leukemia HL-60 cell differentiation via a PKC/ERK pathway by helenalin, a pseudoguainolide sesquiterpene lactone. *European Journal of Pharmacology*, *511*(2), 89-97.

Kim, S. I., Roh, J. Y., Kim, D. H., Lee, H. S., & Ahn, Y. J. (2003). Insecticidal activities of aromatic plant extracts and essential oils against Sitophilus oryzae and Callosobruchus chinensis. *Journal of Stored Products Research*, *39*(3), 293-303.

Khanuja, S. P. S. (2004). United state patent; No. US 6,824,795 b2.

Khumbhar, P. P., & Dewang, P. M. (2001). Eco-friendly pest management using monoterpenoids. I. Antifungal efficacy of thymol derivatives. *Journal of Scientific and Industrial Research*, *60*(8), 645-648

Kong, J. O., Lee, S. M., Moon, Y. S., Lee, S. G., & Ahn, Y. J. (2006). Nematicidal activity of plant essential oils against Bursaphelenchus xylophilus (Nematoda: Aphelenchoididae). *Journal of Asia-Pacific Entomology*, *9*(2), 173-178.

Konstantopoulou, I., Vassilopoulou, L., Mavragani-Tsipidou, P., & Scouras, Z. G. (1992). Insecticidal effects of essential oils: A study of the effects of essential oils extracted from eleven greek aromatic plants on drosophila auraria. *Cellular and Molecular Life Sciences*, *48*(6), 616-619.

Kordali, S., Cakir, A., Mavi, A., Kilic, H., & Yildirim, A. (2005). Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three turkish artemisia species. *Journal of Agricultural and Food Chemistry*, *53*(5), 1408-1416.

Koshti, S. M., Sonar, J. P., Sonawane, A. E., Pawar, Y. A., Nagle, P. S., Mahulikar, P. P., & More, D. H. (2008). Synthesis of azo compounds containing thymol moiety. *Indian Journal of Chemistry*, 47, 329.

Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N., & Shaaya, E. (2002). Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: Possible mode of action against insect pests. *Pest Management Science*, *58*(11), 1101-1106.

Koutsoudaki, C., Krsek, M., & Rodger, A. (2005). Chemical composition and antibacterial activity of the essential oil and the gum of pistacia lentiscus var chia. *Journal of Agricultural and Food Chemistry*, *53*(20), 7681-7685.

Koul, O. (2005). *Insect antifeedants*. Bota Racon, FL: CRC Press.

Koul, O., Walia, S., & Dhaliwal, G. S. (2008). Essential oils as green pesticides: potential and constraints. *Biopestic Int*, *4*(1), 63-84.

Krishna, Badhwar.(1966;1953). Jour. Sci. indu. Res, 12A, 2, IP, 32, 288,

Kuaban, C., Bercion, R., Jifon, G., Cunin, P., & Ngu Blackett, K. (2000). Acquired anti-tuberculosis drug resistance in yaounde, cameroon. *The International Journal of Tuberculosis and Lung Disease*, *4*(5), 427-432.

Kuaban, C., Bercion, R., Noeske, J., Cunin, P., Nkamsse, P., & Ngo Niobe, S. (2000). Anti-tuberculosis drug resistance in the west province of cameroon. *The International Journal of Tuberculosis and Lung Disease*, *4*(4), 356-360.

Kupchan, S. M., Eakin, M. A., & Thomas, A. M. (1971). Tumor inhibitors. 69. structure-cytotoxicity relations among the sesquiterpene lactones. *Journal of Medicinal Chemistry*, *14*(12), 1147-1152.

Kim, B. S., Moon, S. S., & Hwang, B. K. (1999). Isolation, antifungal Activity, and structure elucidation of the glutarimide antibiotic, streptimidone, and produced by micromonospora coerulea. *Journal of Agricultural and Food Chemistry*, *47*(8), 3372-3380.

Kintzios, S. E. (2002). Profile of the multifaceted prince of the herbs. *Oregano: The Genera Origanum and Lippia*, 3-10.

Kubo, A., Lunde, C. S., & Kubo, I. (1995). Antimicrobial activity of the olive oil flavor compounds. *Journal of Agricultural and Food Chemistry*, *43*(6), 1629-1633.

Kumar, S., Warikoo, R., & Wahab, N. (2010). Larvicidal potential of ethanolic extracts of dried fruits of three species of peppercorns against different

instars of an Indian strain of dengue fever mosquito, aedes aegypti L.(diptera: culicidae). *Parasitology Research*, *107*(4), 901-907.

Kyung, K. H. (2011). Antimicrobial properties of allium species. *Current Opinion in Biotechnology*, *23*(2), 142-147.

Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, *91*(3), 453-462.

Larhsini, M., Oumoulid, L., & Lazrek, H. B. (2001). Antibacterial activity of some moroccan medicinal plants. *Phytotherapy Research, 15*(3), 250–252.

Lawerence, B. M. & Reynolds, R.J. (2001). Progress in essential oils. *Perfumer and Flavourist, 26,* 44–52.

Leonardi, A., Riva, C., De Toma, C., Boi, C., Pennini, R., & Sironi, G. (1994). Synthesis and pharmacological evaluation of new indole derivatives structurally related to thymoxamine. *European Journal of Medicinal Chemistry*, *29*(7-8), 551-559.

Lee, K. H., Wu, Y. S., & Hall, I. H. (1977). Antitumor agents. 25. synthesis and antitumor activity of uracil and thymine alpha-methylene-gamma-lactones and related derivatives. *Journal of Medicinal Chemistry, 20*(7), 911-914.

Liang, H., Bao, F., Dong, X., Tan, R., Zhang, C., Lu, Q., & Cheng, Y. (2007). Antibacterial thymol derivatives isolated from centipeda minima. *Molecules*, *12*(8), 1606-1613.

Liu, Z., Zhu, Q., Li, F., Zhang, L., Leng, Y., & Zhang, A. (2011). N-(5-substituted thiazol-2- yl)-2-aryl-3-(tetrahydro-2H-pyran-4-yl) propanamides as glucokinase activators. *Medicinal Chemistry Communication*, *2*(6), 531-535.

Lupo, A. T., Nakatsu, T. J., Caldwell, J., Kang, R. K. L., Cilia, A. T., Loveren, A. G. V., & Villamaria L. (2000) US Patent; No. 6,110,888.

Lv, F., Liang, H., Yuan, Q., & Li, C. (2011). In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International, 44*(9), 3057-3064.

Madadi, M., & Rahimi, R. (2010). Synthesis and characterization of manganese (III) tetra (4-methoxyphenyl) porphyrin encapsulated in zeolite–X as efficient catalyst for the hydroxylation of thymol. *The 14th International Electronic Conference on Synthetic Organic Chemistry*. In J. A. Seijas & M. P. Pilar Vázquez Tato (Ed.). *General Organic Synthesis* (pp. 331-334). Basel, Switzerland: MDPI.

Magyar, J. (2003). *Effect of thymol on cardiac and skeletal muscle* (PhD thesis). University of Debrecen, Debrecen Egyetem, Hungary.

Mann, C. M., Cox, S. D., & Markham, J. L. (2000). The outer membrane of pseudomonas aeruginosa NCTC 6749 contributes to its tolerance to the essential oil of melaleuca alternifolia (tea tree oil). *Letters in Applied Microbiology, 30*(4), 294-297.

Martinez, E. P., Hío, J. C., Osoriol, J. A., & Torres, M. F. (2009). Identification of colletotrichum species causing anthracnose on tahiti lime, tree tomato and mango. *Agronomía Colombiana, 27*(2), 211-218.

Marino, M., Bersani, C., & Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from lamiaceae and compositae. International *Journal of Food Microbiology, 67*(3), 187-195.

Mathela, C. S., Tiwari, A., Padalia, R. C., & Chanotiya, C. S. (2008). Chemical composition of Inula cuspidata CB Clarke. *Indian Journal of Chemistry, 47B(8),* 1249-1253.

Mathela, C. S., Singh, K. K., & Gupta, V. K. (2010). Synthesis and in vitro antibacterial activity of thymol and carvacrol derivatives. *Acta Poloniae Pharmaceutica, 67*(4), 375.

Mathew, V., Keshavayya, J., Vaidya, V. P., & Giles, D. (2007). Studies on synthesis and pharmacological activities of 3, 6-disubstituted-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-thiadiazoles and their dihydro analogues. *European Journal of Medicinal Chemistry, 42*(6), 823-840.

Mezzoug, N., Elhadri, A., Dallouh, A., Amkiss, S., Skali, N. S., Abrini, J., . . . Idaomar, M. (2007). Investigation of the mutagenic and antimutagenic effects of origanum compactum essential oil and some of its constituents. *Mut.Res.-Genetic Toxicology and Environmental Mutagenesis, 629*(2), 100-110.

Mehmood, S., Lateef, M., Omer, M. O., Anjum, A. A., Rashid, M. I., & Shehzad, W. (2014). Adulticidal and larvicidal activity of cassia fistula and piper nigrumagainst malaria vector. *Science International (Lahore), 26*, 331-4.

Mi, J. L., Wu, J., & Zhou, C. H. (2008). Progress in anti-tumor agents: triazoles. *West China Journal of Pharmaceutical Sciences, 23*, 84-86.

Mi, J., Zhou, C., & Bai, X. (2007). Advances in triazole antimicrobial agents. *Chinese Journal of Antibiotics, 32,* 587-593.

McGovern, S. L., Caselli, E., Grigorieff, N., & Shoichet, B. K. (2002). A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. *Journal of Medicinal Chemistry, 45*(8), 1712-1722.

Modzelewska, A., Sur, S., Kumar, S. K., & Khan, S. R. (2005). Sesquiterpenes: natural products that decrease cancer growth. *Current Medicinal Chemistry-Anti-Cancer Agents, 5*(5), 477-499.

More, D. H., Pawar, N. S., Dewang, P. M., Patil, S. L., & Mahulikar, P. P. (2004). Microwave-assisted sinthesis of thymyl ethers and esters in aqueous medium. *Russian Journal of General Chemistry, 74*(2), 217-218.

Monza, C. G., Belli, A., & Novara (1982). US patent; No. 4,336, 396 dated jun 22.

Moszner, N., Salz, U. & Rheinberger, (1994) V. Polymer Bulletin 33: p. 7-12

Murthy, P. S., Borse, B. B., Khanum, H., & Srinivas, P. (2009). Inhibitory effects of ajowan (trachyspermum ammi) ethanolic extract on A. ochraceus growth and ochratoxin production. *Turkish Journal of Biology, 33*(3), 211-217.

Mwangi, I., Berkley, J., Lowe, B., Peshu, N., Marsh, K., & Newton, Charles R J C. (2002). Acute bacterial meningitis in children admitted to a rural kenyan hospital: Increasing antibiotic resistance and outcome. *The Pediatric Infectious Disease Journal, 21*(11), 1042.

Nagle, P. S., Pawar, Y. A., Sonawane, A. E., Nikum, A. P., Patil, U. D., & More, D. H. (2013). Thymol: Synthesis, reactions & its spectrum of pharmacological and chemical applications. *Indo American Journal of Pharmaceutical Research*, *3*, 7549-7561.

Nagle, P. S., Pawar, Y. A., Sonawane, A. E., Bhosale, S. M., & More, D. H. (2012). Synthesis and evaluation of antioxidant and antimicrobial properties of thymol containing pyridone moieties. *Medicinal Chemistry Research, 21*(7), 1395-1402.

Nakagawa, Y., Iinuma, M., Matsuura, N., Yi, K., Naoi, M., Nakayama, T., . . . Akao, Y. (2005). A potent apoptosis-inducing activity of a sesquiterpene lactone, arucanolide, in HL60 cells: A crucial role of apoptosis-inducing factor. *Journal of Pharmacological Sciences, 97*(2), 242.

Nath, D., Sethi, N., Srivastava, S., Jain, A. K., & Srivastava, R. (1997). Survey on indigenous medicinal plants used for abortion in some districts of uttar pradesh. *Fitoterapia*, *68*(3), 223-225.

Nath, D. R., Bhuyan, M., & Goswami, S. (2006). Botanicals as mosquito larvicides. *Defence Science Journal*, *56*(4), 507.

Nguefack, J., Tamgue, O., Dongmo, J. L., Dakole, C. D., Leth, V., Vismer, H. F., & Nkengfack, A. E. (2012). Synergistic action between fractions of essential oils from cymbopogon citratus, ocimum gratissimum and thymus vulgaris against penicillium expansum. *Food Control*, *23*(2), 377-383.

Numpaque, M. A., Oviedo, L. A., Gil, J. H., García, C. M., & Durango, D. L. (2011). Thymol and carvacrol: Biotransformation and antifungal

activity against the plant pathogenic fungi colletotrichum acutatum and botryodiplodia theobromae. *Tropical Plant Pathology*, *36*(1), 3-13.

Odds, F. C. (2003). Synergy, antagonism, and what the chequerboard puts between them. *Journal of Antimicrobial Chemotherapy*, *52*(1), 11.

Ouellette, W., Jones, S., & Zubieta, J. (2011). Solid state coordination chemistry of metal-1,2, 4-triazolates and the related metal-4-pyridyltetrazolates. *Royal Society of Chemistry*, *13*(14), 4457-4485.

Ormancey, X., Sisalli, S., & Coutiere, P. (2001). Formulation of essential oils in functional perfumery. *Parfums, Cosmetiques, Actualites*, *157*(1), 30-40.

Osorio, E., Arango, G., Robledo, S., Munoz, D., Jaramillo, L., & Velez, I. (2007). Antileishmanial and cytotoxic activity of synthetic aromatic monoterpens. *Acta Farmaceutica Bonaerense*, *25*(3), 405.

Ok, P.Z, Young, H.J, Lee, K.R, Moon, H.I, Laek, H.G, Lee, M.J & Kim, D.K, US Patent 2001,6, 255, 517B1.

Oussalah, M., Caillet, S., Saucier, L., & Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, salmonella typhimurium, staphylococcus aureus and listeria monocytogenes. *Food Control, 18*(5), 414-420.

Park, I. K., Kim, J., Lee, S. G., & Shin, S. C. (2007). Nematicidal activity of plant essential oils and components from ajowan (trachyspermum ammi), allspice (pimenta dioica) and litsea (litsea cubeba) essential oils against pine wood nematode (bursaphelenchus xylophilus). *Journal of Nematology*, *39*(3), 275.

Pathak, A. K., Nainwal, N., Goyal, B. M., Singh, R., Mishra, V., Nayak, S., & Gupta, V. (2010). Pharmacological activity of trachyspermum ammi: A review. *Journal of Pharmacy Research*, *3*(4), 895-899.

Palaniappan, K., & Holley, R. A. (2010). Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *International Journal of Food Microbiology*, *140*(2), 164-168.

Pelczar, M. J., Chan, E. C. S., & Krieg, N. R. (1988). Control of microorganisms, the control of microorganisms by physical agents. *Microbiology*, *469*, 509.

Pei, R., Zhou, F., Ji, B., & Xu, J. (2009). Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against E. coli with an improved method. *Journal of Food Science, 74*(7), M379-M383.

Pelissari, F. M., Grossmann, M. V., Yamashita, F., & Pineda, E. A. G. (2009). Antimicrobial, mechanical, and barrier properties of cassava starch–chitosan films incorporated with oregano essential oil. *Journal of Agricultural and Food Chemistry*, *57*(16), 7499-7504.

Phillips, M. J. (1920). Synthesis of thymol from p-cymene in seven steps. *Industrial and Engineering Chemistry,* 12, 733.

Phillips, C., & Laird, K. (2011). *Vapour of a citrus essential oil blend and its antimicrobial properties*. U.S. Patent 20110136761.

Platel, K., & Srinivasan, K. (2001). Studies on the influence of dietary spices on food transit time in experimental rats. *Nutrition Research*, *21*(9), 1309-1314.

Polo, L. M., Castro, C. M., Cruzado, M. C., Collino, C. J., Cuello-Carrión, F. D., Ciocca, D. R., & López, L. A. (2007). 11, 13-dihydro-dehydroleucodine, a derivative of dehydroleucodine with an inactivated alkylating function conserves the anti-proliferative activity in G2 but does not cause cytotoxicity. *European Journal of Pharmacology*, *556*(1), 19-26.

Poulose, A. J., & Croteau, R. (1978). Biosynthesis of aromatic monoterpenes: Conversion of gamma-terpinene to p-cymene and thymol in thymus vulgaris L. *Archives of Biochemistry and Biophysics, 187*(2), 307-314.

Prajapati, S., Goswami, K., & Patal, A. (2013). Synthesis and characterisation of 4-Aryl thiazole ring system and its antimicrobial activity. *International Journal of Pharmacy and Biological Sciences, 4*(1), 803-808.

Priestley, C. M., Williamson, E. M., Wafford, K. A., & Sattelle, D. B. (2003). Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABAA receptors and a homo-oligomeric GABA receptor from drosophila melanogaster. *British Journal of Pharmacology, 140*(8), 1363-1372.

Qiu, J., Wang, D., Xiang, H., Feng, H., Jiang, Y., Xia, L., . . . Deng, X. (2010). Subinhibitory concentrations of thymol reduce enterotoxins A and B and alpha-hemolysin production in staphylococcus aureus isolates. *PloS One, 5*(3), e9736.

Rahuman, A. A., Venkatesan, P., & Gopalakrishnan, G. (2008). Mosquito larvicidal activity of oleic and linoleic acids isolated from citrullus colocynthis (linn.) schrad. *Parasitology Research, 103*(6), 1383-1390.

Rajkumar, S., Jebanesan, A., & Nagarajan, R. (2011). Effect of leaf essential oil of coccinia indica on egg hatchability and different larval instars of malarial mosquito anopheles stephensi. *Asian Pacific Journal of Tropical Medicine*, *4*(12), 948-951.

Ramos, A., Rivero, R., Victoria, M. C., Visozo, A., Piloto, J., & Garcıa, A. (2001). Assessment of mutagenicity in parthenium hysterophorus L. *Journal of Ethnopharmacology*, *77*(1), 25-30.

Rao, A., Zhang, Y., Muend, S., & Rao, R. (2010). Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. *Antimicrobial Agents and Chemotherapy*, *54*(12), 5062-5069.

Rasooli, I., Fakoor, M. H., Allameh, A. A., Rezaee, M. B., & Owlia, P. (2009). Phytoprevention of aflatoxin production. *Journal of Medicinal Plants*, *8*(5), 97-104.

Rattanachaikunsopon, P., & Phumkhachorn, P. (2010). Assessment of factors influencing antimicrobial activity of carvacrol and cymene against vibrio cholerae in food. *Journal of Bioscience and Bioengineering*, *110*(5), 614-619.

Reddy, P. B., Agrawal, S. K., Singh, S., Bhat, B. A., Saxena, A. K., Kumar, H. M. S., & Qazi, G. N. (2008). Synthesis and biological evaluation of 4β-[(4-substituted)-1, 2, 3-triazol-1-yl] podophyllotoxins as potential anticancer agents. *Chemistry and Biodiversity*, *5*(9), 1792-1802.

Reddy, D. M., Qazi, N. A., Sawant, S. D., Bandey, A. H., Srinivas, J., Shankar, M., & Mondhe, D. (2011). Design and synthesis of spiro

derivatives of parthenin as novel anti-cancer agents. *European Journal of Medicinal Chemistry*, *46*(8), 3210-3217.

Rhayour, K., Bouchikhi, T., Tantaoui-Elaraki, A., Sendide, K., & Remmal, A. (2003). The mechanism of bactericidal action of oregano and clove essential oils and of their phenolic major components on escherichia coli and bacillus subtilis. *Journal of Essential Oil Research*, *15*(4), 286-292.

Robledo, S., Osorio, E., Munoz, D., Jaramillo, L. M., Restrepo, A., Arango, G., & Vélez, I. (2005). In vitro and in vivo cytotoxicities and antileishmanial activities of thymol and hemisynthetic derivatives. *Antimicrobial Agents and Chemotherapy*, *49*(4), 1652-1655.

Rodriguez-Fernandez, E., Manzano, J. L., Benito, J. J., Hermosa, R., Monte, E., & Criado, J.J. (2005). Thiourea, triazole and thiadiazine compounds and their metal complexes as antifungal agents. *Journal of Inorganic Biochemistry*, *99*(8), 1558-1572.

Roda, K. P., & Vansdadia R. N. (1988). Indian J. Chem. Soc. LXV, Vol. 44, p. 807.

Rojano, B., Saez, J., Schinella, G., Quijano, J., Vélez, E., Gil, A., & Notario, R. (2008). Experimental and theoretical determination of the antioxidant properties of isoespintanol (2-Isopropyl-3, 6-dimethoxy-5-methylphenol). *Journal of Molecular Structure*, *877*(1), 1-6.

Roller, S. (Ed.). (2003). *Natural antimicrobials for the minimal processing of foods*. Elsevier, Cambridge: Woodhead Publishing Limited.

Rostovtsev, V. V., Green, L. G., Fokin, V. V., & Sharpless, K. B. (2002). A stepwise huisgen cycloaddition process: copper (I)-catalyzed

regioselective "ligation" of azides and terminal alkynes. *Angewandte Chemie*, *114*(14), 2708-2711.

Różalski, M., Krajewska, U., Panczyk, M., Mirowski, M., Różalska, B., Wąsek, T., & Janecki, T. (2007). Synthesis and biological evaluation of 4-methylideneisoxazolidin-5-ones–a new class of highly cytotoxic α-methylidene-γ-lactones. *European Journal of Medicinal Chemistry*, *42*(2), 248-255.

Saini, M. S., & Dwivedi, J. (2013). Synthesis and biological significances of 1, 2, 4-triazole and its derivatives: A review. *International Journal of Pharmaceutical Sciences and Research*, *4*(8), 2866.

Sallaud, C., Rontein, D., Onillon, S., Jabès, F., Duffé, P., Giacalone, C., . . . Tissier, A. (2009). A novel pathway for sesquiterpene biosynthesis from Z,Z-farnesyl pyrophosphate in the wild tomato solanum habrochaites. *The Plant Cell, 21*(1), 301-317.

Sánchez, E., García, S., & Heredia, N. (2010). Extracts of edible and medicinal plants damage membranes of vibrio cholerae. *Applied and Environmental Microbiology*, *76*(20), 6888-6894.

Sánchez-González, L., Vargas, M., González-Martínez, C., Chiralt, A., & Cháfer, M. (2011). Use of essential oils in bioactive edible coatings: A review. *Food Engineering Reviews*, *3*(1), 1-16.

Sapir-Mir, M., Mett, A., Belausov, E., Tal-Meshulam, S., Frydman, A., Gidoni, D., & Eyal, Y. (2008). Peroxisomal localization of arabidopsis isopentenyl diphosphate isomerases suggests that part of the plant isoprenoid mevalonic acid pathway is compartmentalized to peroxisomes. *Plant Physiology*, *148*(3), 1219-1228.

Satzinger, G. & Herramann, M. F. (1976). US Patent. 3,966,779, June 29.

Saxena, B. P. (1989). Insecticides from neem. In Arnason, J. T., Philogene, B. J. R. & Morand, P. (Eds.), *Insecticides of Plant Origin*, (pp. 387). Phillipines: American Chemistry Society Symposium Series.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S. L., . . . Griffin, P. M. (2011). Foodborne illness acquired in the united states--major pathogens. *Emerging Infectious Diseases,* 17(1), 7.

Schmidt, T. J. (1999). Toxic activities of sesquiterpene lactones: Structural and biochemical aspects. *Current Organic Chemistry*, *3*(577-608), 4.

Sedy, K. A., & Koschier, E. H. (2003). Bioactivity of carvacrol and thymol against frankliniella occidentalis and thrips tabaci. *Journal of Applied Entomology*, *127*(6), 313-316.

Šegvić Klarić, M., Kosalec, I., Mastelić, J., Piecková, E., & Pepeljnak, S. (2007). Antifungal activity of thyme (thymus vulgaris L.) essential oil and thymol against moulds from damp dwellings. *Letters in Applied Microbiology*, *44*(1), 36-42.

Sethi, N., & Singh, R. K. (1989). Teratological evaluation of some commonly used indigenous antifertility plants in rats. *International Journal of Crude Drug Research*, *27*(2), 118-120.

Sfaei-Ghomi, J., Meshkatalsadat, M. H., Shamai, S., Hasheminejad, M. & Hassani, A. (2009). Chemical characterization of bioactive volatile molecules of four thymus species using nanoscale injection method. *Digest Journal of Nanomaterials and Biostructures*, *4*(4), 835-841.

Shapira, R., & Mimran, E. (2007). Isolation and characterization of escherichia coli mutants exhibiting altered response to thymol. *Microbial Drug Resistance*, *13*(3), 157-165.

Shah, B. A., Kaur, R., Gupta, P., Kumar, A., Sethi, V. K., Andotra, S. S., & Taneja, S. C. (2009). Structure–activity relationship (SAR) of parthenin analogues with pro-apoptotic activity: Development of novel anti-cancer leads. *Bioorganic & Medicinal Chemistry Letters*, *19*(15), 4394-4398.

Shen, A. Y., Huang, M. H., Liao, L. F., & Wang, T. S. (2005). Thymol analogues with antioxidant and L-type calcium current inhibitory activity. *Drug Development Research*, *64*(4), 195-202.

Sikkema, J., De Bont, J. A., & Poolman, B. (1995). Mechanisms of membrane toxicity of hydrocarbons. *Microbiological Reviews*, *59*(2), 201-222.

Silva, F., Ferreira, S., Duarte, A., Mendonça, D. I., & Domingues, F. C. (2011). Antifungal activity of coriandrum sativum essential oil, its mode of action against candida species and potential synergism with amphotericin B. *Phytomedicine*, *19*(1), 42-47.

Singh, I., Kaur, H., Kumar, S., & Kumar, A. (2010). Synthesis and antimicrobial activity of some new pyridinyl/ quinazolinyl/azetidinyl/ thiazolidinonyl triazoles. *International Journal of Pharmacy and Biological Sciences*, *1*, 1-17.

Singh, I. P., Shukla, V. K., Dwivedi, A. K., & Khanna, N. M. (1989). Synthesis of some aromatic aldehydes and phenols as potential male antifertility agents. *ChemiInform*, *20*(49).

Singh, G., Maurya, S., Catalan, C., & De Lampasona, M. P. (2004). Chemical constituents, antifungal and antioxidative effects of ajwain essential oil and its acetone extract. *Journal of Agricultural and Food Chemistry, 52*(11), 3292-3296.

Skoula, M., & Harborne, J. B. (2002). The taxonomy and chemistry of *Origanum*. In S. E. Kintzios, (Eds.), *The genera origanum and lippie* (pp. 67-108). London: Taylor and Francis.

Srivastava, K. C. (1988). Extract of a spice—omum (trachyspermum ammi)-shows antiaggregatory effects and alters arachidonic acid metabolism in human platelets. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *33*(1), 1-6.

Sokovic, M., Tzakou, O., Pitarokili, D., & Couladis, M. (2002). Antifungal activities of selected aromatic plants growing wild in Greece. *Nahrung-Food*, *46*(5), 317-320.

Soylu, S., Yigitbas, H., Soylu, E. M., & Kurt, Ş. (2007). Antifungal effects of essential oils from oregano and fennel on sclerotinia sclerotiorum. *Journal of Applied Microbiology*, *103*(4), 1021-1030.

Stahl-Biskup, E. (2002). Essential oil chemistry of the genus thymus - a global view. In E. StahlBiskup & Saez, F., (Eds.), *Thyme: The genus thymus,* (pp. 75-124). London: Taylor & Francis.

Stroh, J., Wan, M. T., Isman, M. B., & Moul, D. J. (1998). Evaluation of the acute toxicity to juvenile pacific coho salmon and rainbow trout of some plant essential oils, a formulated product, and the carrier. *Bulletin of Environmental Contamination and Toxicology*, *60*(6), 923-930.

Stojakowska, A., Kędzia, B., & Kisiel, W. (2005). Antimicrobial activity of 10-isobutyryloxy-8, 9-epoxythymol isobutyrate. *Fitoterapia*, *76*(7), 687-690.

Suau, R., Torres, G., & Valpuesta, M. (1995). The photo-fries rearrangement of 2, 5-disubstituted phenyl acetates. *Tetrahedron Letters*, *36*(8), 1311-1314.

Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*, *21*(9), 1199-1218.

Talakal, T. S., Dwivedi, S. K., & Sharma, S. R. (1995). In vitro and in vivo therapeutic activity of parthenium hysterophorus against trypanosoma evansi. *Indian Journal of Experimental Biology*, *33*(11), 894-896.

Talalay, P., De Long, M. J., & Prochaska, H. J. (1988). Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proceedings of the National Academy of Sciences*, *85*(21), 8261-8265.

Tamura, T., & Iwamoto, H. (2004). Thymol: a classical small-molecule compound that has a dual effect (potentiating and inhibitory) on myosin. *Biochemical and Biophysical Research Communications*, *318*(3), 786-791.

Tassou, C., Koutsoumanis, K., & Nychas, G. J. (2000). Inhibition of salmonella enteritidis and staphylococcus aureus in nutrient broth by mint essential oil. *Food Research International*, *33*(3), 273-280.

Tauxe, R.V. (1990). Emerging foodborne diseases: An evolving public health challenge. *Dairy Food Environm. Sanitation*, 17, 788-795.

Tawata, S., Taira, S., Kobamoto, N., Ishihara, M., & Toyama, S. (1996). Synthesis and fungicidal activity of new thiophosphorylated monoterpenoids and related compounds. *Journal of Pesticide Science, 21,* 141–146.

Theis, N., & Lerdau, M. (2003). The evolution of function in plant secondary metabolites. *International Journal of Plant Sciences, 164*(S3), S93-S102.

Tholl, D. (2006). Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology*, *9*(3), 297-304.

Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biology and Technology*, *32*(3), 235-245.

Tripathi, P., & Shukla, A. K. (2007). Emerging non-conventional technologies for control of post harvest diseases of perishables. *Fresh Produce*, *1*(2), 111-120.

Tripathi, A. K., Veena, P., Aggarwal, K. K., & Sushil, K. (2000). Effect of volatile oil constituents of mentha species against the stored grain pests, callosobruchus maculatus and tribolium castaneum. *Journal of Medicinal and Aromatic Plant Sciences*, *22*(1B), 549-556.

Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C. & Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy*, *49*(6), 2474-2478.

Tserennadmid, R., Takó, M., Galgóczy, L., Papp, T., Pesti, M., Vágvölgyi, C., & Krisch, J. (2011). Anti yeast activities of some essential oils in growth medium, fruit juices and milk. *International Journal of Food Microbiology*, *144*(3), 480-486.

Turner, G., Gershenzon, J., Nielson, E. E., Froehlich, J. E., & Croteau, R. (1999). Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. *Plant Physiology*, *120*(3), 879-886.

Turina, A. D. V., Nolan, M. V., Zygadlo, J. A., & Perillo, M. A. (2006). Natural terpenes: Self-assembly and membrane partitioning. *Biophysical Chemistry*, *122*(2), 101-113.

Ultee, A., Bennik, M. H. J., & Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen bacillus cereus. *Applied and Environmental Microbiology*, *68*(4), 1561-1568.

Ündeğer, Ü. Başaran, A., Degen, G. H., & Başaran, N. (2009). Antioxidant activities of major thyme ingredients and lack of (oxidative) DNA damage in V79 chinese hamster lung fibroblast cells at low levels of carvacrol and thymol. *Food and Chemical Toxicology*, *47*(8), 2037-2043.

(USEPA) Environmental Protection Agency. (1996). *Exemption of certain pesticide substances from federal insecticide, fungicide, and rodenticide act requirements.* Washington, DC: Environmental Protection Agency.

(USEPA) Environmental Protection Agency. (2009). *Minimum risk pesticides*. Washington, DC: Environmental Protection Agency.

Varma, R. L., & Narayanan, C. S. (1985). Synthesis of monocyclic B-lactams with a terpenoid moiety. *ChemInform*, *16*(34).

Vashi, B. S., & Shah, V. H. (1996). Synthesis and biological screening of substituted thymolylthiazolidinones and thymolylazetidinones. *Journal of the Indian Chemical Society*, *73*(9), 491-492.

Vashi B. S, Mehta D. S, & Shah V. H. (1995). Synthesis and biological screening of substituted thymolyl thiazolidinones and thymolylazetidinones. *Journal of the Indian Chemical Society*, *34*, 802.

Vazquez, B. I., Fente, C., Franco, C. M., Vazquez, M. J., & Cepeda, A. (2001). Inhibitory effects of eugenol and thymol on penicillium citrinum strains in culture media and cheese. *International Journal of Food Microbiology*, *67*(1), 157-163.

Velazhahan, R., Vijayanandraj, S., Vijayasamundeeswari, A., Paranidharan, V., Samiyappan, R., Iwamoto, T., & Muthukrishnan, S. (2010). Detoxification of aflatoxins by seed extracts of the medicinal plant, trachyspermum ammi (L.) sprague ex turrill–structural analysis and biological toxicity of degradation product of aflatoxin G1. *Food Control*, *21*(5), 719-725.

Venturini, M. E., Blanco, D., & Oria, R. (2002). In vitro antifungal activity of several antimicrobial compounds against penicillium expansum. *Journal of Food Protection*, *65*(5), 834-839.

Veldhuizen, E. J., Tjeerdsma-van Bokhoven, J. L., Zweijtzer, C., Burt, S. A., & Haagsman, H. P. (2006). Structural requirements for the

antimicrobial activity of carvacrol. *Journal of Agricultural and Food Chemistry*, *54*(5), 1874-1879.

Vrushali, T. (2001). Bioactivity of some medicinal plants against chosen insect pests/vectors. *Journal of Medicinal and Aromatic Plant Sciences*, *4A*, 120-124.

Wachira, S. W., Omar, S., Jacob, J. W., Wahome, M., Alborn, H. T., Spring, D. R. & Torto, B. (2014). Toxicity of six plant extracts and two pyridone alkaloids from ricinus communis against the malaria vector anopheles gambiae. *Parasites & Vectors*, *7*(1), 312.

Walsh, S. E., Maillard, J. Y., Russell, A. D., Catrenich, C. E., Charbonneau, D. L., & Bartolo, R. G. (2003). Activity and mechanisms of action of selected biocidal agents on gram-positive and-negative bacteria. *Journal of Applied Microbiology*, *94*(2), 240-247.

Waghamale, S., & Piste, P. (2013). Pharmacological activities of triazole, oxadiazole and thiadiazole. *International Journal of Pharmacy and Biological Sciences*, *4*(3), 310-332.

Wang, Y., & Zhou, C. (2011). Recent advances in the researches of triazole compounds as medicinal drugs. *Scientia Sinica Chimica*, *41*(9), 1429-1456.

Wagner, S., Suter, A., & Merfort, I. (2004). Skin penetration studies of arnica preparations and of their sesquiterpene lactones. *Planta Medica*, *70*(10), 897-903.

Wahidullah, S., & Paknikar, S. K. (1988). Synthesis of 2, 3-dimethoxy-p-cymene. *ChemInform*, *19*(13).

Weremczuk-Jeżyna, I., Kisiel, W., & Wysokińska, H. (2006). Thymol derivatives from hairy roots of arnica montana. *Plant Cell Reports*, *25*(9), 993-996.

Whiting, M., Muldoon, J., Lin, Y. C., Silverman, S. M., Lindstrom, W., Olson, A. J., & Fokin, V. V. (2006). Inhibitors of HIV-1 protease by using in situ click chemistry. *Angewandte Chemie International Edition*, *45*(9), 1435-1439.

Wimmer. P, & Buysch H. J. US Patent 1991, 5, 030,770.

Wienkötter, N., Begrow, F., Kinzinger, U., Schierstedt, D., & Verspohl, E. J. (2007). The effect of thyme extract on β2-receptors and mucociliary clearance. *Planta Medica*, *73*(07), 629-635.

World Health Organization. (2005). *Guidelines for laboratory and field testing of mosquito larvicides.* Geneva: World Health Organization.

World Health Organization. (2013). *Malaria fact sheet.* Geneva: World Health Organization.

World Health Organization. (2000-2010). *Global Report on Antimalarial Efficacy and Drug Resistance.* Geneva: World Health Organization.

Xu, J., Zhou, F., Ji, B. P., Pei, R. S., & Xu, N. (2008). The antibacterial mechanism of carvacrol and thymol against escherichia coli. *Letters in Applied Microbiology*, *47*(3), 174-179.

Yamanaka, T. (1976). Catalytic properties of metal sulfates supported on γ-Al_2O_3 in the liquid-phase isopropylation of m-cresol with propylene. *Bulletin of the Chemical Society of Japan*, *49*(10), 2669-2673.

Yoshida, T., Mori, K., & Guangxin, H. (1995). Inulavosin, a new thymol dimer with piscicidal activity from inula nervosa. *Heterocycles*, *9*(41), 1923-1926.

Zaridah, M. Z., Nor Azah, M. A., Abu Said, A., & Mohd Faridz, Z. P. (2003). Larvicidal properties of citronellal and cymbopogon nardus essential oils from two different localities. *Trop Biomed*, *20*(2), 169-174.

Zhou, C., Gan, L., Zhang, Y., Zhang, F., Wang, G., Jin, L., & Geng, R. (2009). Review on supermolecules as chemical drugs. *Science in China Series B: Chemistry*, *52*(4), 415-458.

Zhou, C. H., Zhang, F. F., Gan, L. L., Zhang, Y. Y., & Geng, R. X. (2009). Research in supramolecular chemical drugs. *Sci. China, Ser B: Chem*, *39*, 208-252.

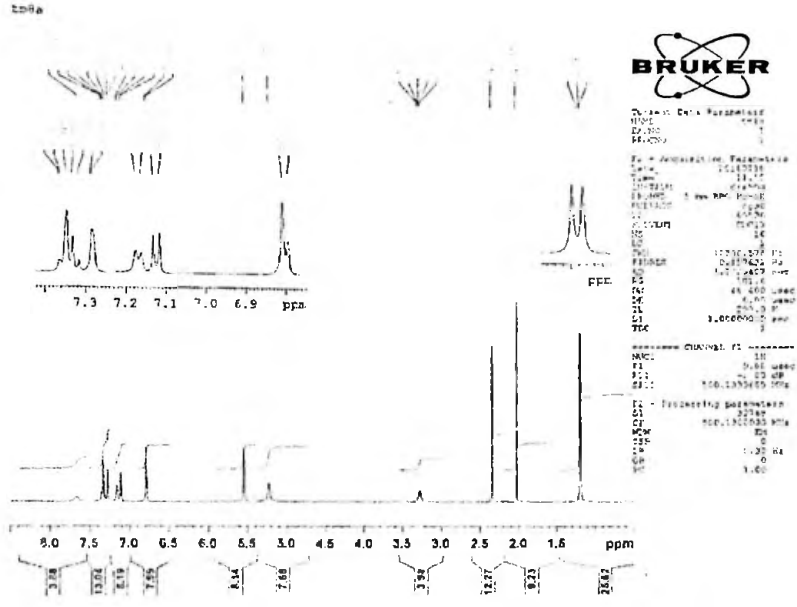
Zhou, C. H., Zhang, Y. Y., Yan, C. Y., Wan, K., Gan, L. L., & Shi, Y. (2010). Recent researches in metal supramolecular complexes as anticancer agents. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *10*(5), 371-395.

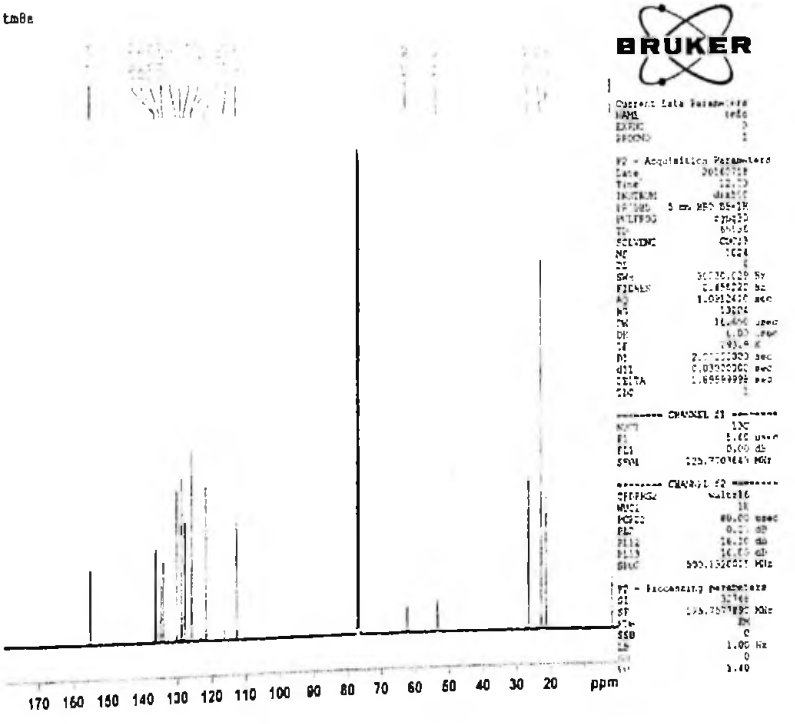
Zink, D. L. (1997). The impact of consumer demands and trends on food processing. *Emerging infectious diseases*, *3*(4), 467.

APPENDICES

APPENDIX A-1

^{1}H & ^{13}C NMR FOR TM 8A





APPENDIX A-2

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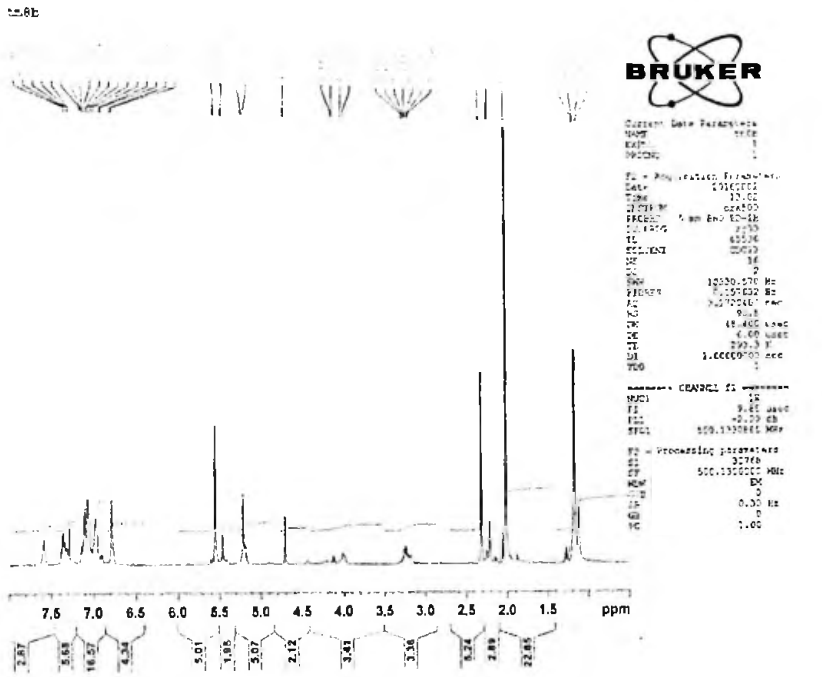

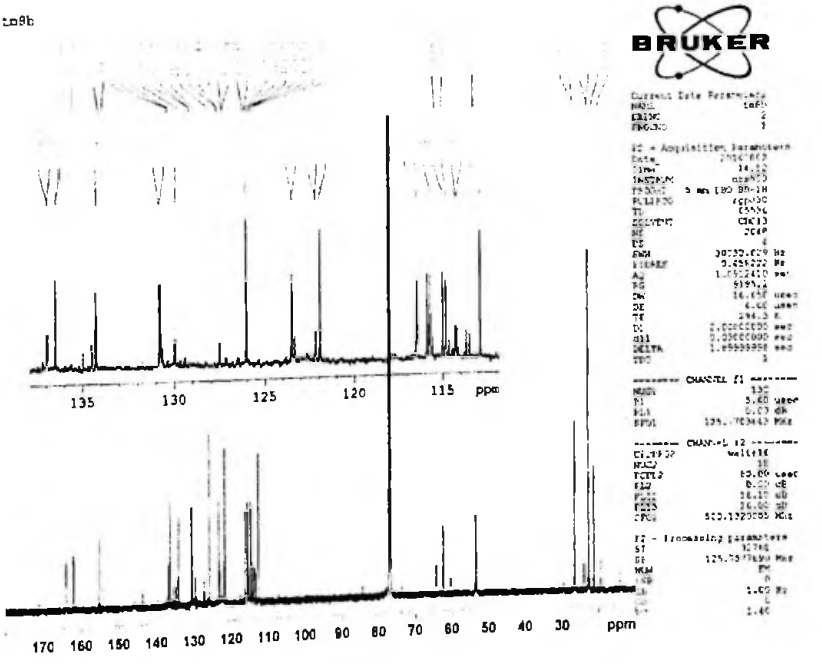

APPENDIX A-3

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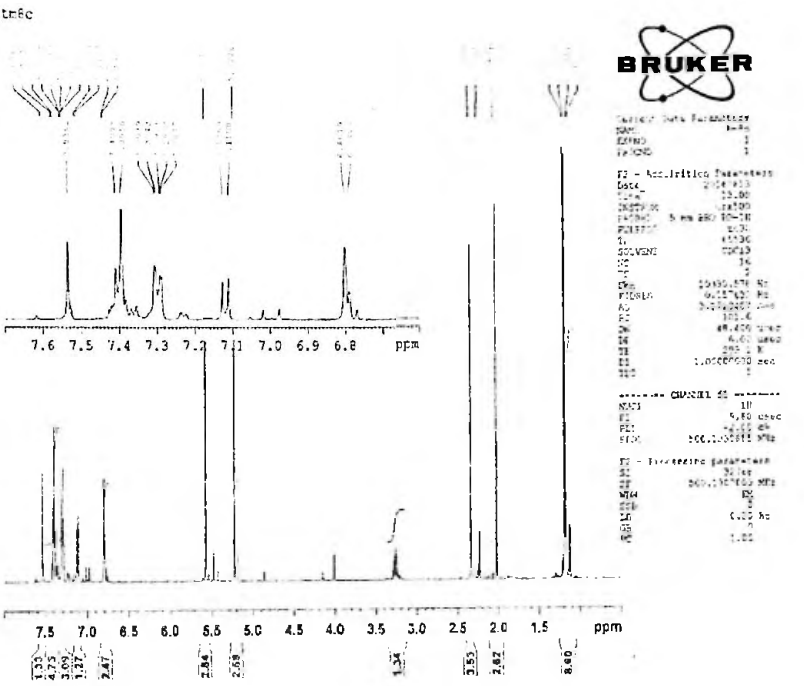

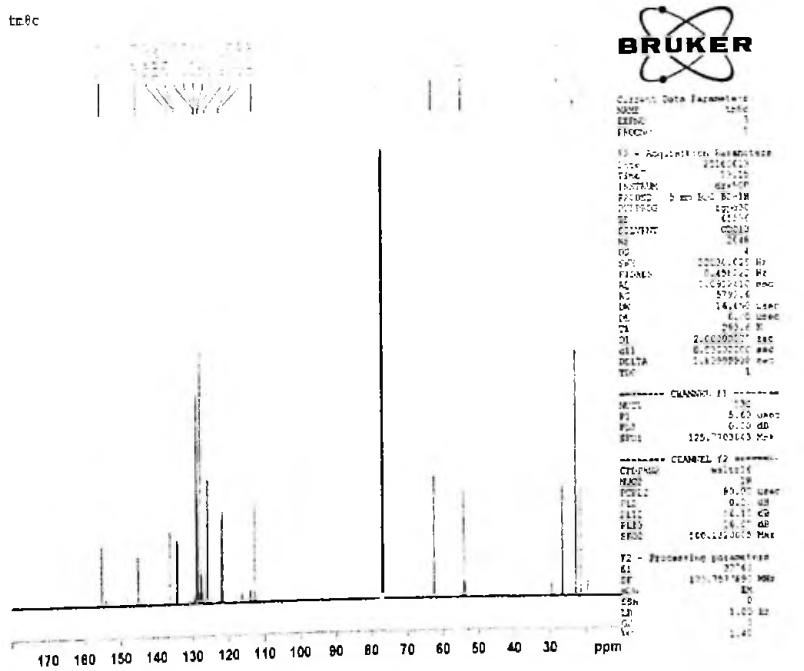

APPENDIX A-4

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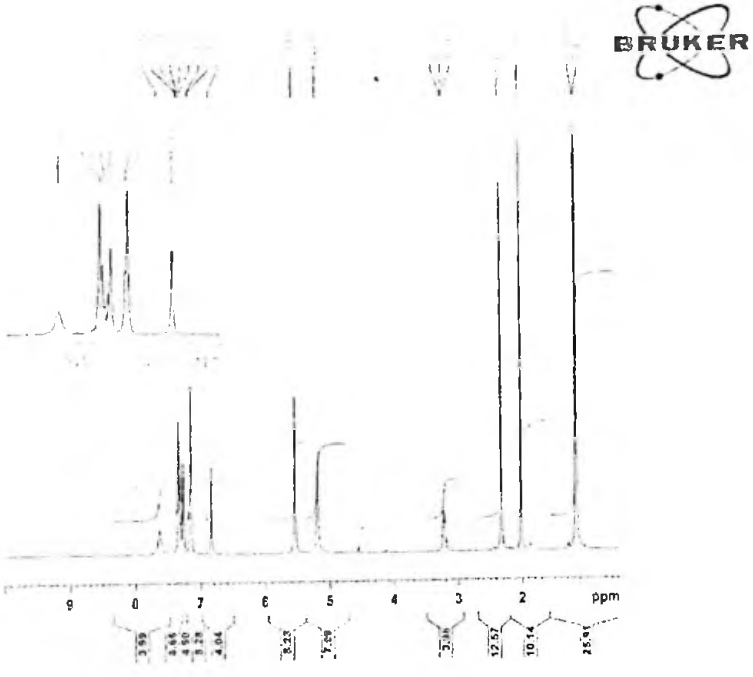

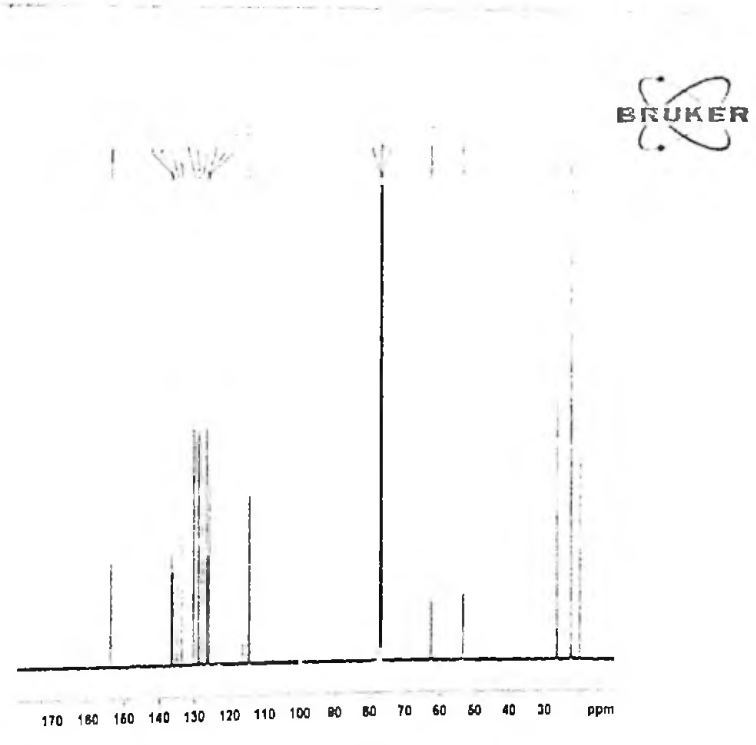

APPENDIX A-5

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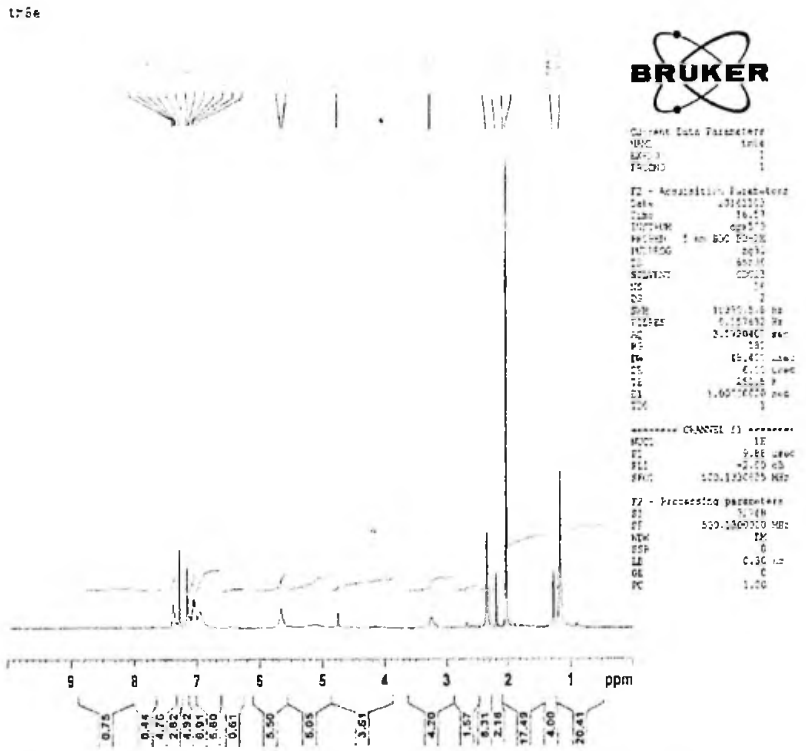
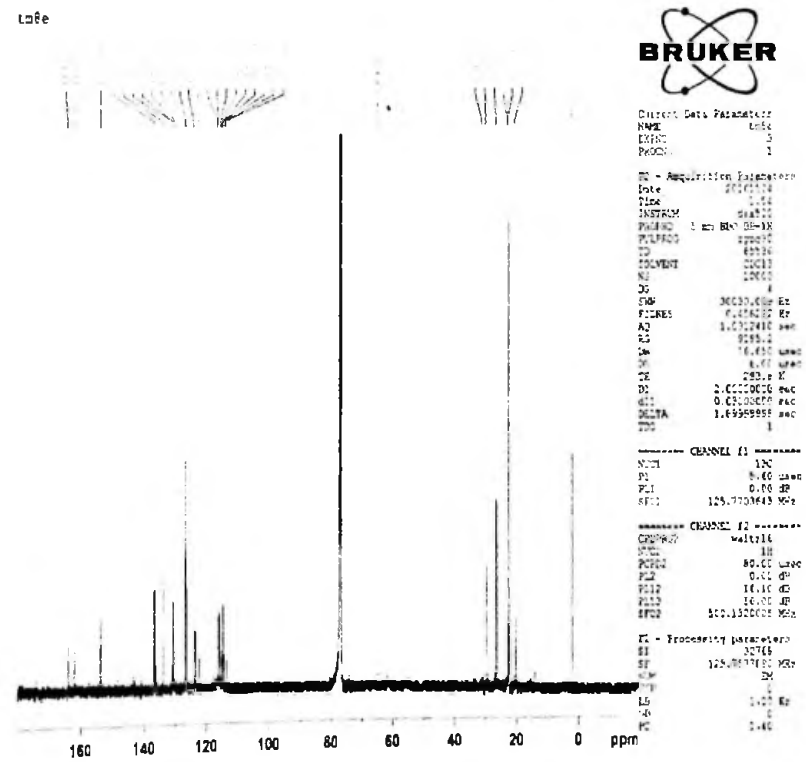
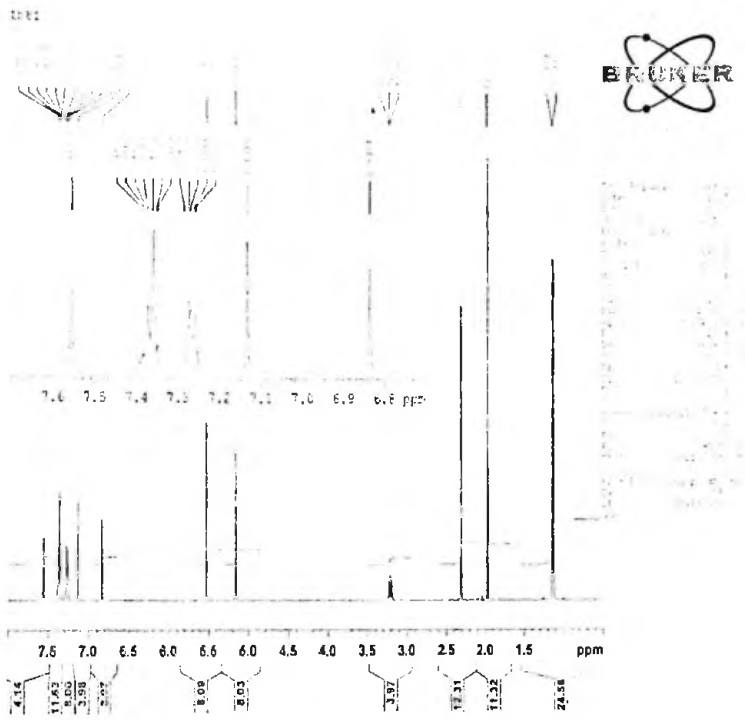
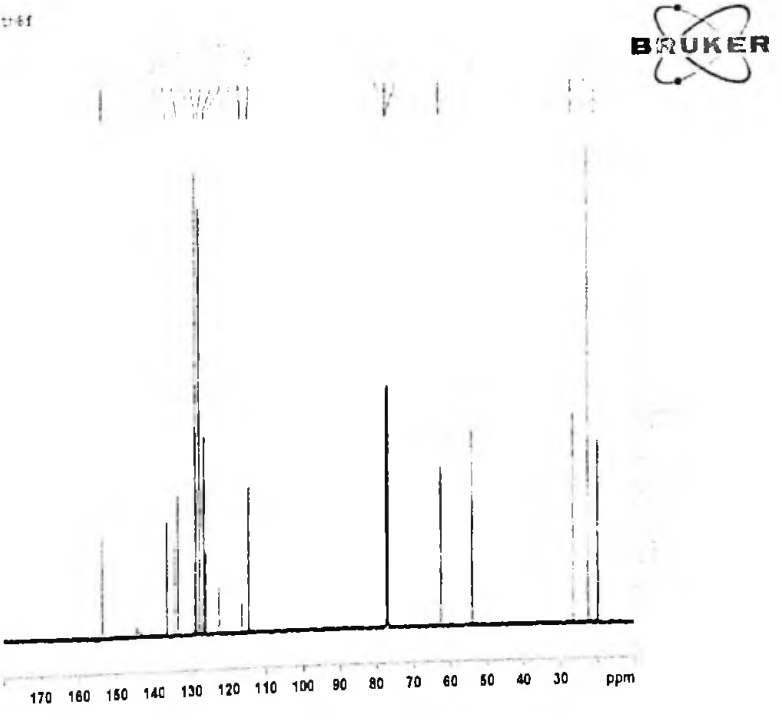




APPENDIX A-6

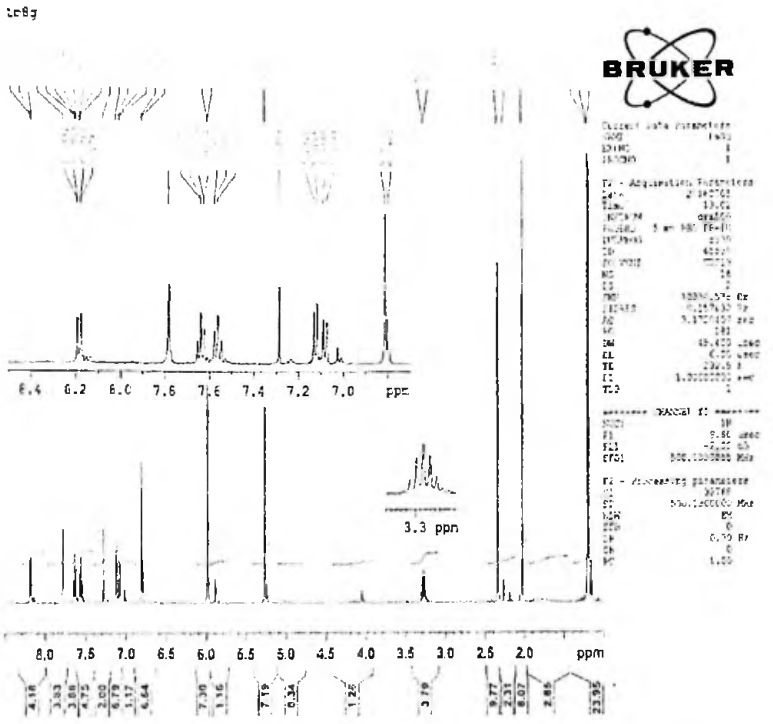
^{1}H & ^{13}C NMR FOR TM 8F

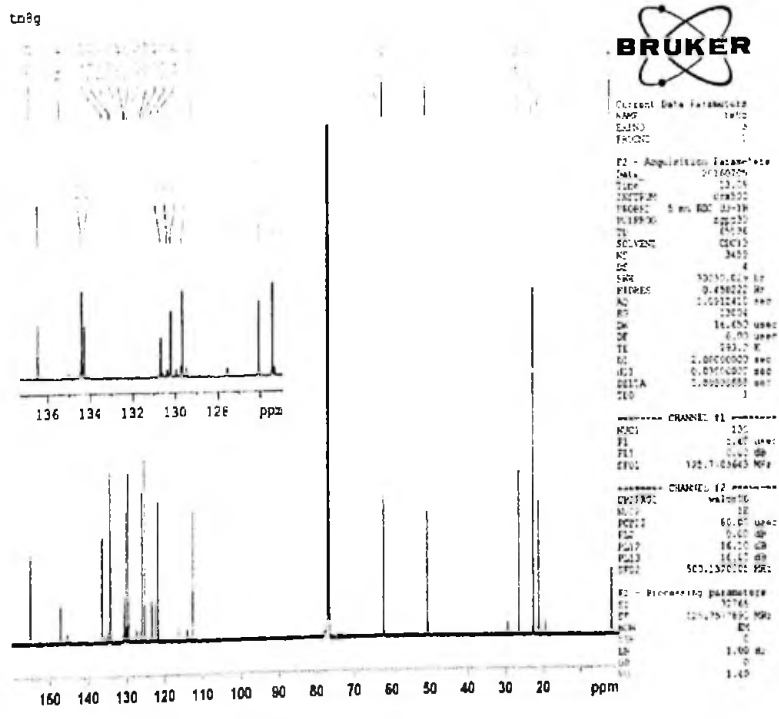




APPENDIX A-7

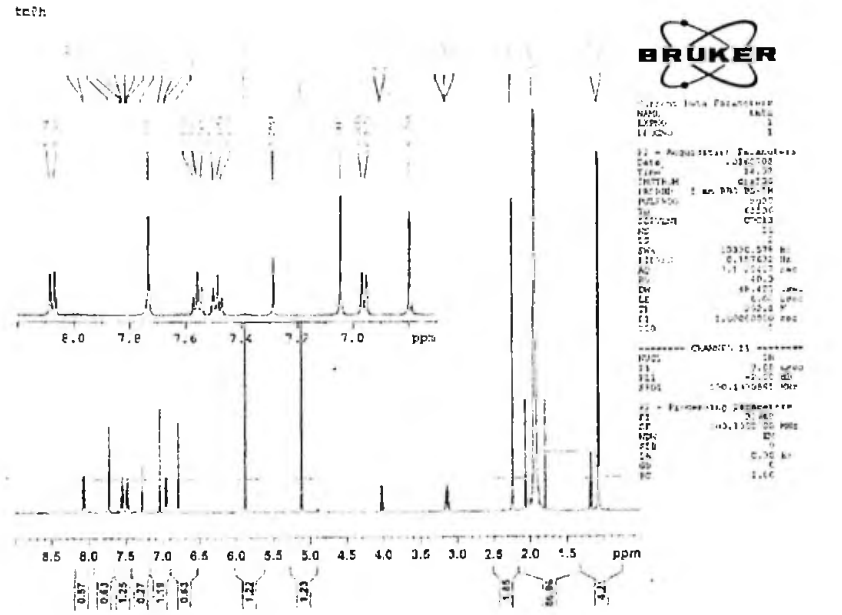
^{1}H & ^{13}C NMR FOR TM 8G

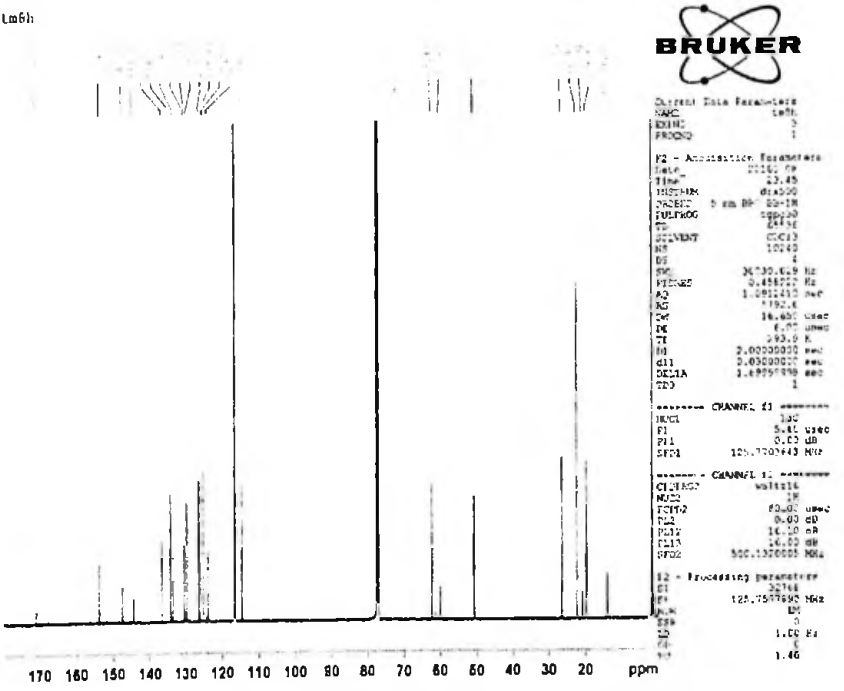




APPENDIX A-8

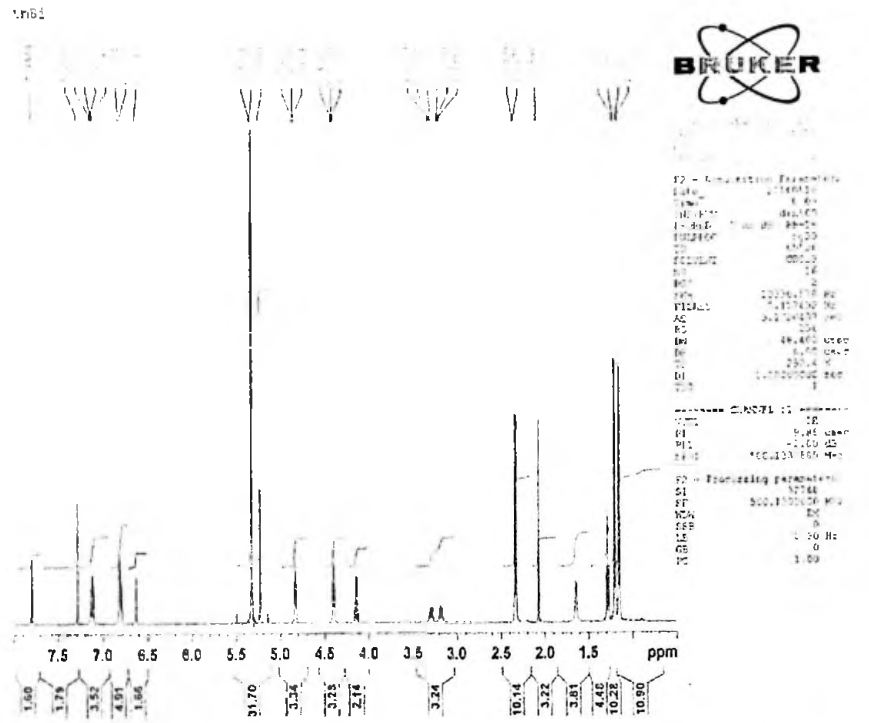
^{1}H & ^{13}C NMR FOR TM 8H

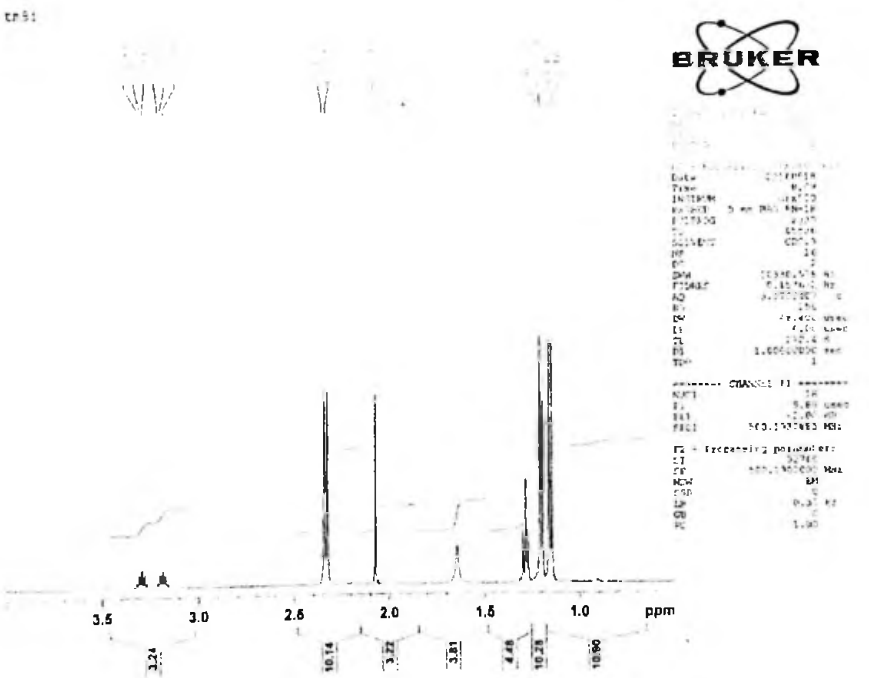




APPENDIX A-9

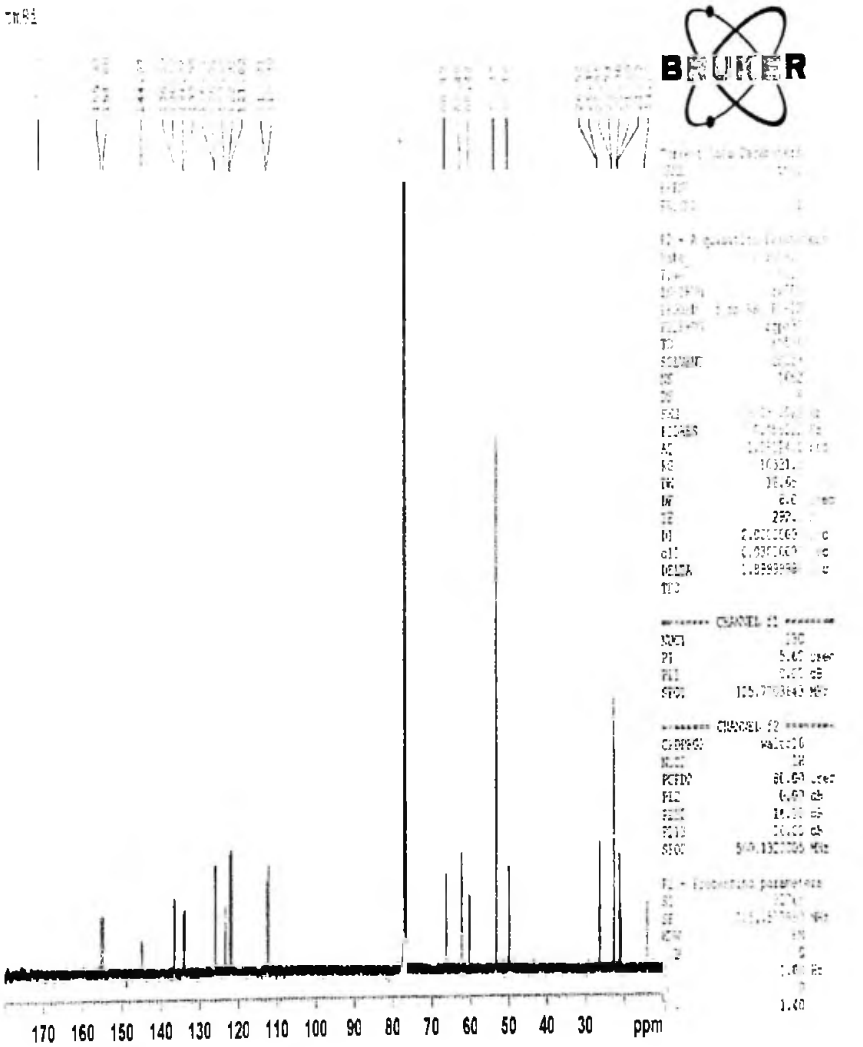
^{1}H- NMR FOR TM 8I





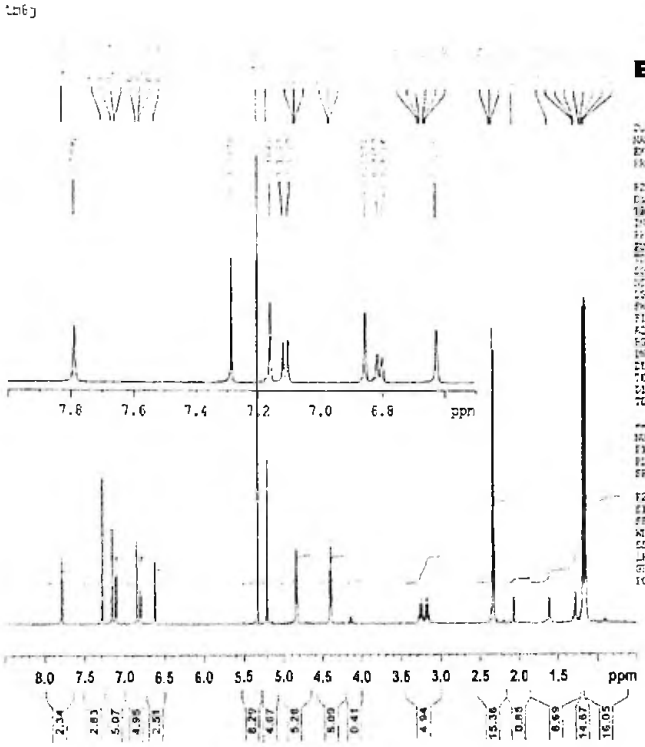
APPENDIX A-10

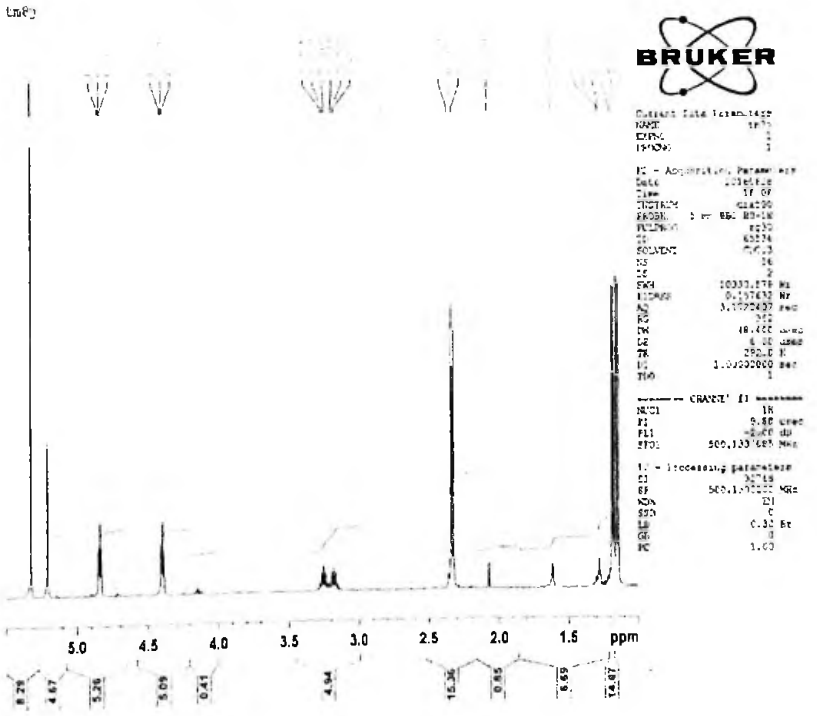
^{13}C-NMR FOR TM 8I



APPENDIX A-11

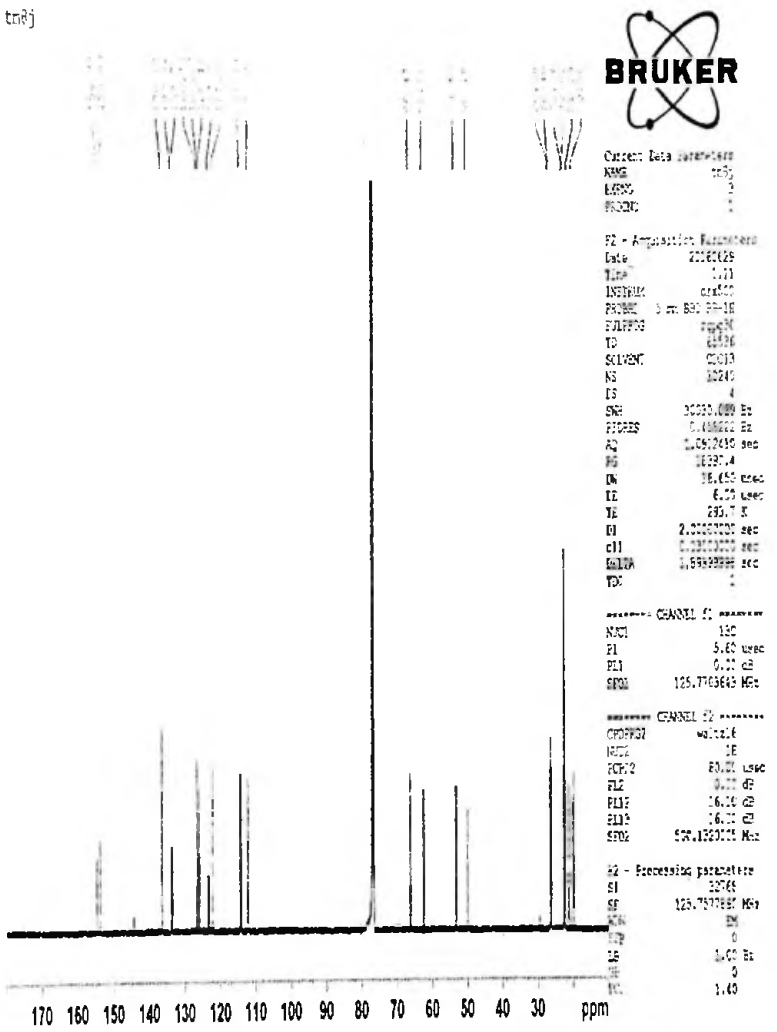
^{1}H-NMR FOR TM 8J



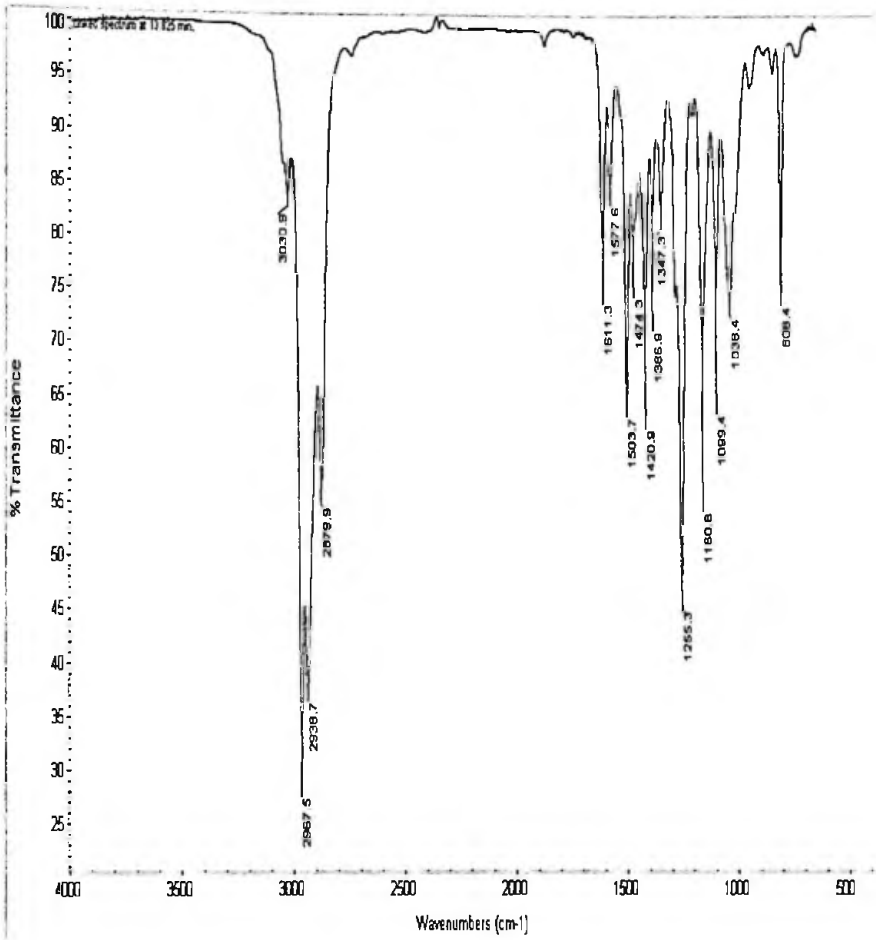


APPENDIX A-12

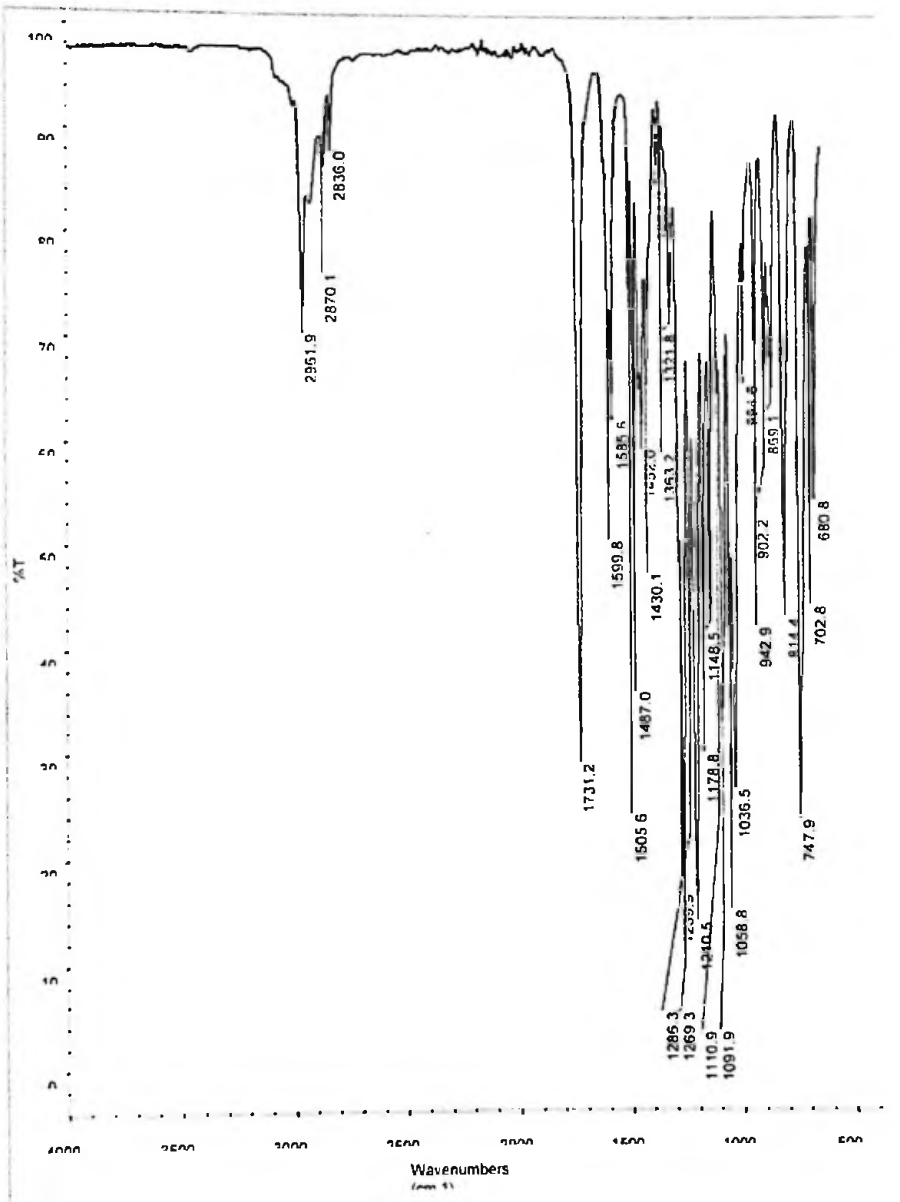
^{13}C-NMR FOR TM 8J



APPENDIX B-1
IR SPECTRUM FOR ETHER DERIVATIVES OF THYMOL

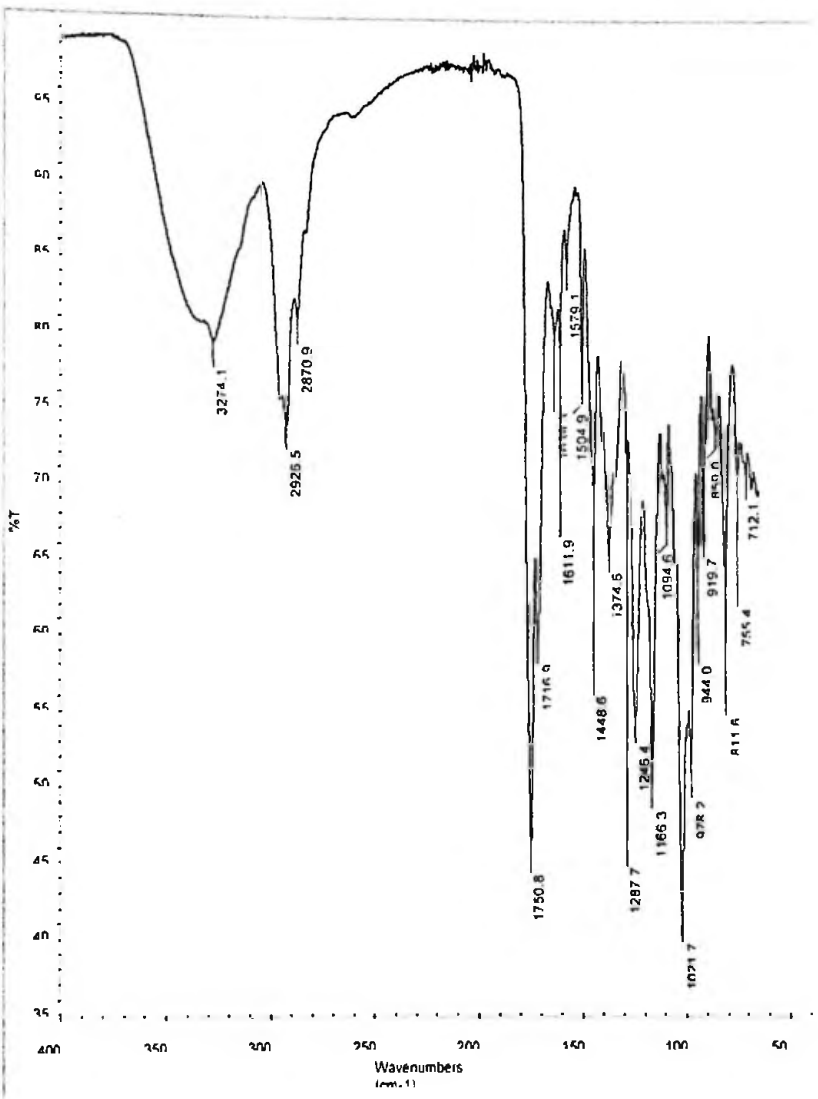


APPENDIX B-2
IR SPECTRUM FOR ESTER DERIVATIVES OF THYMOL



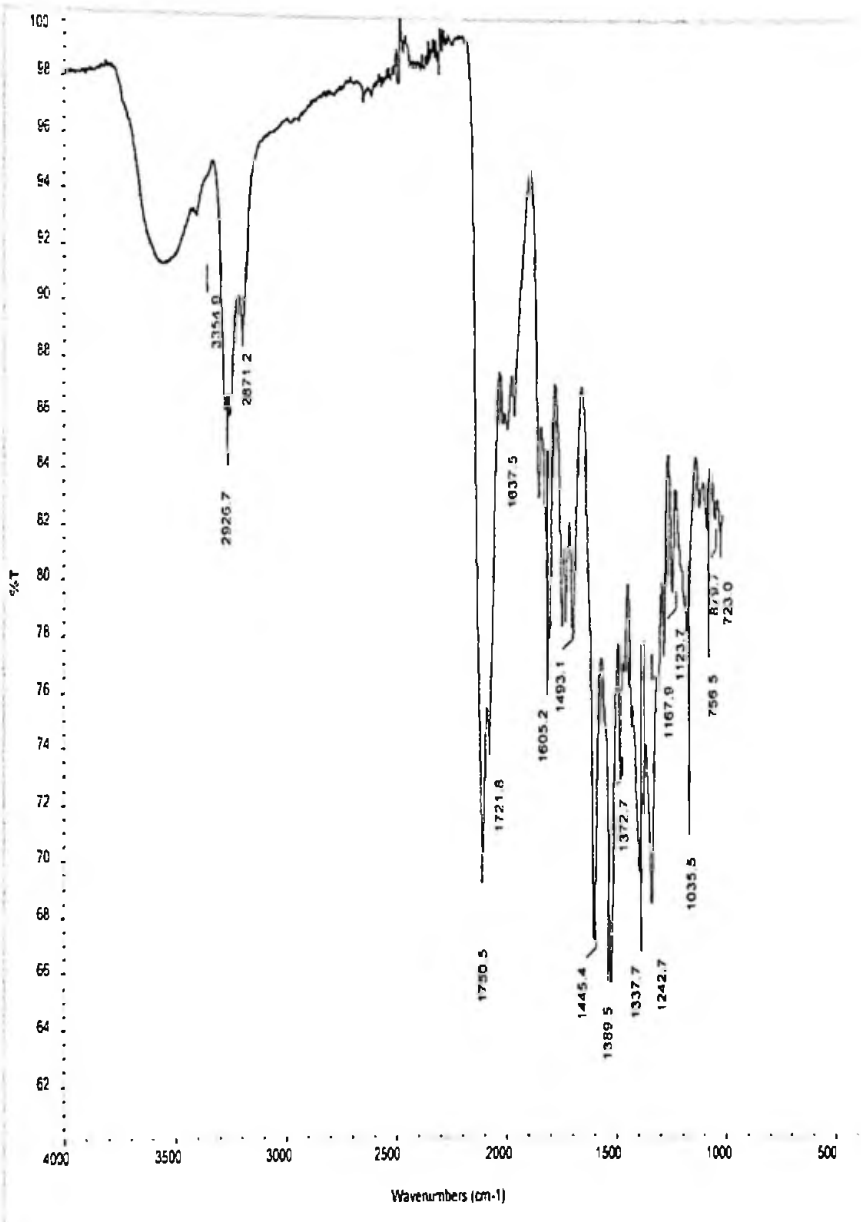
APPENDIX B-3

IR SPECTRUM FOR TM 10A



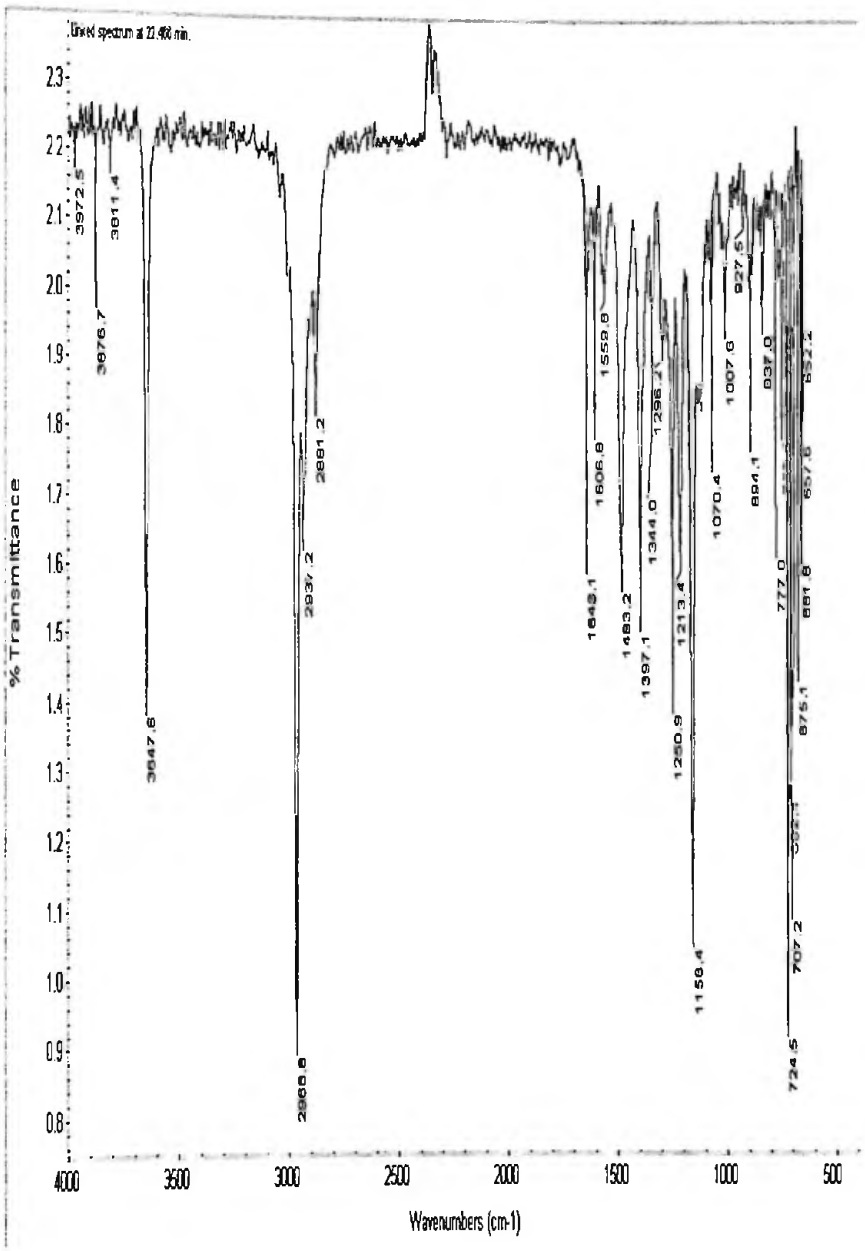
APPENDIX B-4

IR SPECTRUM FOR TM 10B



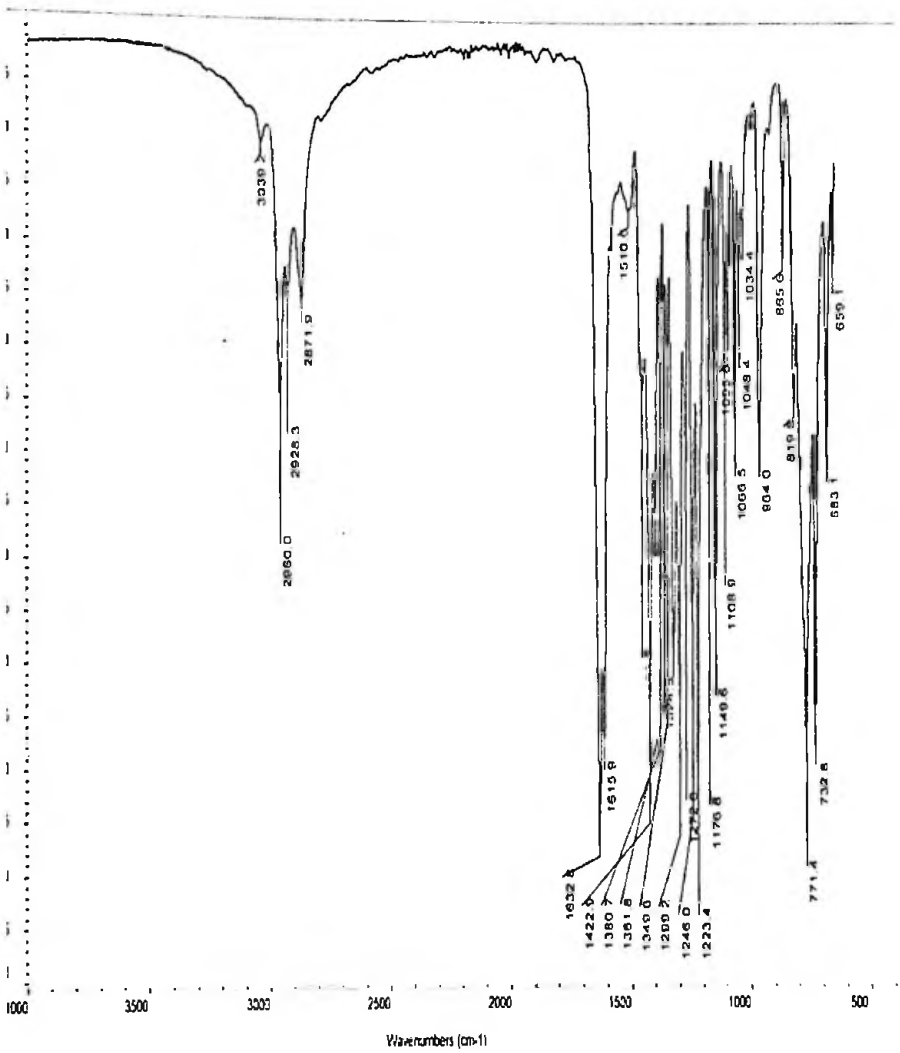
APPENDIX B-5

IR SPECTRUM FOR TM 3A



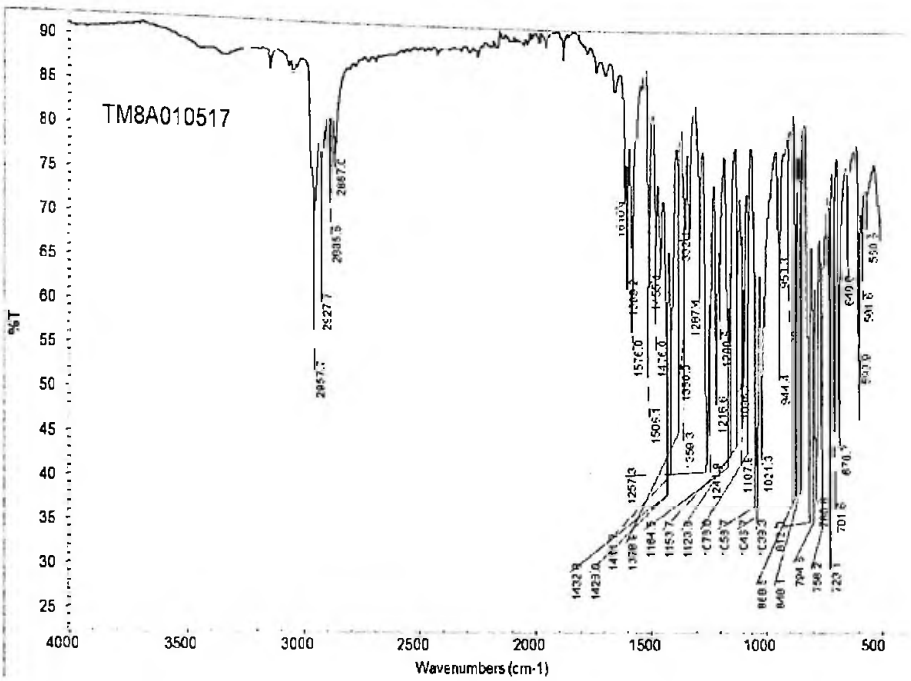
APPENDIX B-6

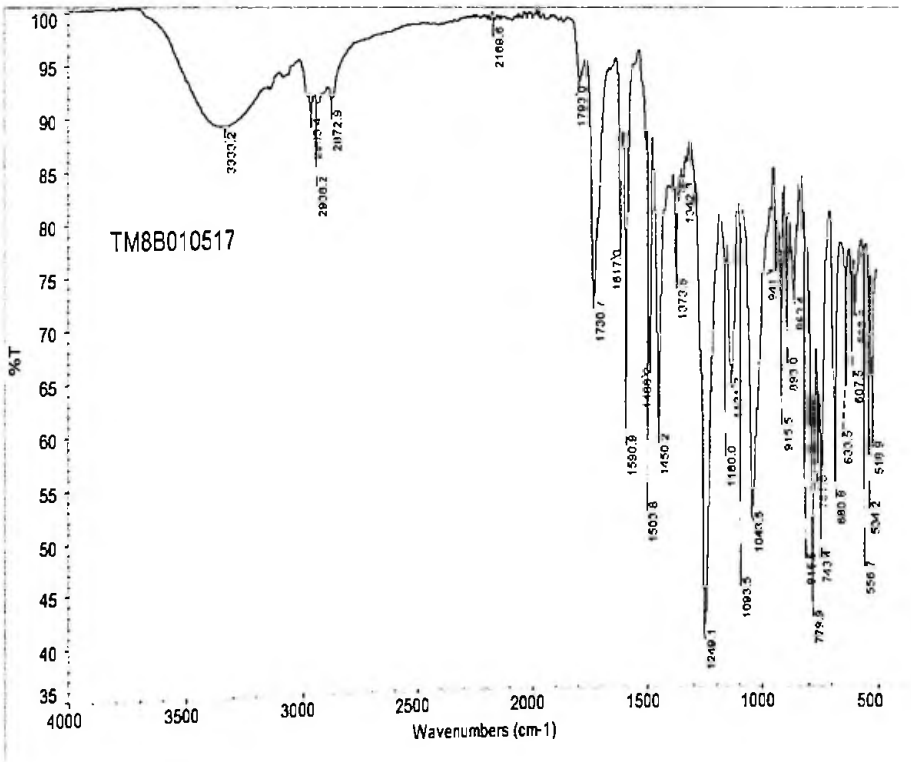
IR SPECTRUM FOR TM 3B



APPENDIX B-7

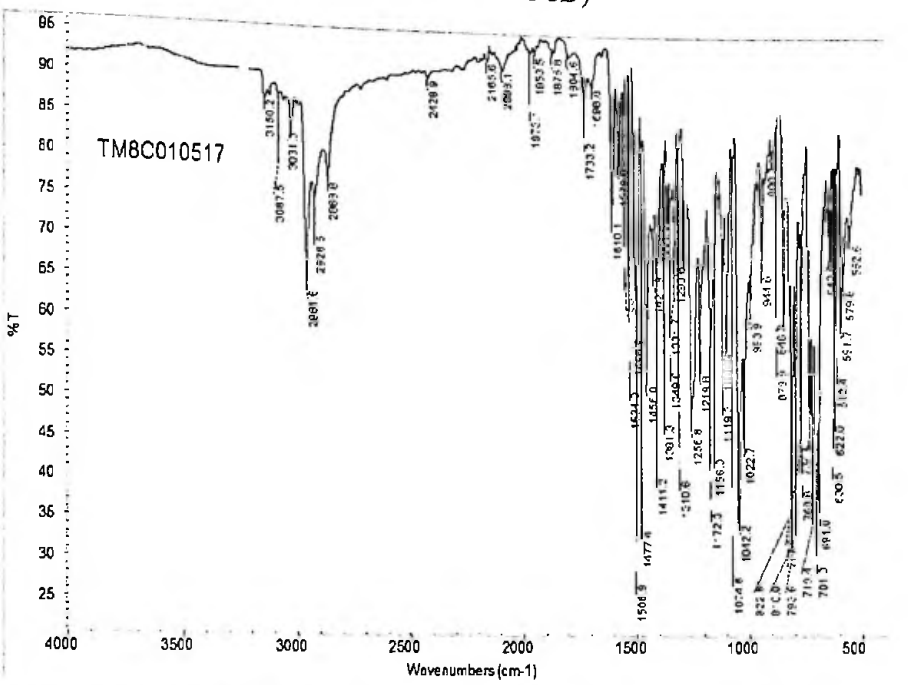
IR SPECTRUM FOR 1, 2, 3-TRIAZOLE DERIVATIVES OF THYMOL
(TM 8A & TM 8B)

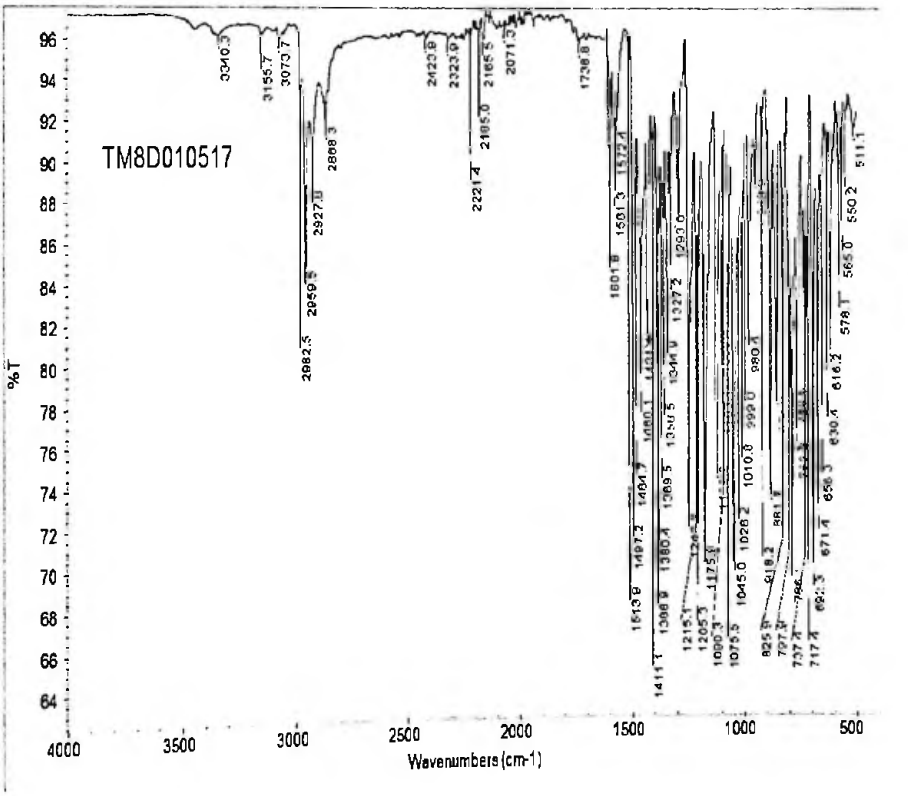




APPENDIX B-8

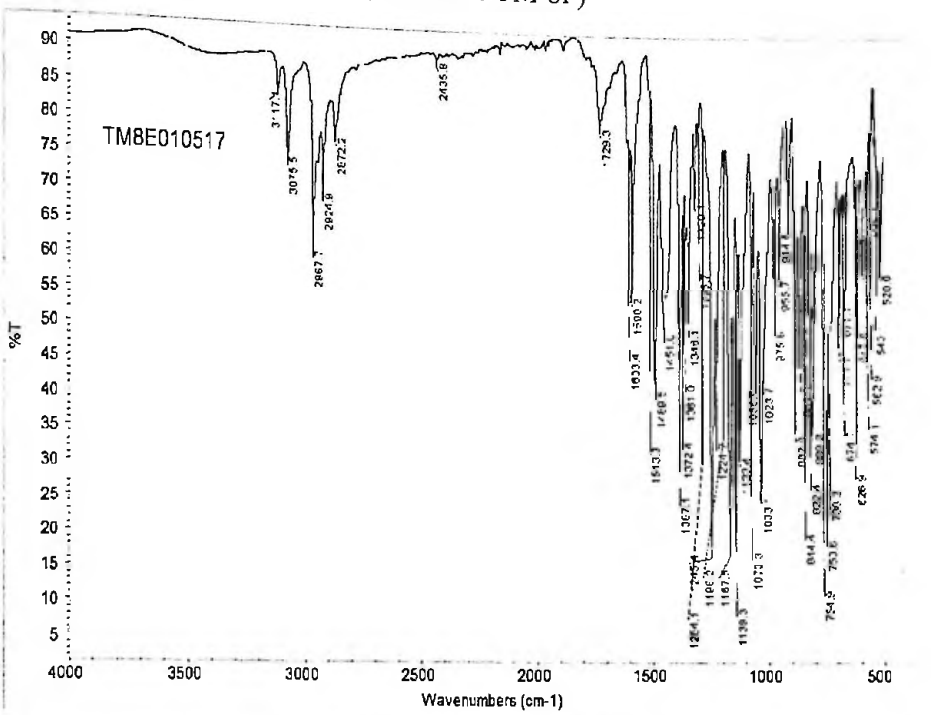
IR SPECTRUM FOR 1, 2, 3-TRIAZOLE DERIVATIVES OF THYMOL
(TM 8C & TM 8D)

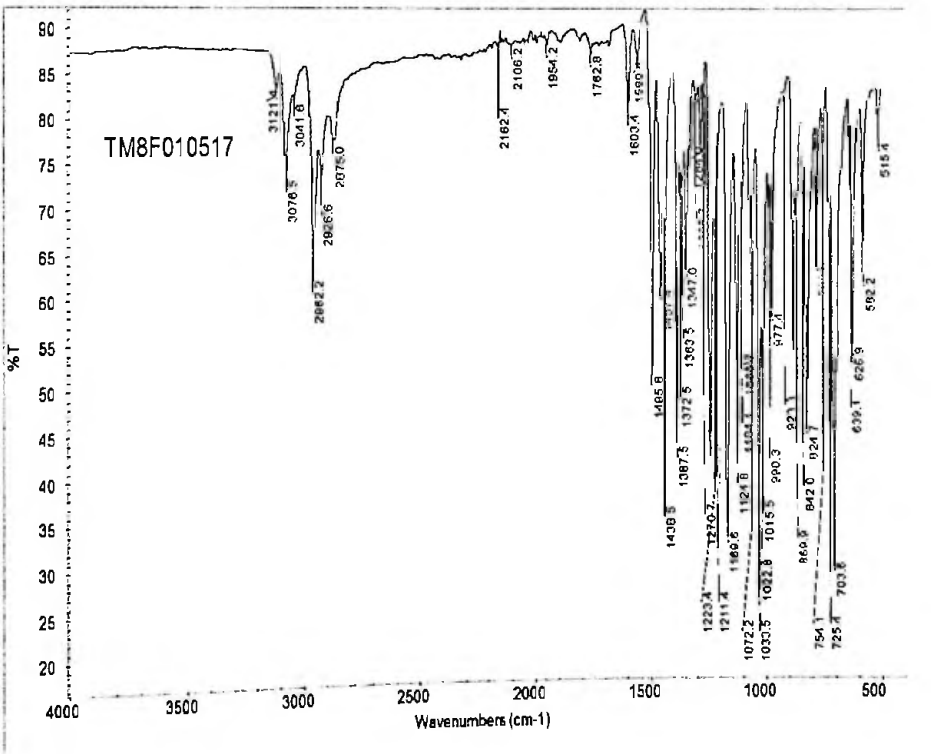




APPENDIX B-9

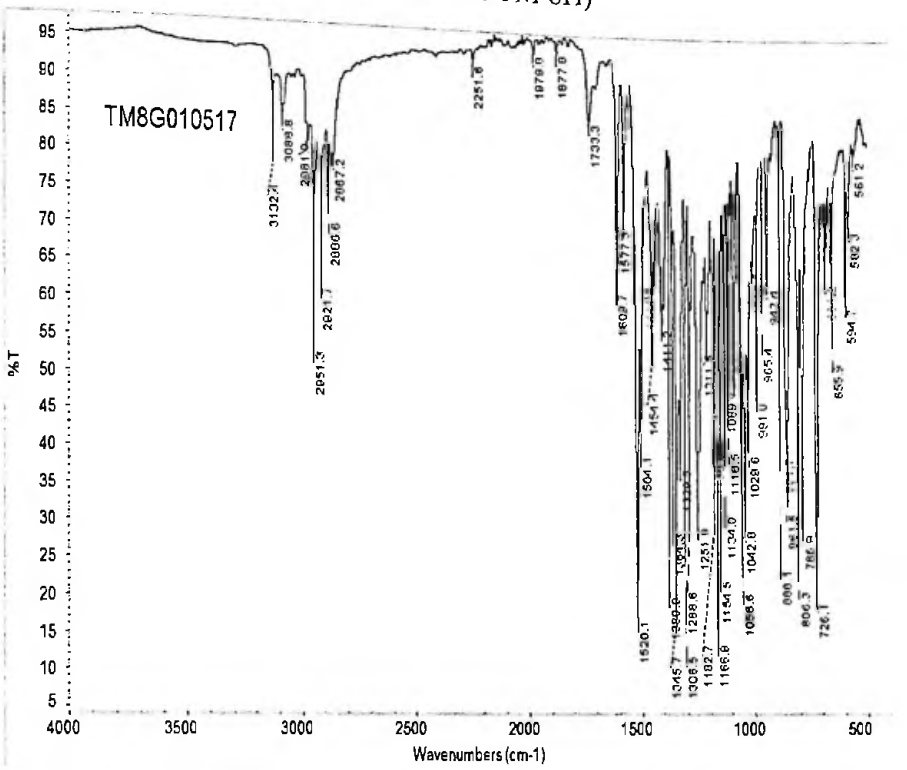
IR SPECTRUM FOR 1, 2, 3-TRIAZOLE DERIVATIVES OF THYMOL
(TM 8E & TM 8F)

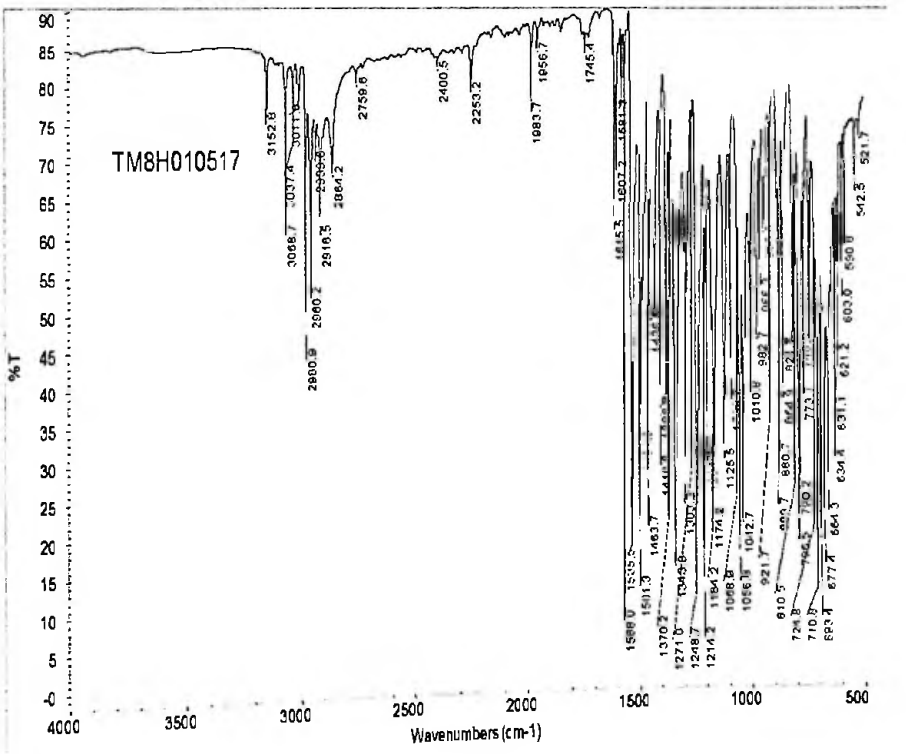




APPENDIX B-10

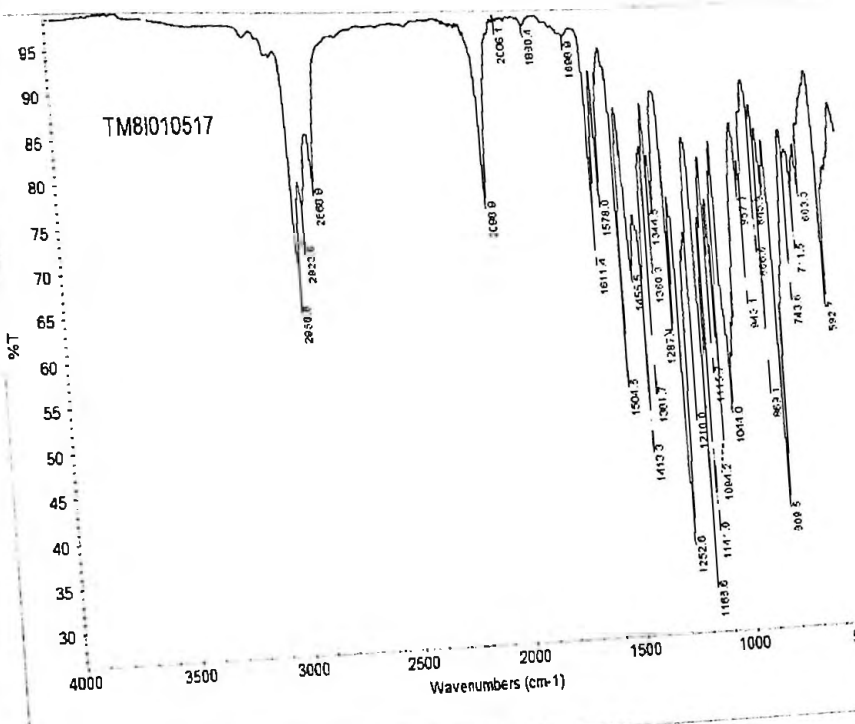
IR SPECTRUM FOR 1, 2, 3-TRIAZOLE DERIVATIVES OF THYMOL
(TM 8G & TM 8H)

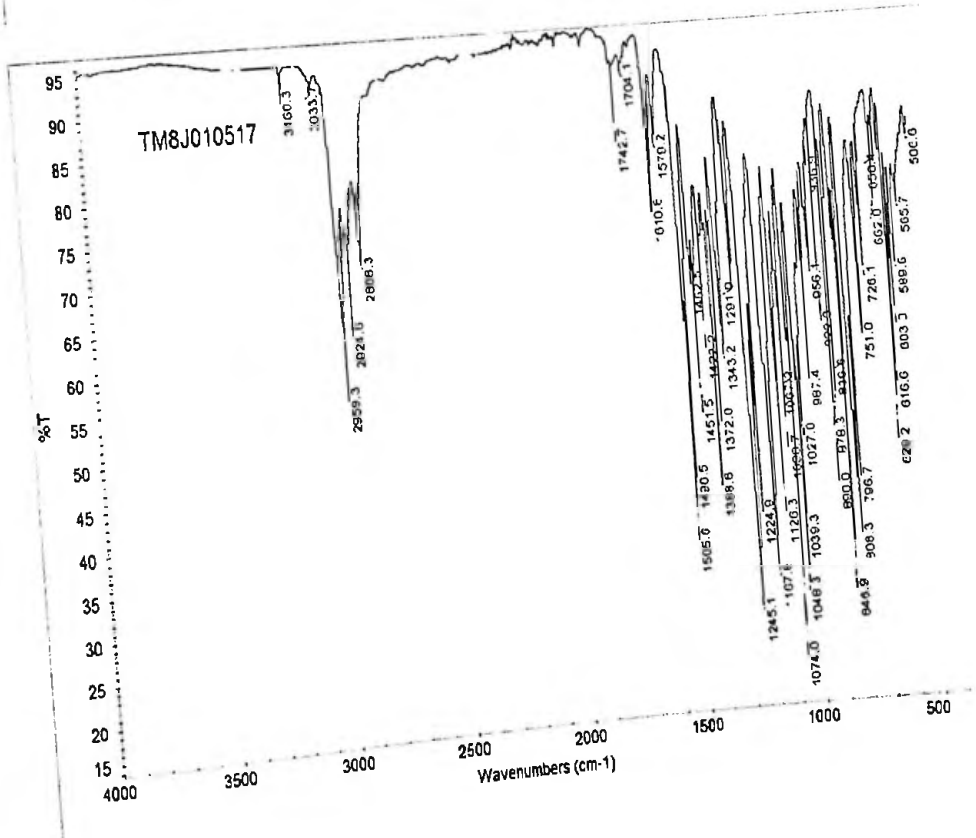




APPENDIX B-11

IR SPECTRA FOR 1, 2, 3-TRIAZOLE DERIVATIVES OF THYMOL
(TM 81 & TM 8J)

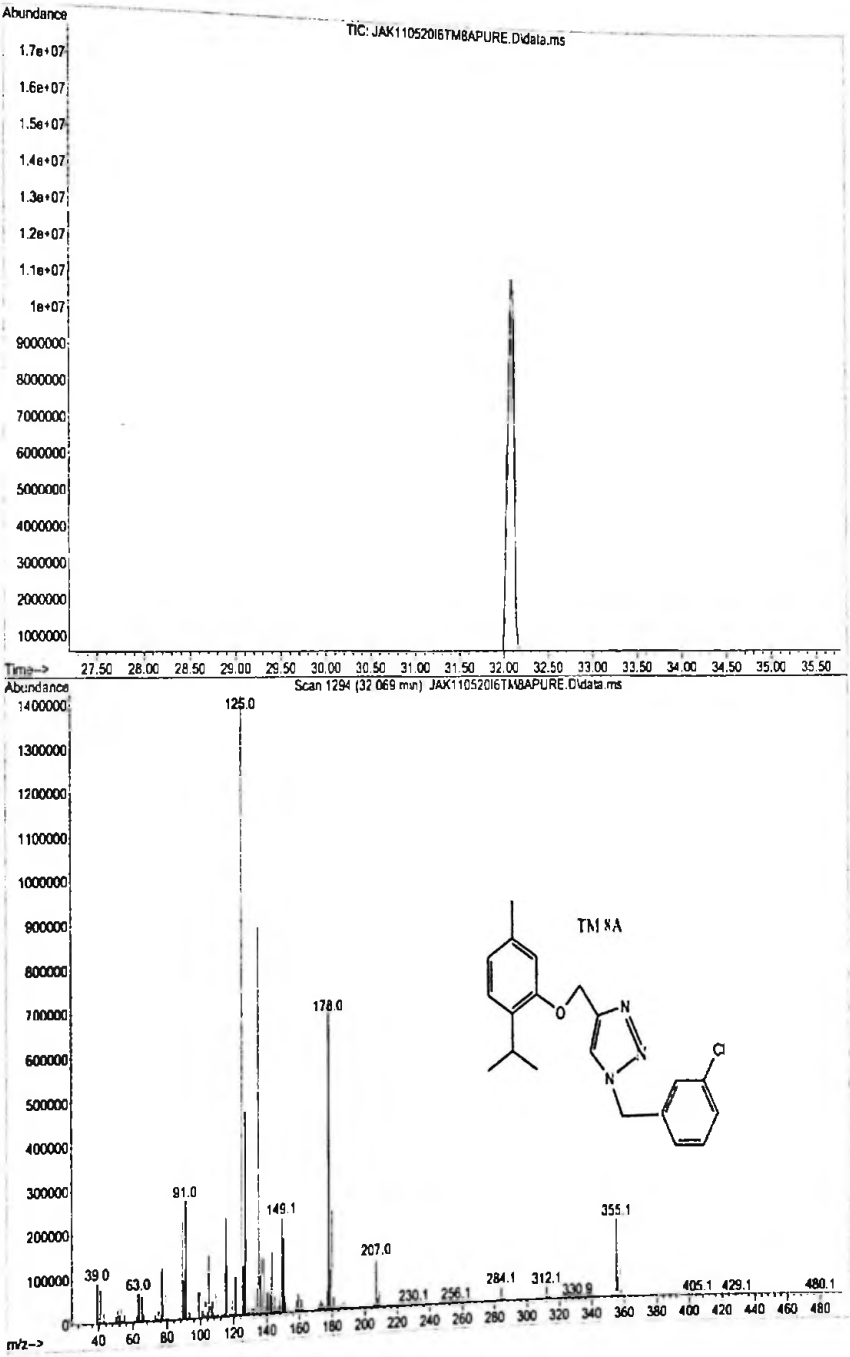




APPENDIX C-1

MASS SPECTRUM (EI) FOR TM 8A

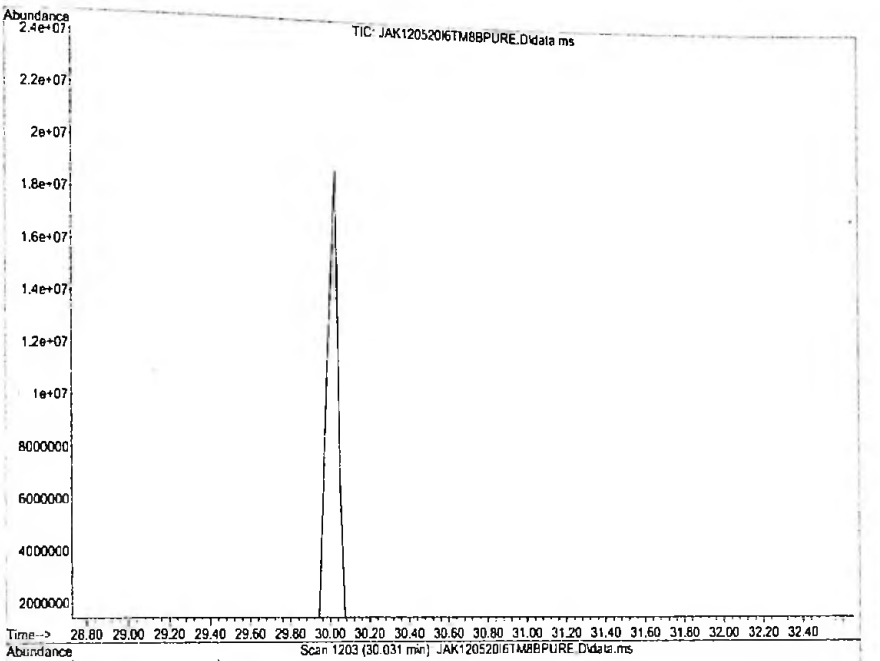
```
File       :D:\JAK02092015\JAK110520I6TM8APURE.D
Operator   : JAK
Acquired   : 12 May 2016  00:40      using AcqMethod DCM VOLATILES 35-280 XTD 40MINUTES .M
Instrument :
    ICIPE MSD
Sample Name: TM 8A
Misc Info  : TM 8A
Vial Number: 20
```

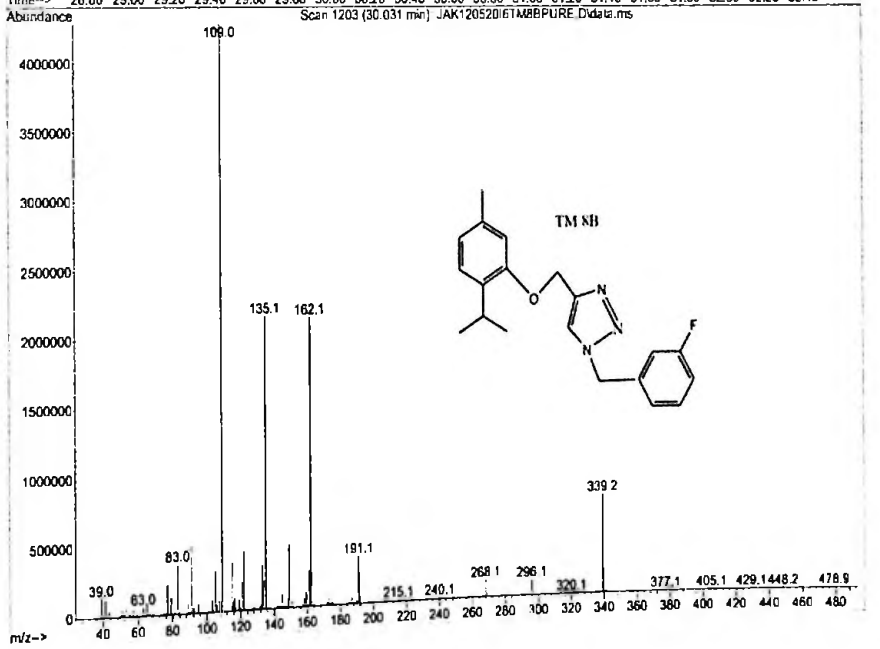


APPENDIX C-2

MASS SPECTRUM (EI) FOR TM 8B

```
File       :D:\JAK02092015\JAK120520I6TM8BPURE.D
Operator   : JAK
Acquired   : 13 May 2016   6:58      using AcqMethod DCM VOLATILES 35-280 XTD 40MINUTES .M
Instrument :    ICIFE MSD
Sample Name: TM 8B PURE
Misc Info  : TM 8B PURE
Vial Number: 20
```

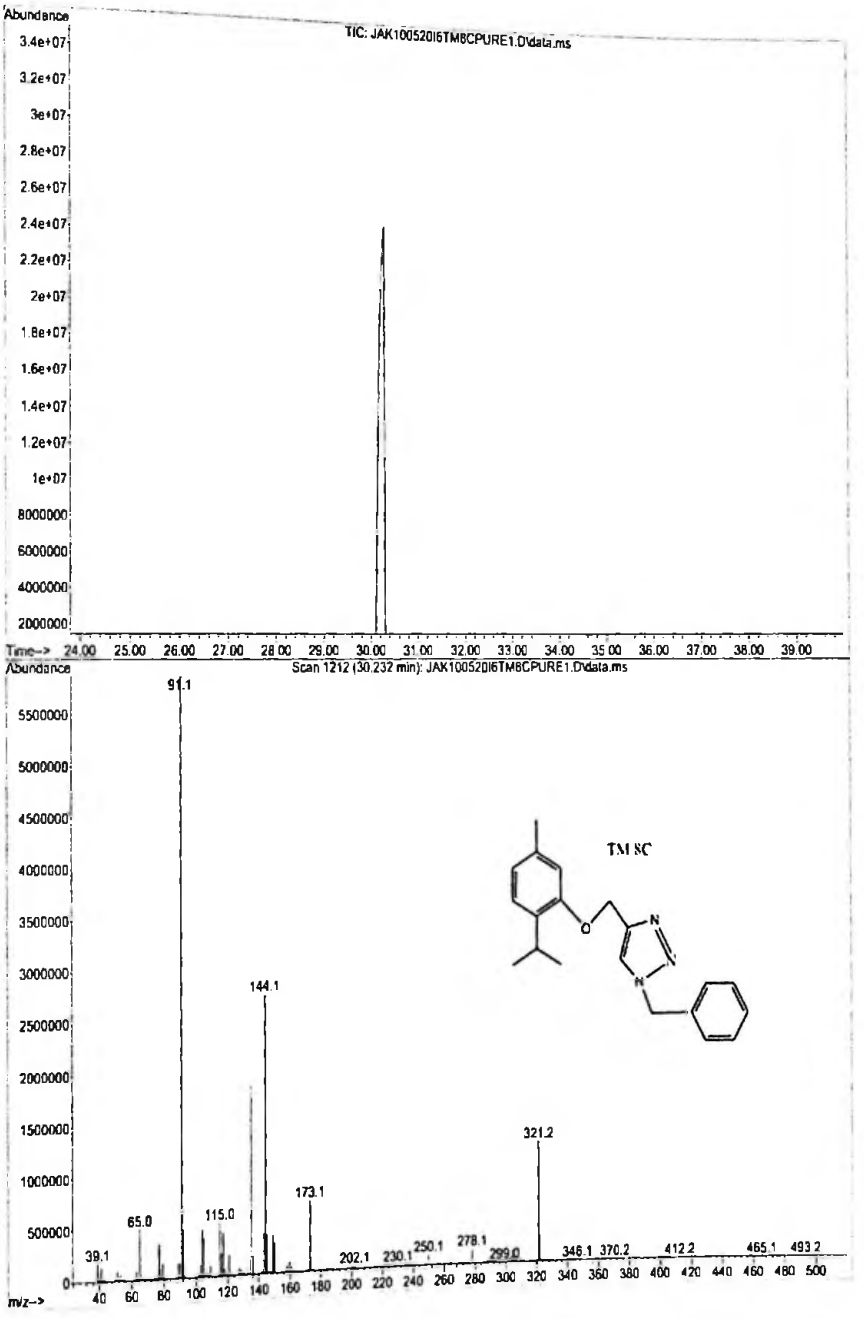




APPENDIX C-3

MASS SPECTRUM (EI) FOR TM 8C

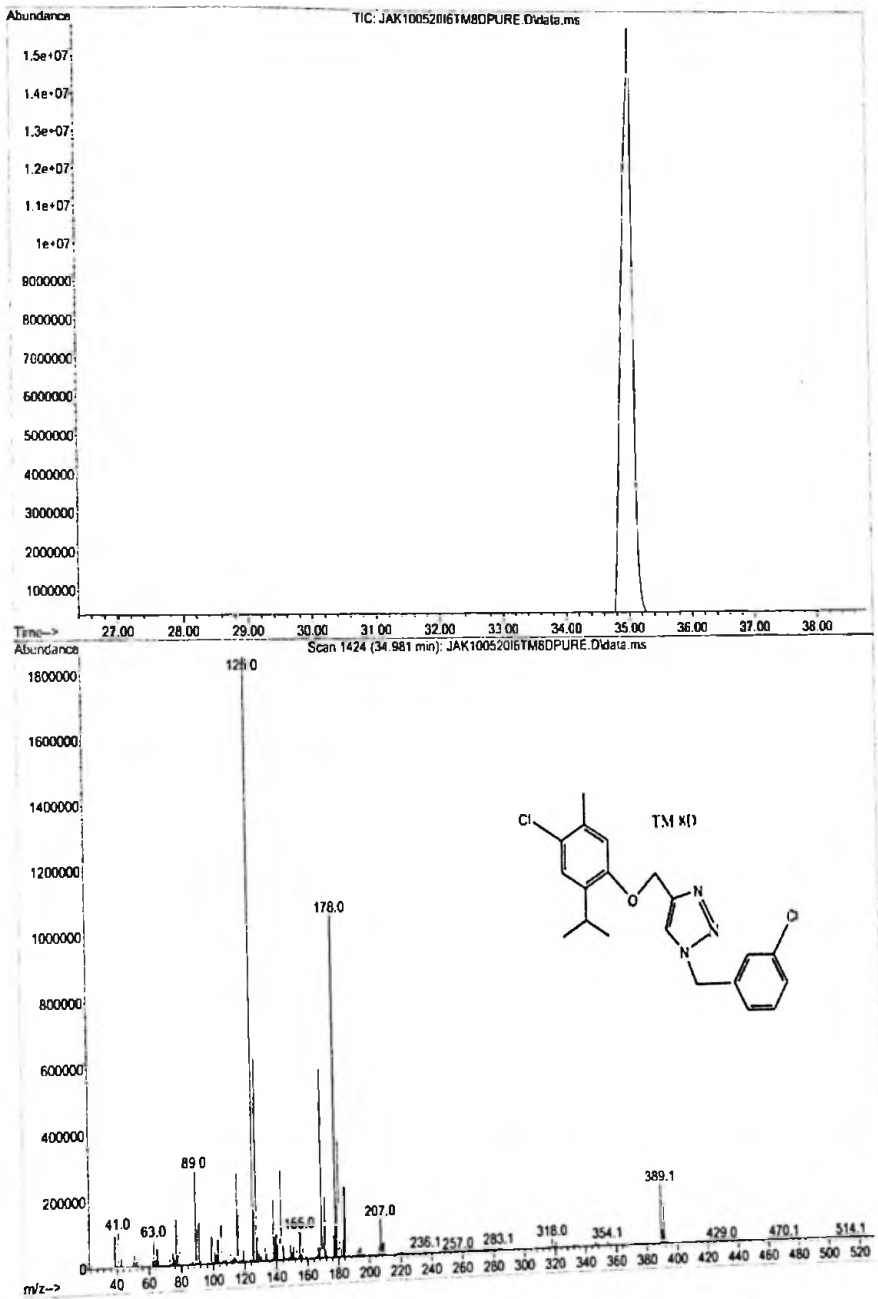
```
File       :D:\JAKC2092015\JAK10052016TM8CPURE1.D
Operator   : JAK
Acquired   : 11 May 2016  00:33      using AcqMethod DCM VOLATILES 35-280 STD 40MINUTES .M
Instrument :    1CIPE MSD
Sample Name: 1M 8C
Misc Info  : TM 8C
Vial Number: 20
```



APPENDIX C-4

MASS SPECTRUM (EI) FOR TM 8D

```
File       :D:\JAK02092015\JAK10052016TM8DPURE.D
Operator   : JAK
Acquired   : 11 May 2016   6:23      using AcqMethod DCM VOLACTLES 35-280 XTD 40MINUTES .M
Instrument :    1CIPE MSD
Sample Name: TM 8D PURE
Misc Info  : TM 8D PURE
Vial Number: 20
```



APPENDIX C-5

MASS SPECTRUM (EI) FOR TM 8E

```
File       :D:\JAK02092015\JAK120520I6TM8E.D
Operator   : JAK
Acquired   : 15 May 2016  00:12      using AcqMethod ECM VOLATILES 35-280 XCD 40MINUTES .M
Instrument :     HCIPE MSD
Sample Name: TM 8E
Misc Info  : TM 8E
Vial Number: 20
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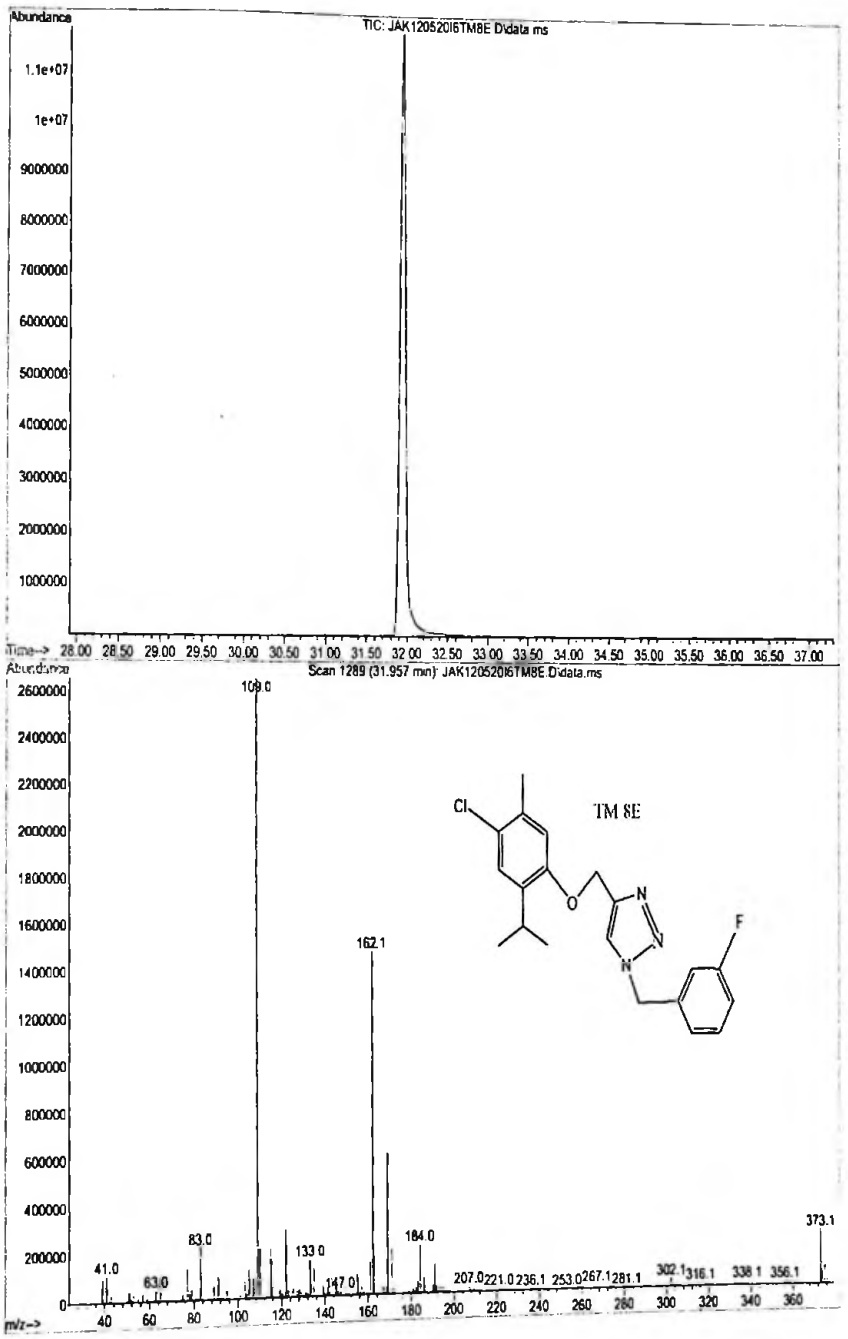
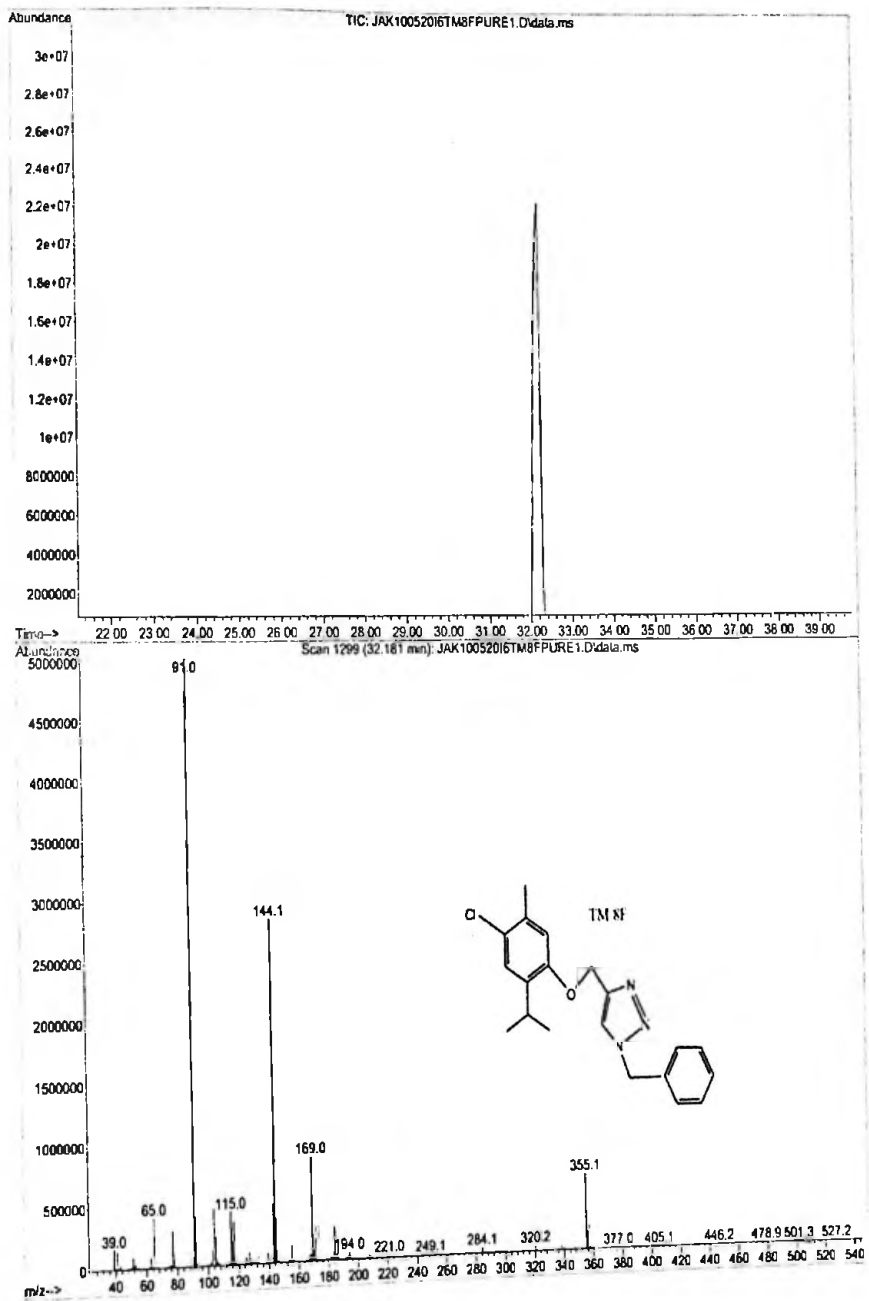


APPENDIX C-6

MASS SPECTRUM (EI) FOR TM 8F

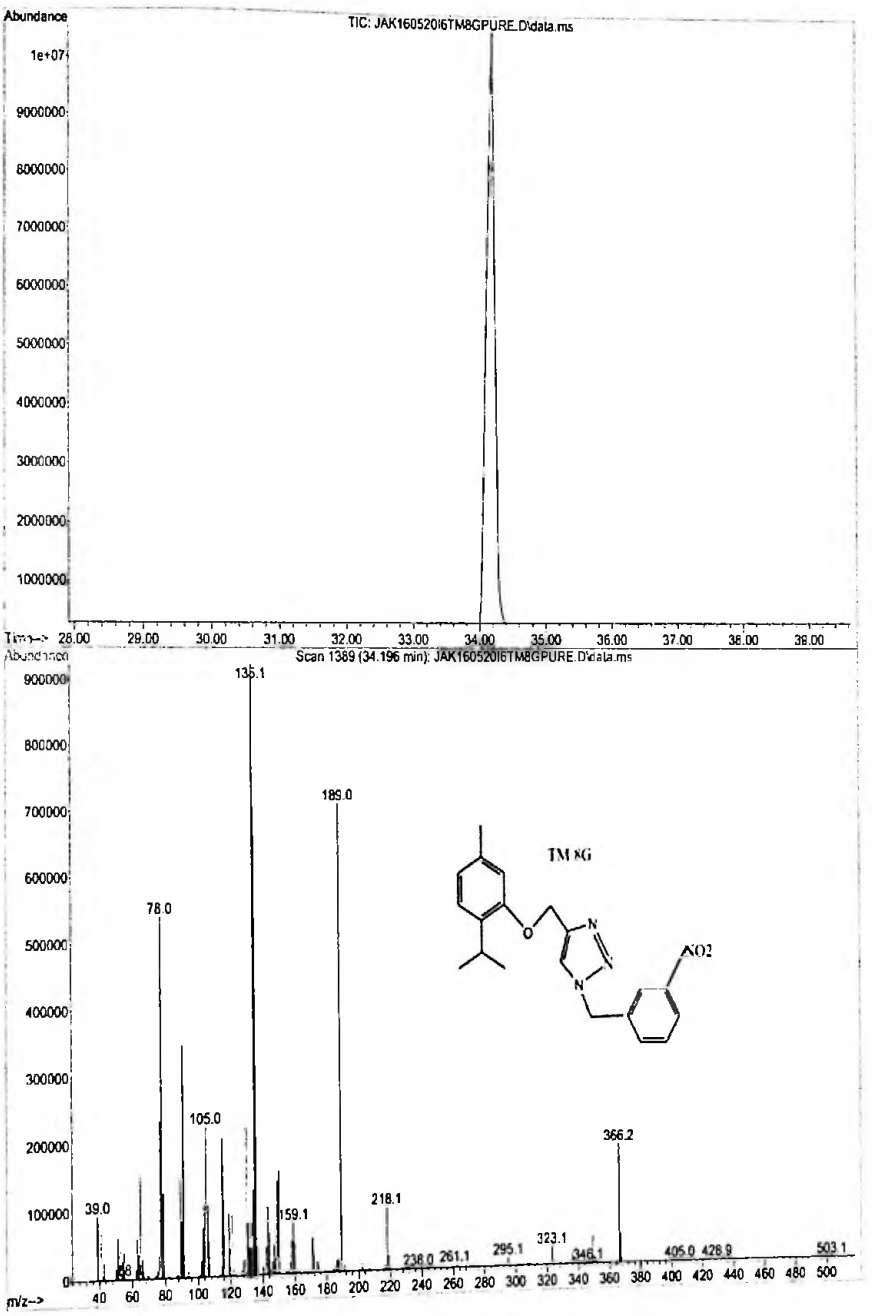
```
File       :D:\JAK02092015\JAK10052016TM8FPURE1.D
Operator   : JAK
Acquired   : 10 May 2016  20:13      using AcqMethod DCM VOLATILES 35-280 XTD 40MINUTES .M
Instrument :    1CIPE MSD
Sample Name: TM 8F PURE
Misc Info  : TM 8F PURE
Vial Number: 25
```



APPENDIX C-7

MASS SPECTRUM (EI) FOR TM 8G

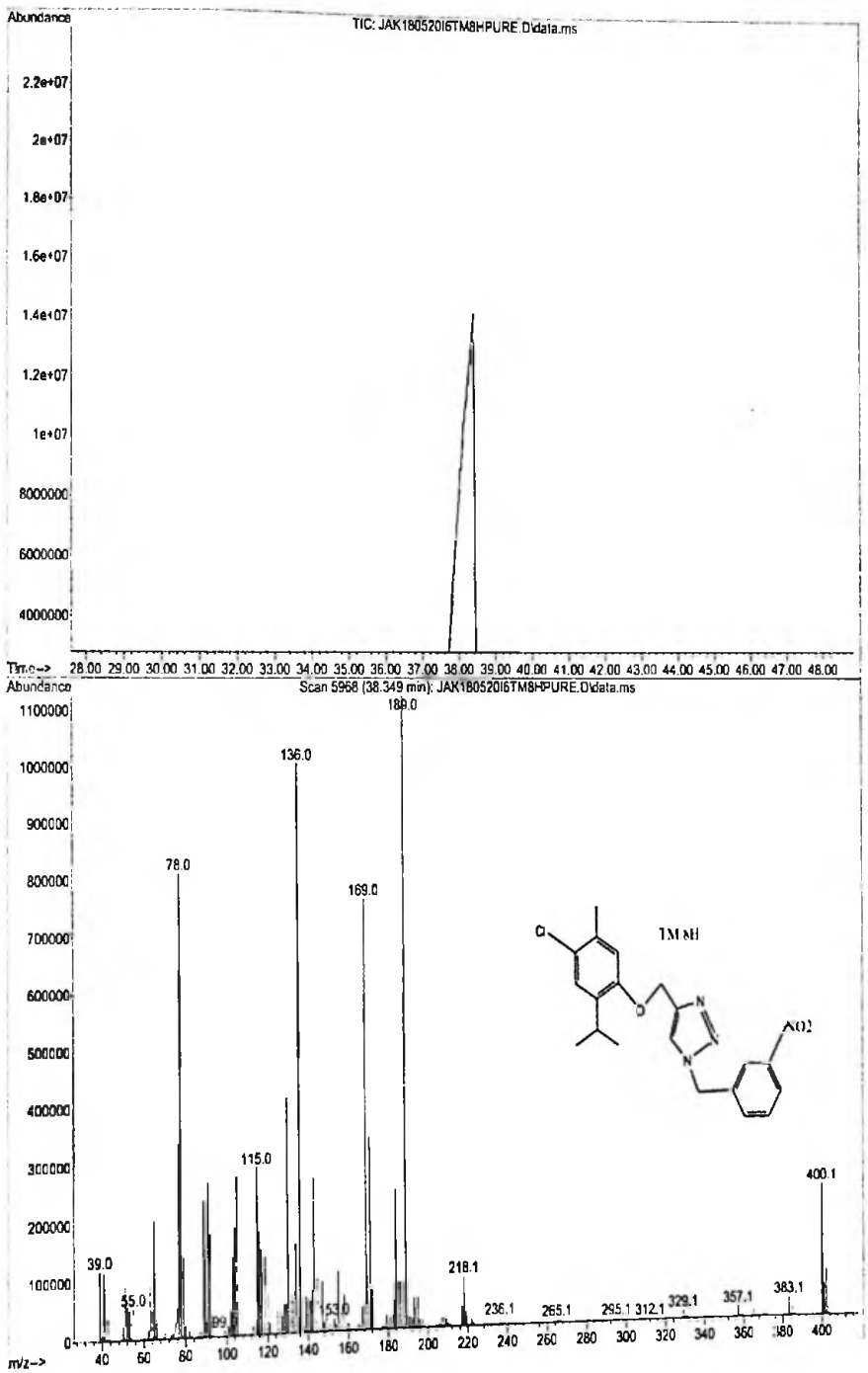
```
File       :D:\JAK02092015\JAK16052016TM8GPURE.D
Operator   : JAK
Acquired   : 16 May 2016   2:36      using AcqMethod DCM VOLATILES 35-280 XTD 40MINUTES .M
Instrument :    1CIPE MSD
Sample Name: TM 8G PURE
Misc Info  : TM 8G PURE
Vial Number: 20
```



APPENDIX C-8

MASS SPECTRUM (EI) FOR TM 8H

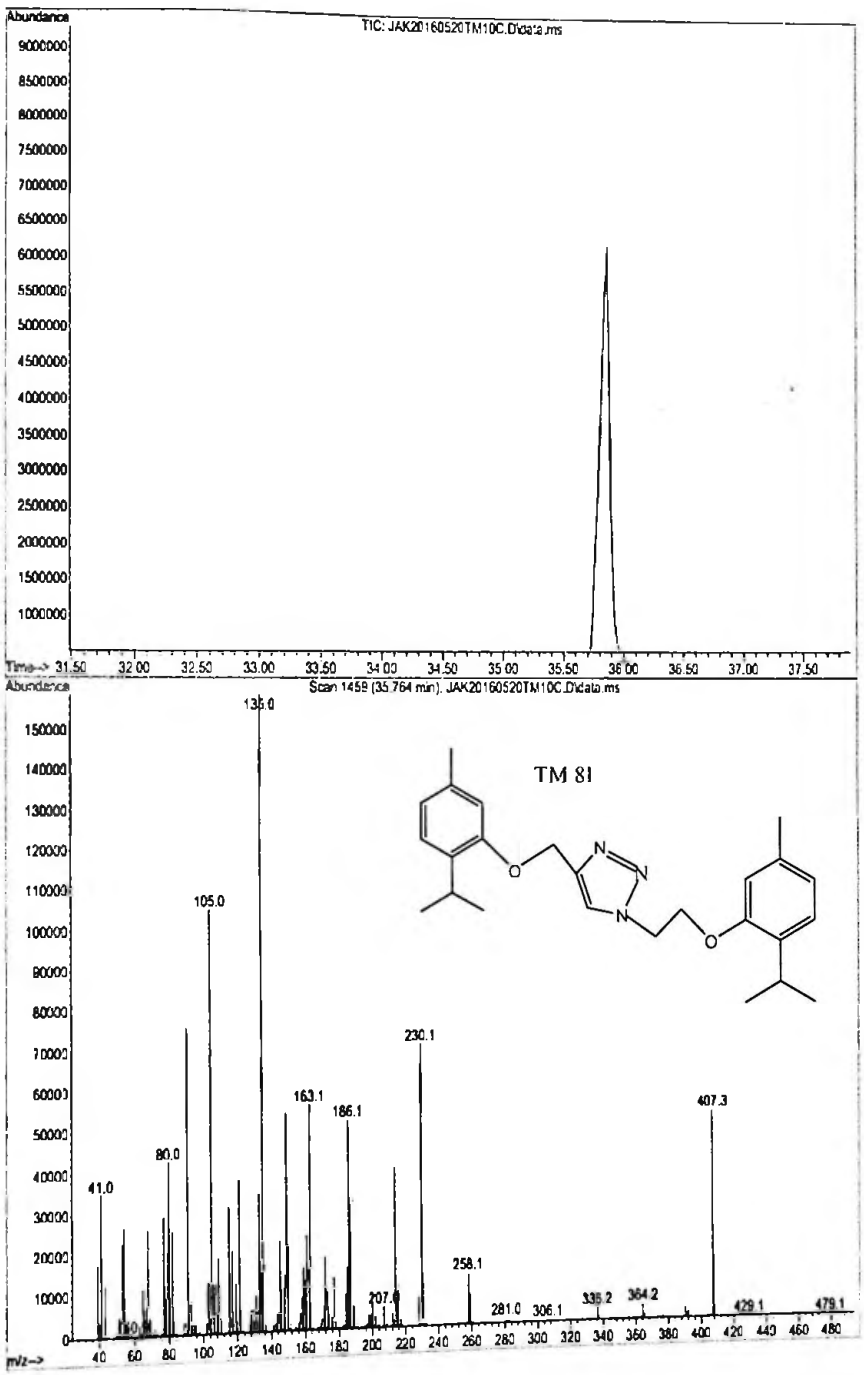
```
File       :D:\JAK02092015\JAK180520I6TM8HPURE.D
Operator   : JAK
Acquired   : 18 May 2016  12:11      using AcqMethod DCM VOLATILES 35-280 XTD 50MINUTES .M
Instrument :    IC1PE MSD
Sample Name: TM 8H PURE
Misc Info  : TM 8H PURE
Vial Number: 20
```



APPENDIX C-9

MASS SPECTRUM (EI) FOR TM 8I

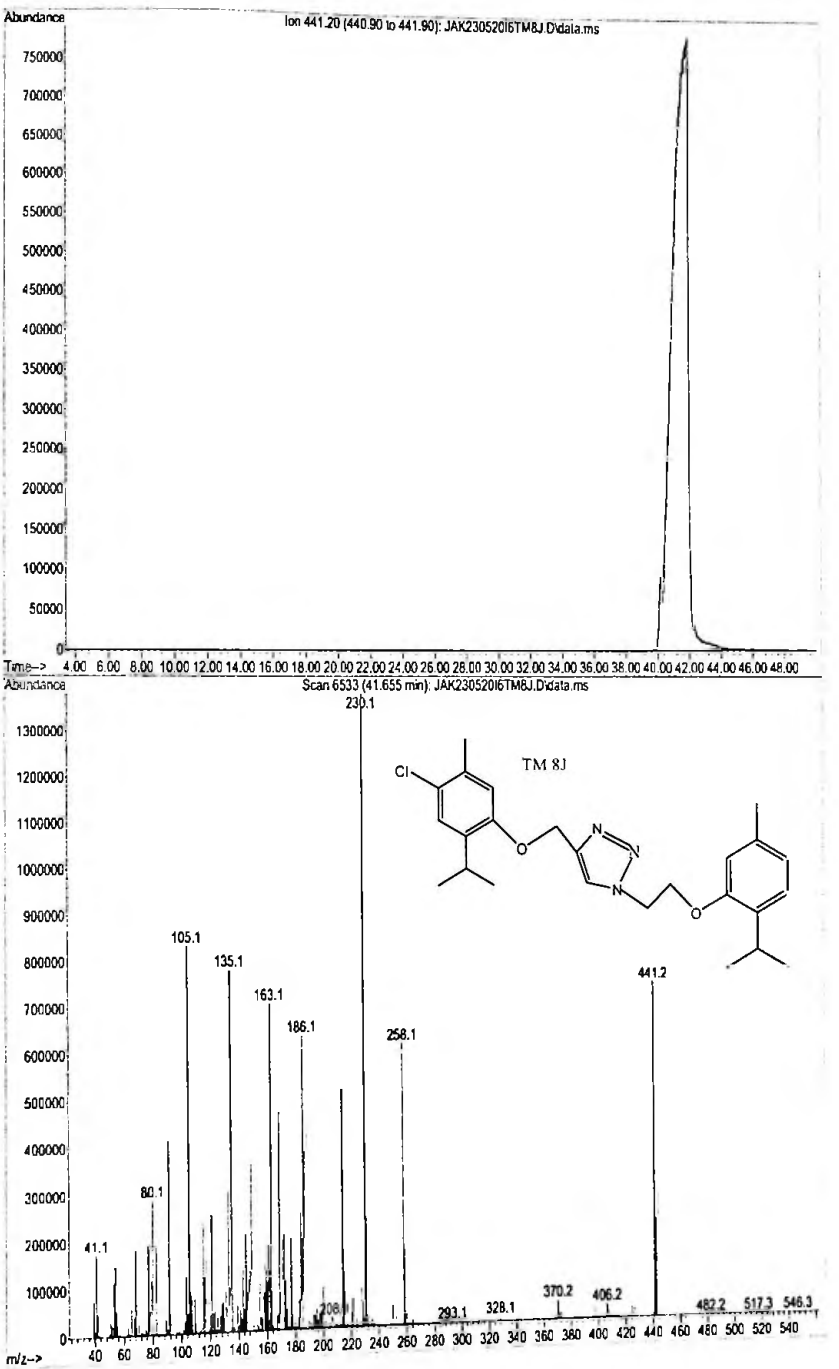
```
File       :D:\JAK02092015\JAK20160520TM10C.D
Operator   : JAK
Acquired   : 21 May 2016  20:27     using AcqMethod DCM VOLATILES 35-280 XTD 40MINUTES .M
Instrument :    ICIPE MSD
Sample Name: TM8I.
Misc Info  : TRIAZOLE DERIVATIVE
Vial Number: 99
```



APPENDIX C-10

MASS SPECTRUM (EI) FOR TM 8J

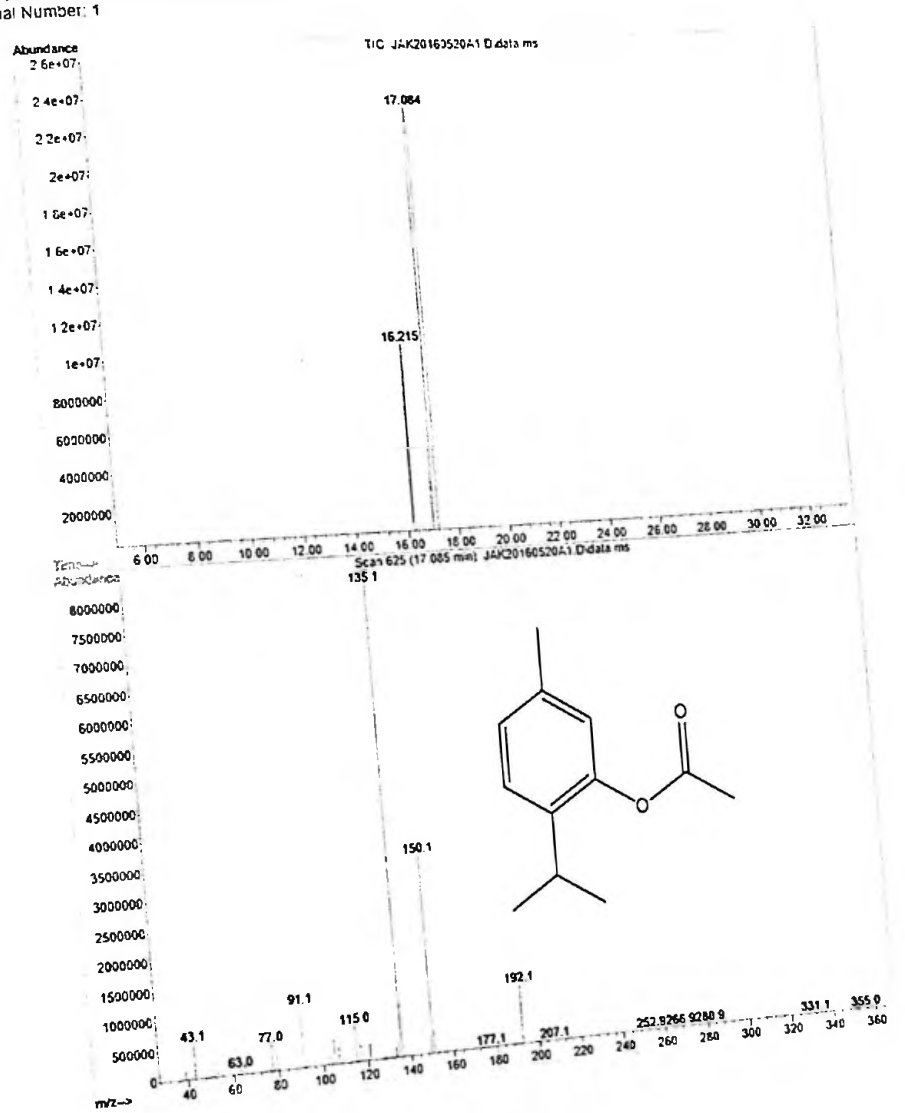
```
File       :D:\JAK02092015\JAK230520165TM8J.D
Operator   : JAK
Acquired   : 23 May 2016  13:23     using AcqMethod DCM VOLATILES 35-280 XTD 50MINUTES .M
Instrument :    1CIFE MSD
Sample Name: TM 8J PURE
Misc Info  : TM 8J PURE
Vial Number: 55
```



APPENDIX D-1

MASS SPECTRUM (EI) FOR TM 1A

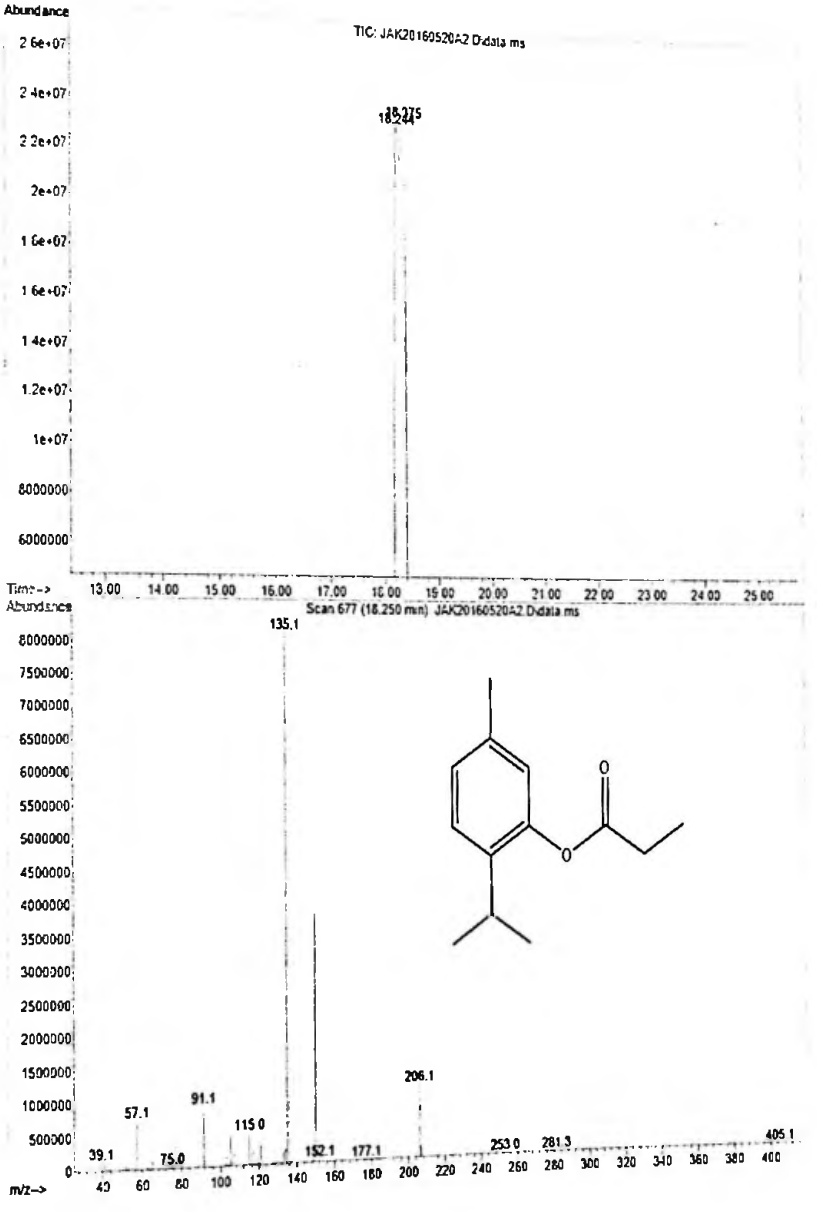
File C:\gcms\EI_data\JAK20160520A1.D
Operator JAK
Acquired 21 May 2016 1:53 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1A
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 1



APPENDIX D-2

MASS SPECTRUM (EI) FOR TM 1B

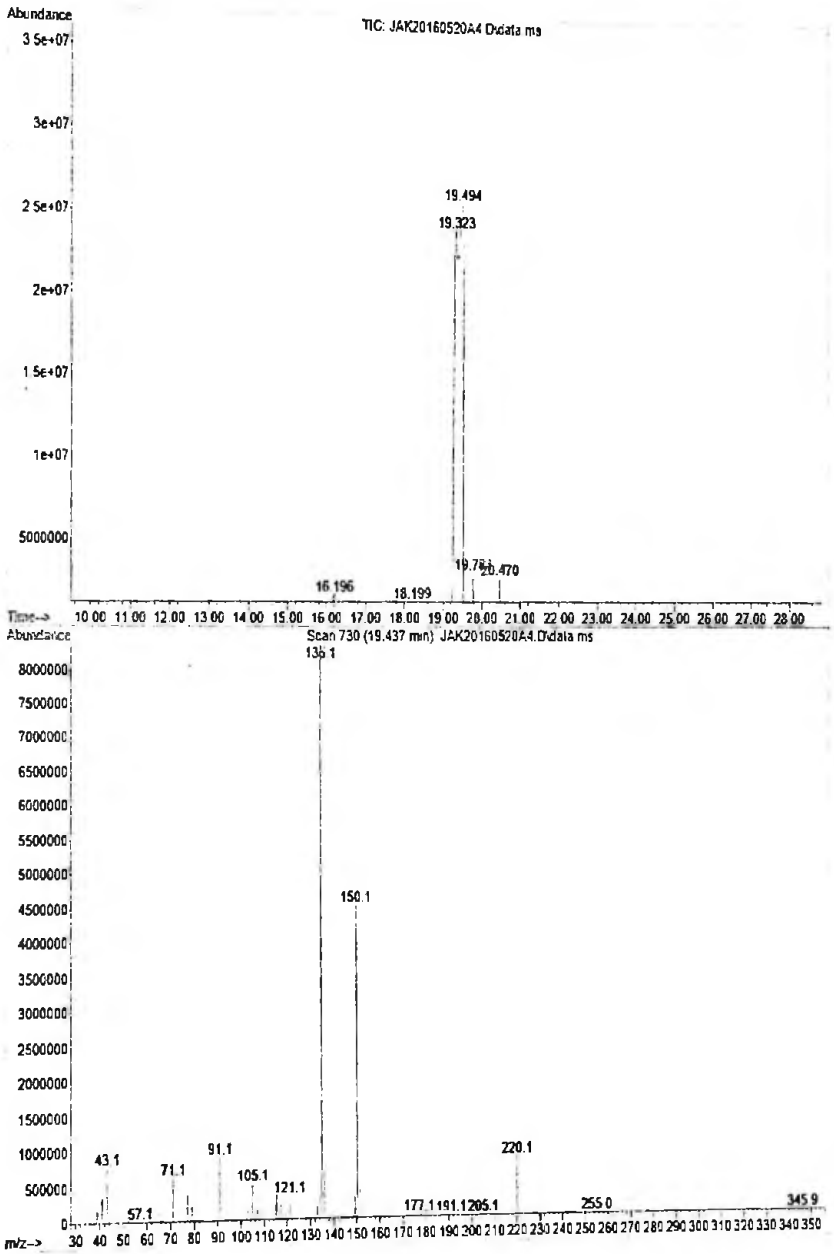
File : C:\gcms\EI_data\JAK20160520A2.D
Operator : JAK
Acquired : 21 May 2016 2:37 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1B
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 2



APPENDIX D-3

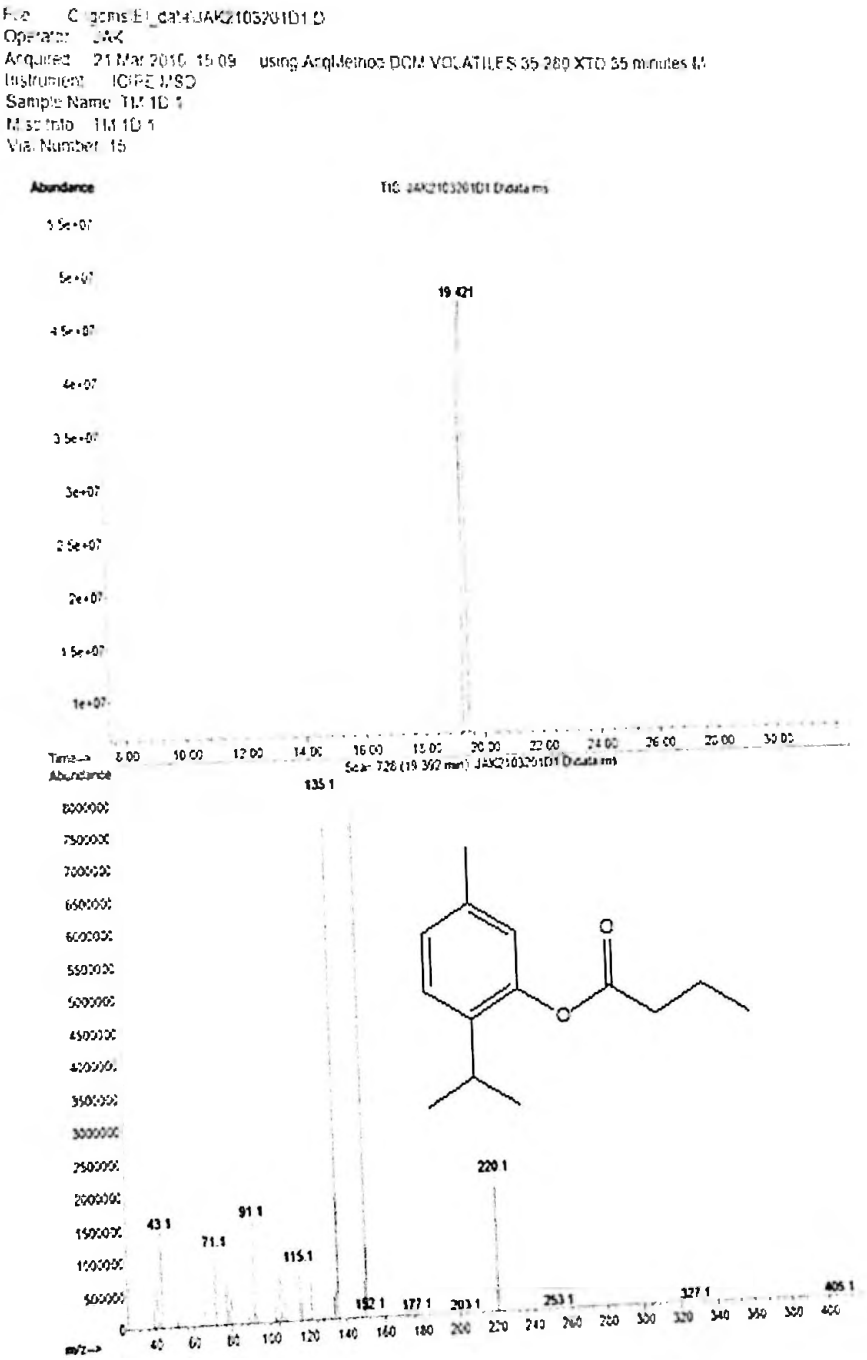
MASS SPECTRUM (EI) FOR TM 1C

File C:\gcms\EI_data\JAK20160520A4.D
Operator : JAK
Acquired : 21 May 2016 4:06 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1D
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 4



APPENDIX D-4

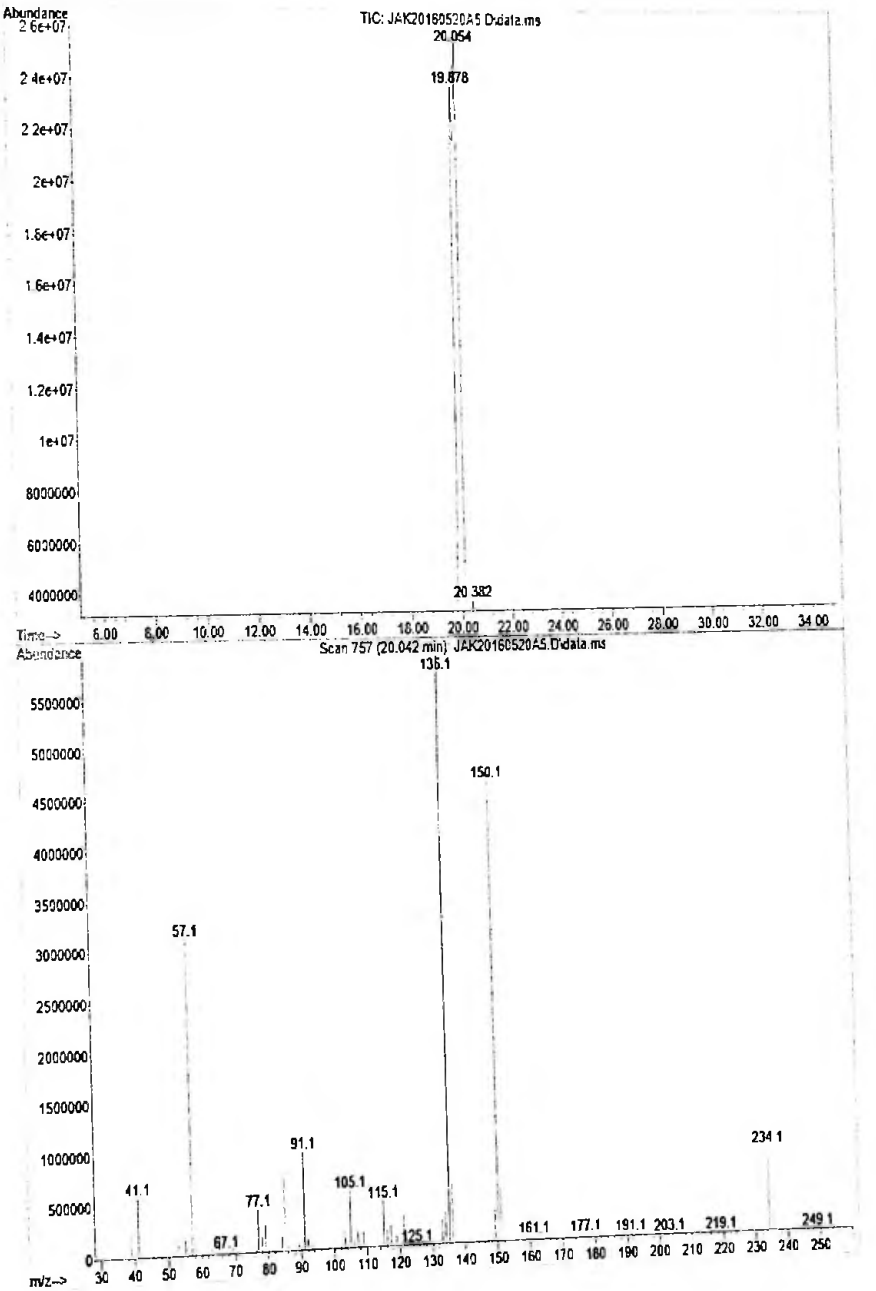
MASS SPECTRUM (EI) FOR TM 1D



APPENDIX D-5

MASS SPECTRUM (EI) FOR TM 1E

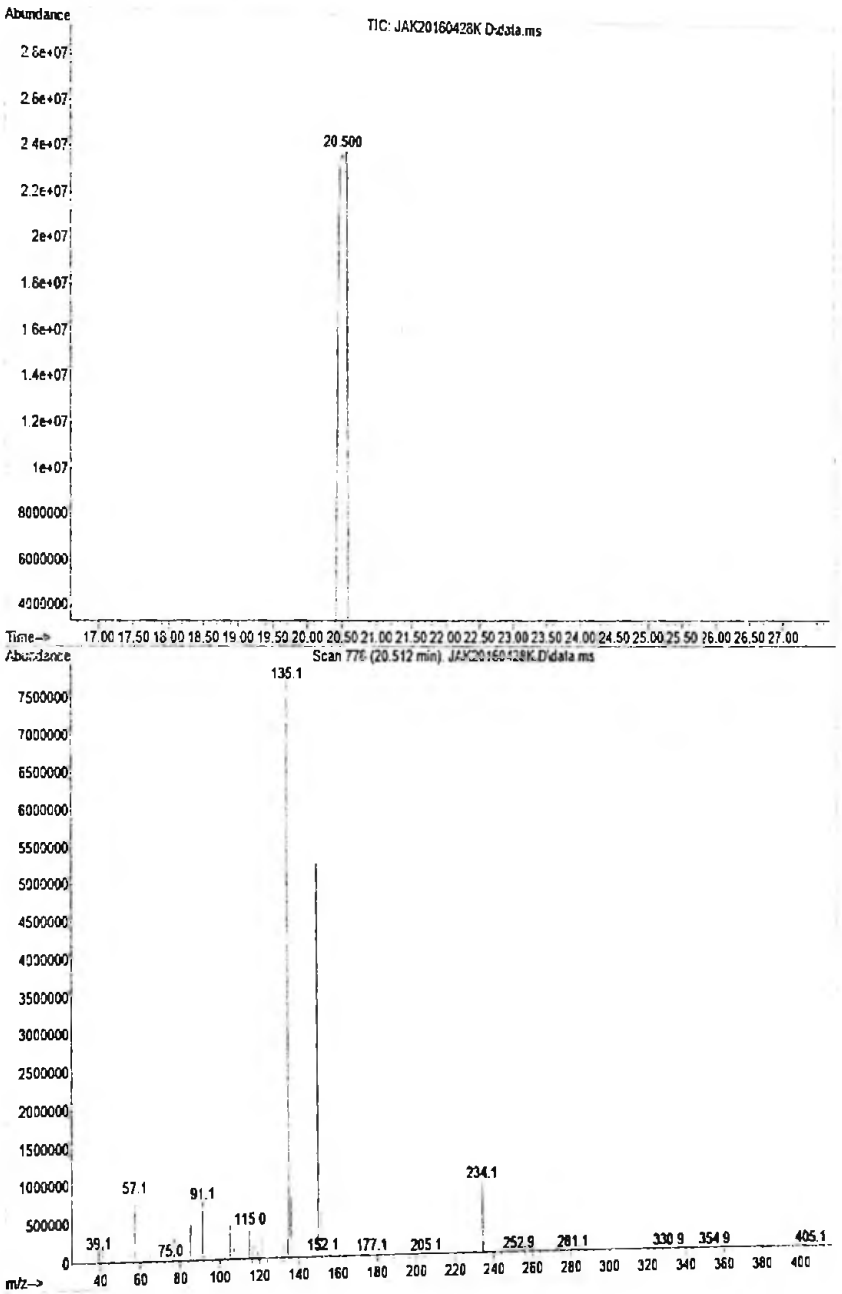
File :C:\gcms\EI_data\JAK20160520A5.D
Operator : JAK
Acquired : 21 May 2016 4:50 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1E
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 5



APPENDIX D-6

MASS SPECTRUM (EI) FOR TM 1F

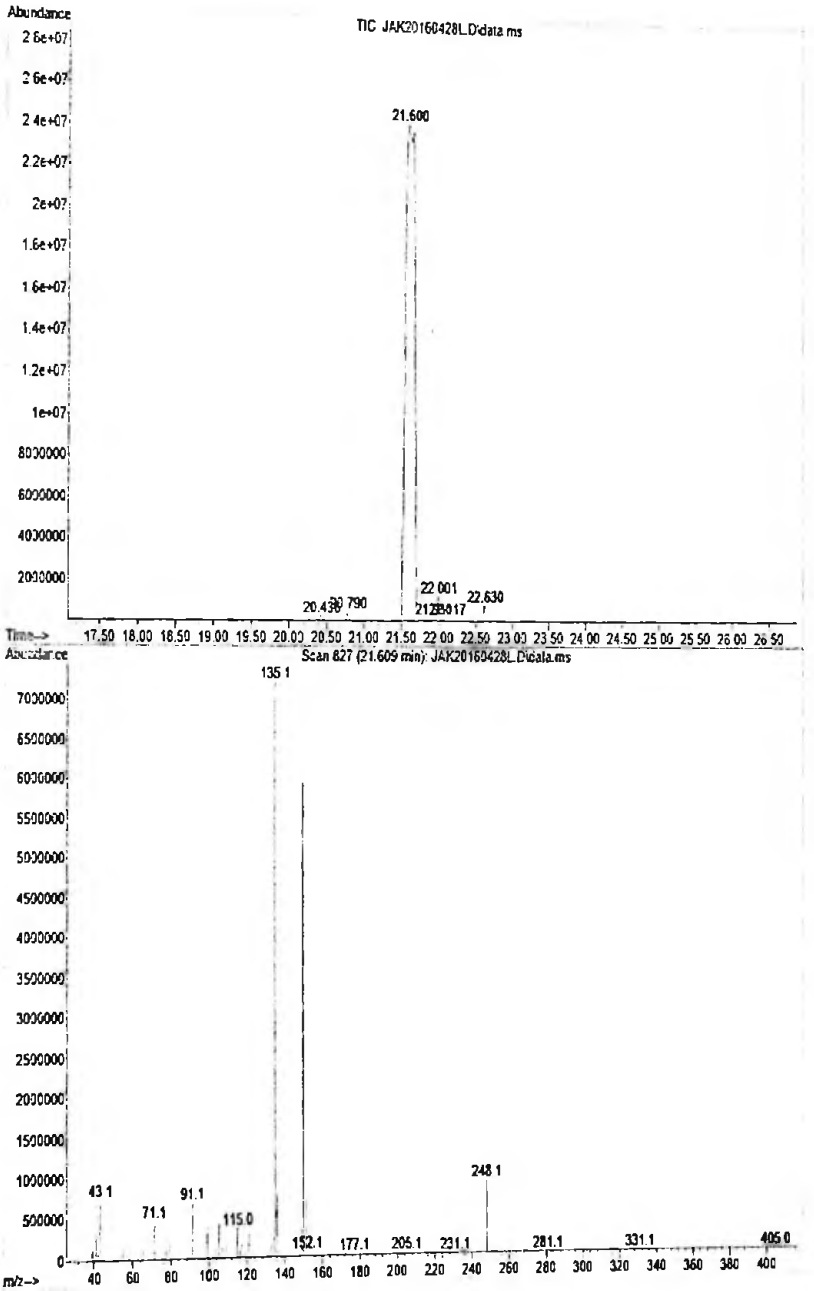
File :C:\gcms\EI_data\JAK20160428K.D
Operator : JAK
Acquired : 29 Apr 2016 5:37 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1F
Misc Info : THYMOL DERIVATIVE ESTER
Vial Number: 21



APPENDIX D-7

MASS SPECTRUM (EI) FOR TM 1G

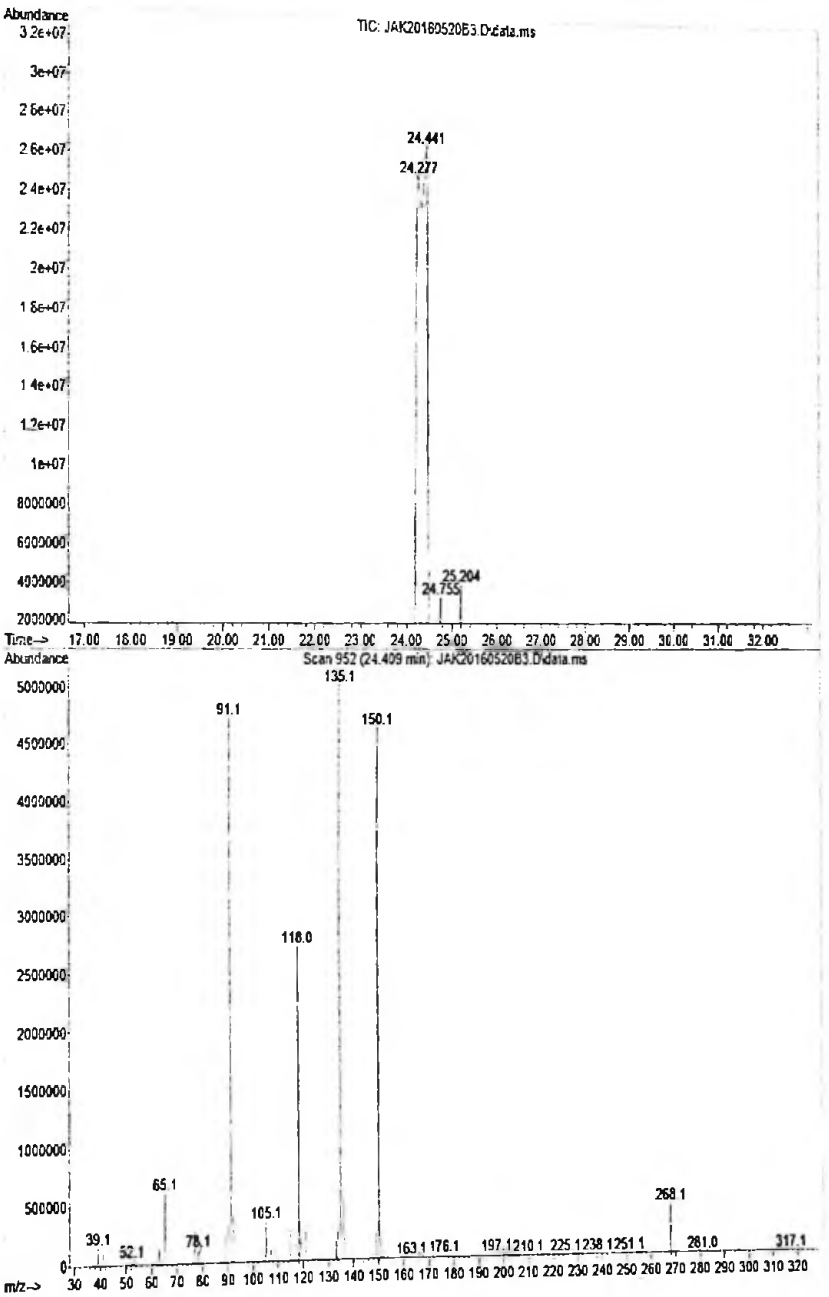
File : C:\gcms\EI_data\JAK20160428L.D
Operator : JAK
Acquired : 29 Apr 2016 6:22 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1G
Misc Info : THYMOL DERIVATIVE ESTER
Vial Number: 22



APPENDIX D-8

MASS SPECTRUM (EI) FOR TM 11

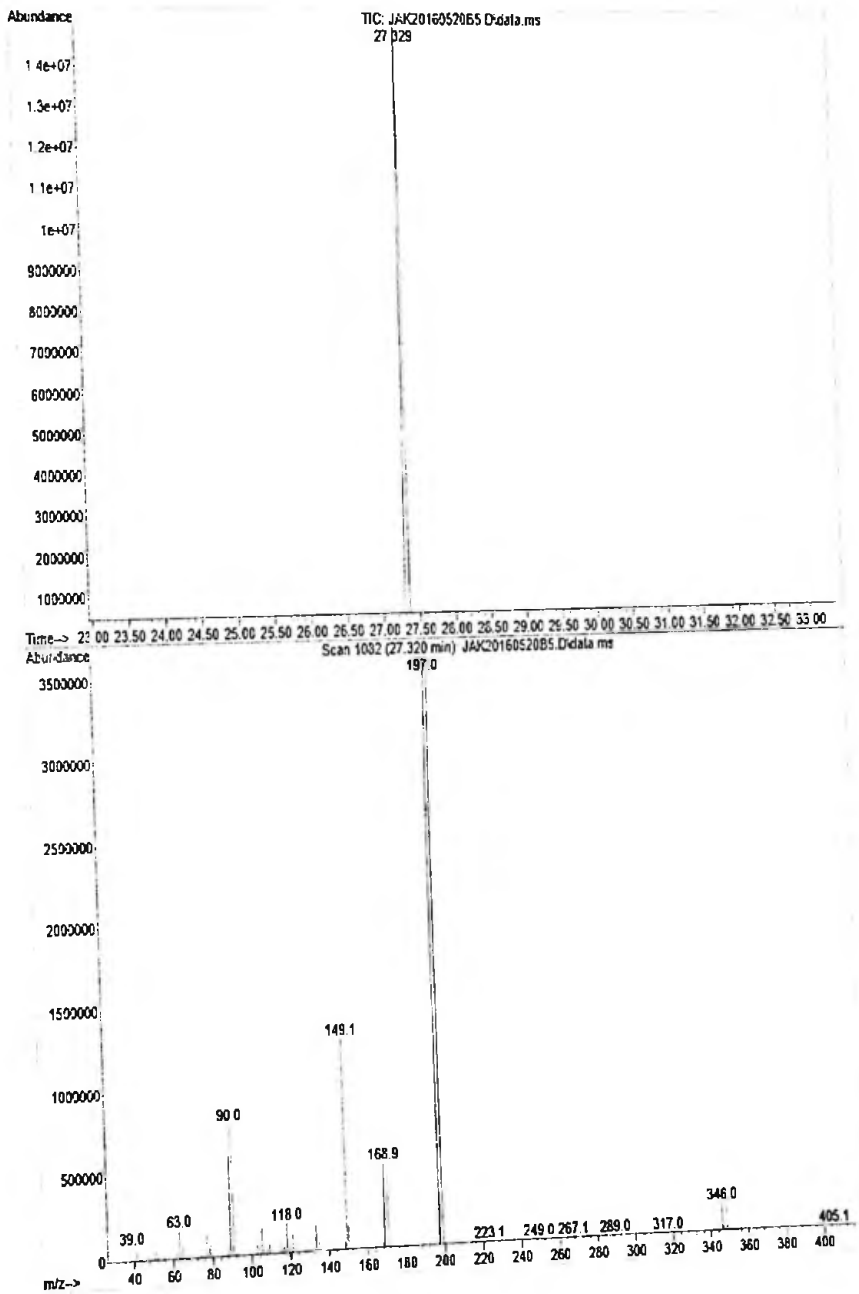
File :C:\gcms\EI_data\JAK20160520B3.D
Operator : JAK
Acquired : 21 May 2016 8:22 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 11
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 9



APPENDIX D-10

MASS SPECTRUM (EI) FOR TM 1L

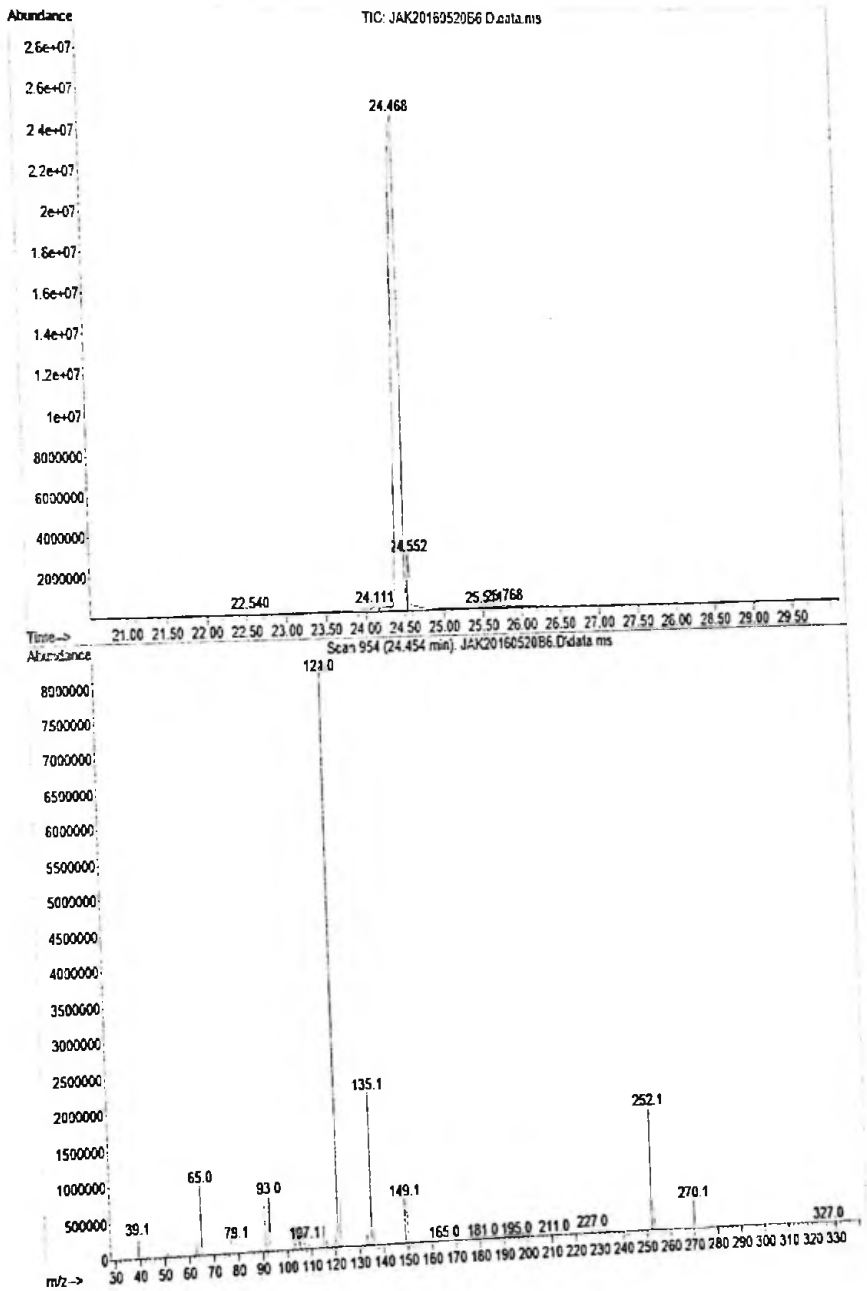
File :C:\gcms\EI_data\JAK20160520B5.D
Operator : JAK
Acquired : 21 May 2016 9:51 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1L
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 11



APPENDIX D-11

MASS SPECTRUM (EI) FOR TM 1M

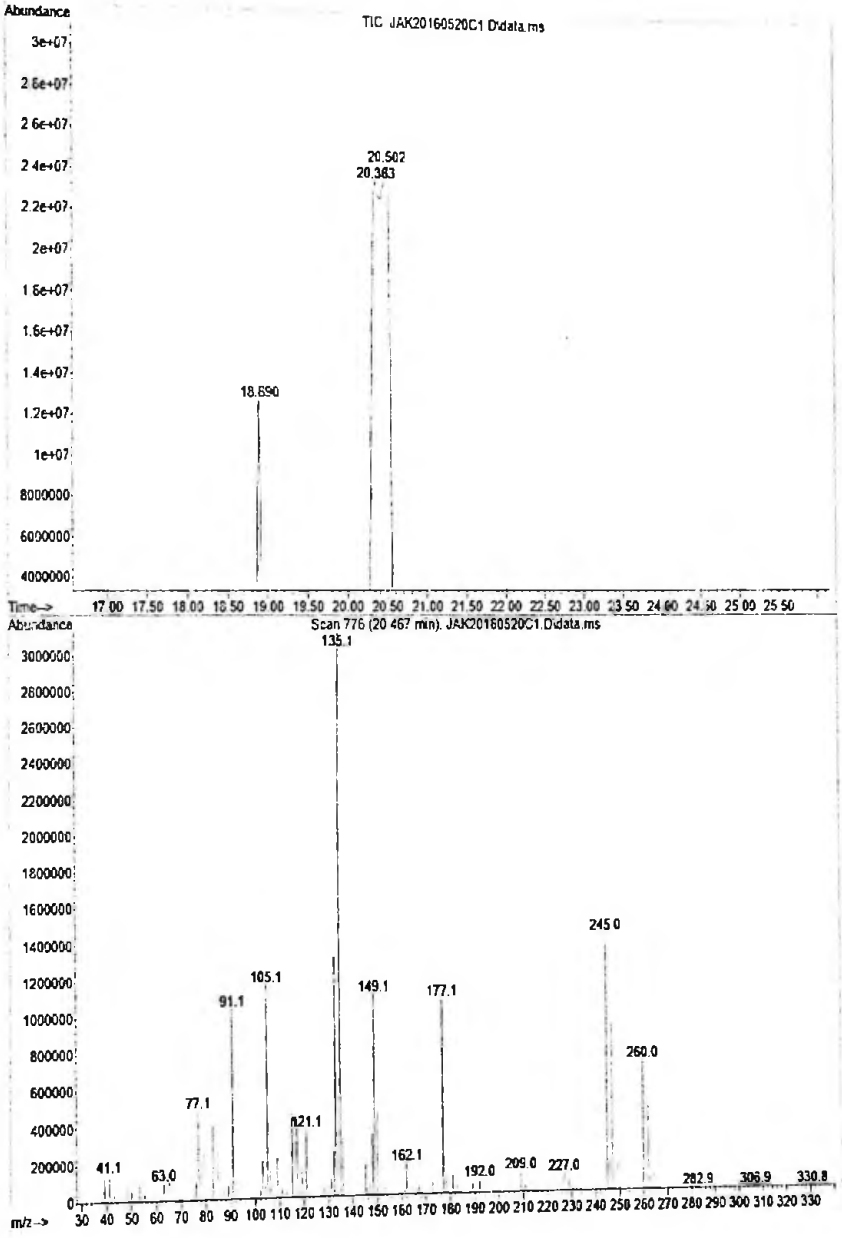
File :C:\gcms\EI_data\JAK20160520B6.D
Operator : JAK
Acquired : 21 May 2016 10:35 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1M
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 12



APPENDIX D-12

MASS SPECTRUM (EI) FOR TM 1N

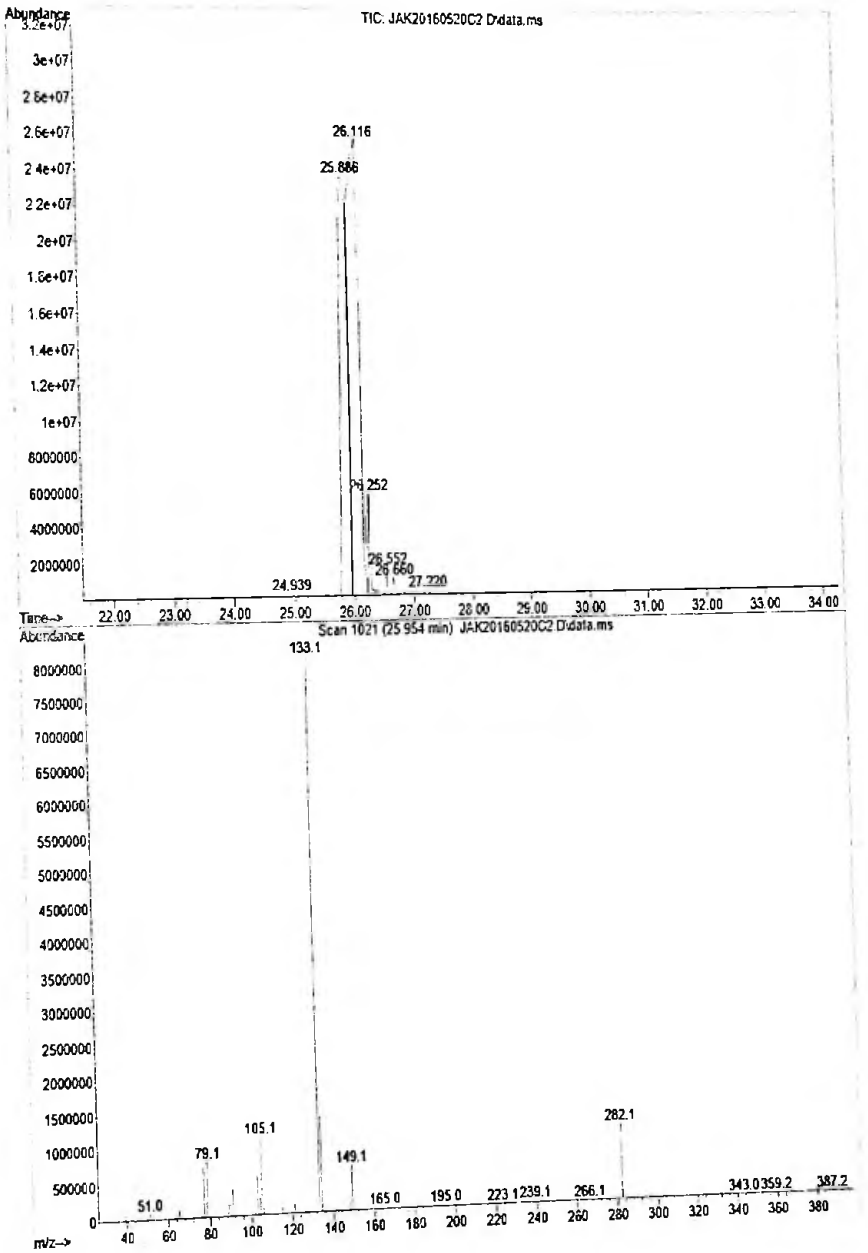
File : C:\gcms\EI_data\JAK20160520C1.D
Operator : JAK
Acquired : 21 May 2016 11:54 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1N
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 13



APPENDIX D-13

MASS SPECTRUM (EI) FOR TM 1P

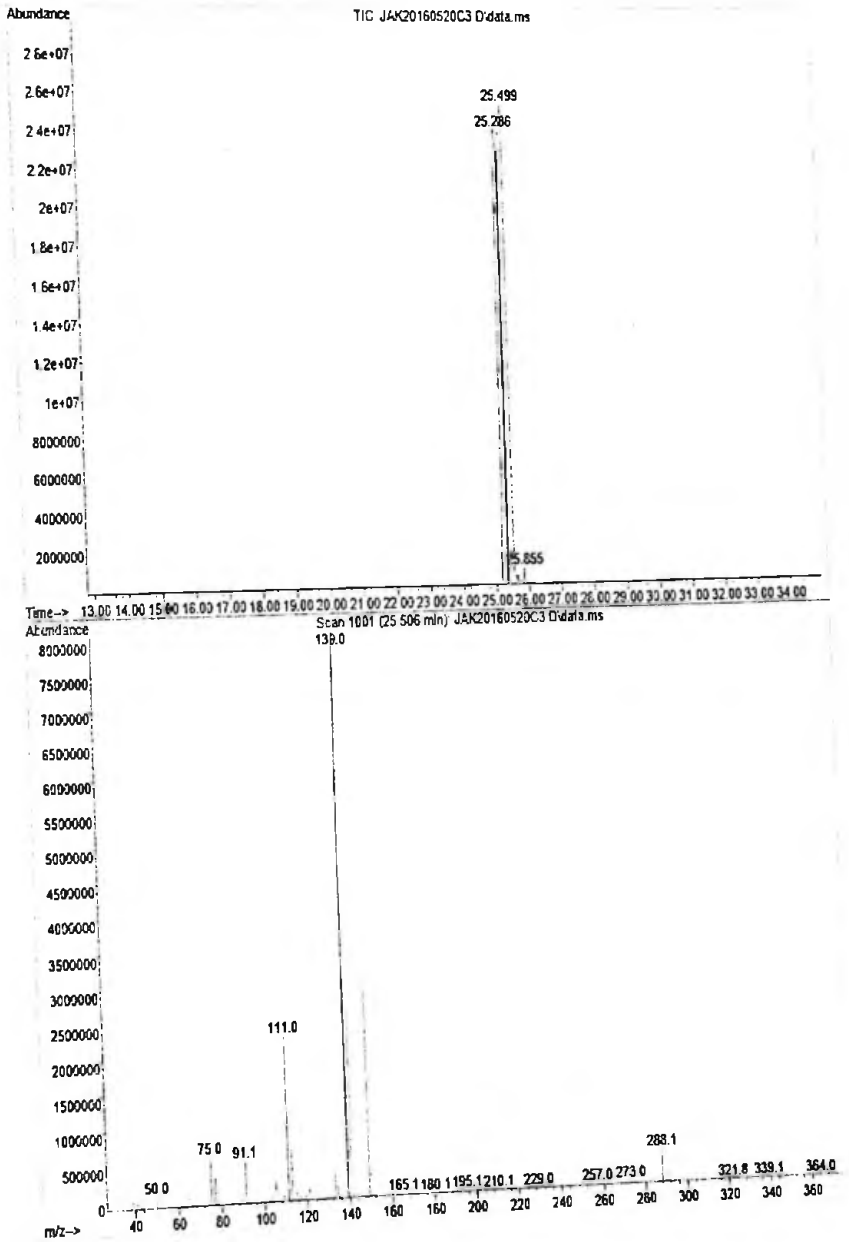
File :C:\gcms\EI_data\JAK20160520C2.D
Operator : JAK
Acquired : 21 May 2016 12:39 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1P
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 14



APPENDIX D-14

MASS SPECTRUM (EI) FOR TM 1Q

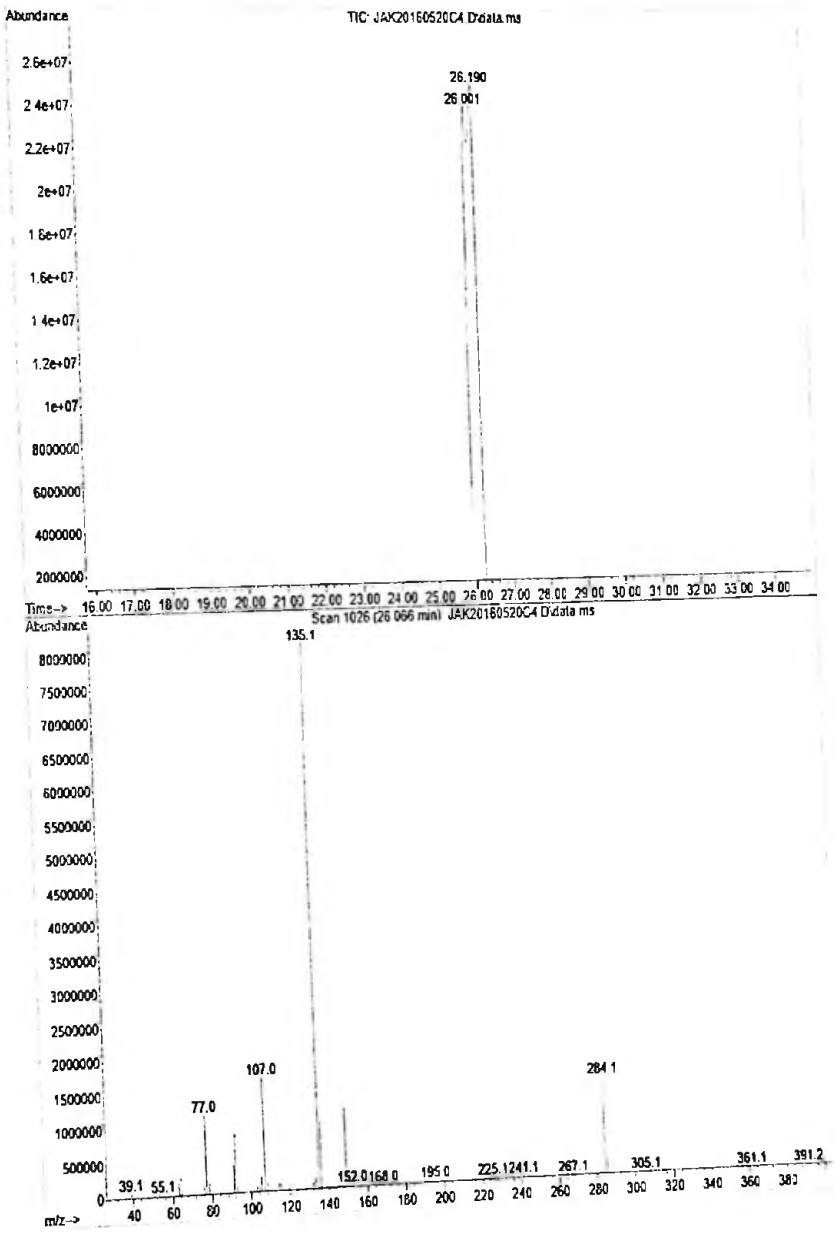
File :C:\gcms\EI_data\JAK20160520C3.D
Operator : JAK
Acquired : 21 May 2016 13:23 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1Q
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 15



APPENDIX D-15

MASS SPECTRUM (EI) FOR TM 1R

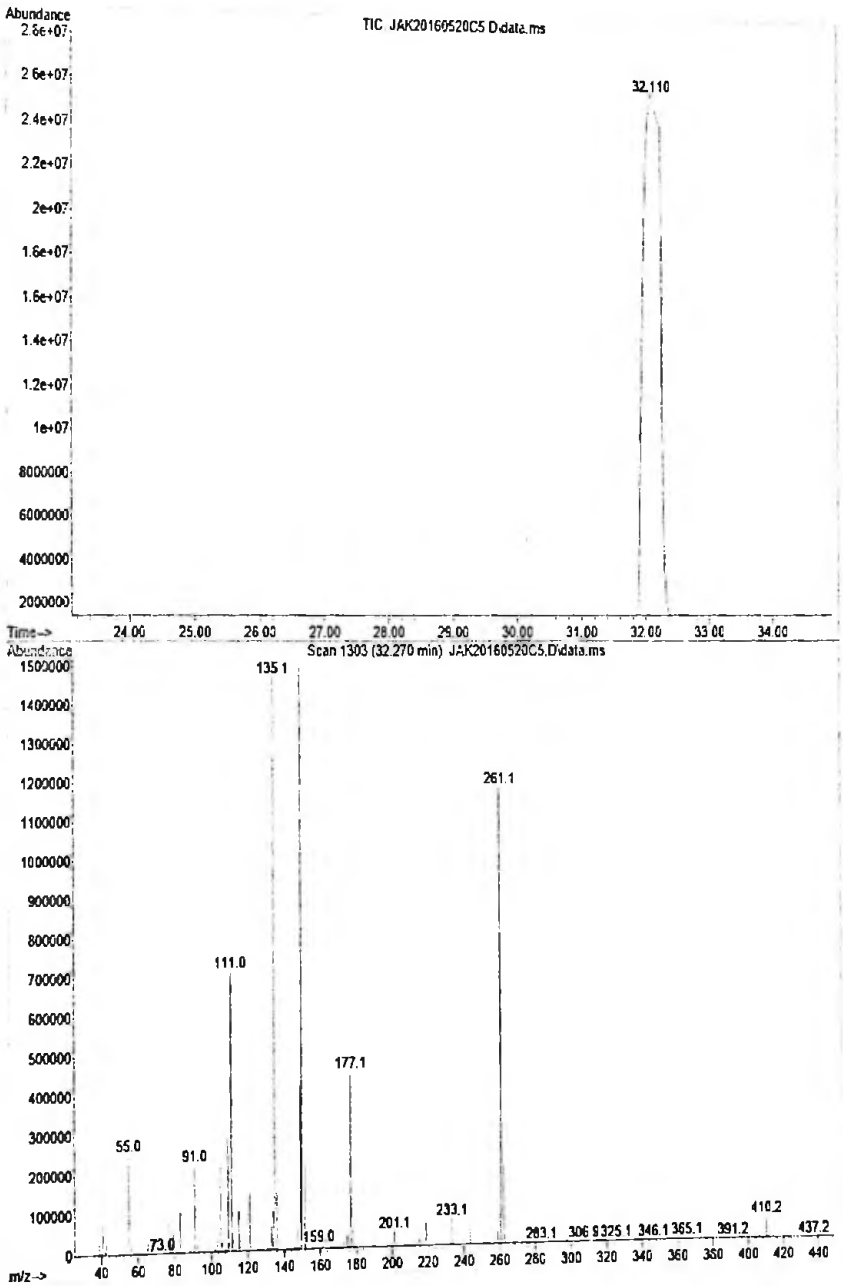
File : C:\gcms\EI_data\JAK20160520C4.D
Operator : JAK
Acquired : 21 May 2016 14:07 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1R
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 16



APPENDIX D-16

MASS SPECTRUM (EI) FOR TM 1U

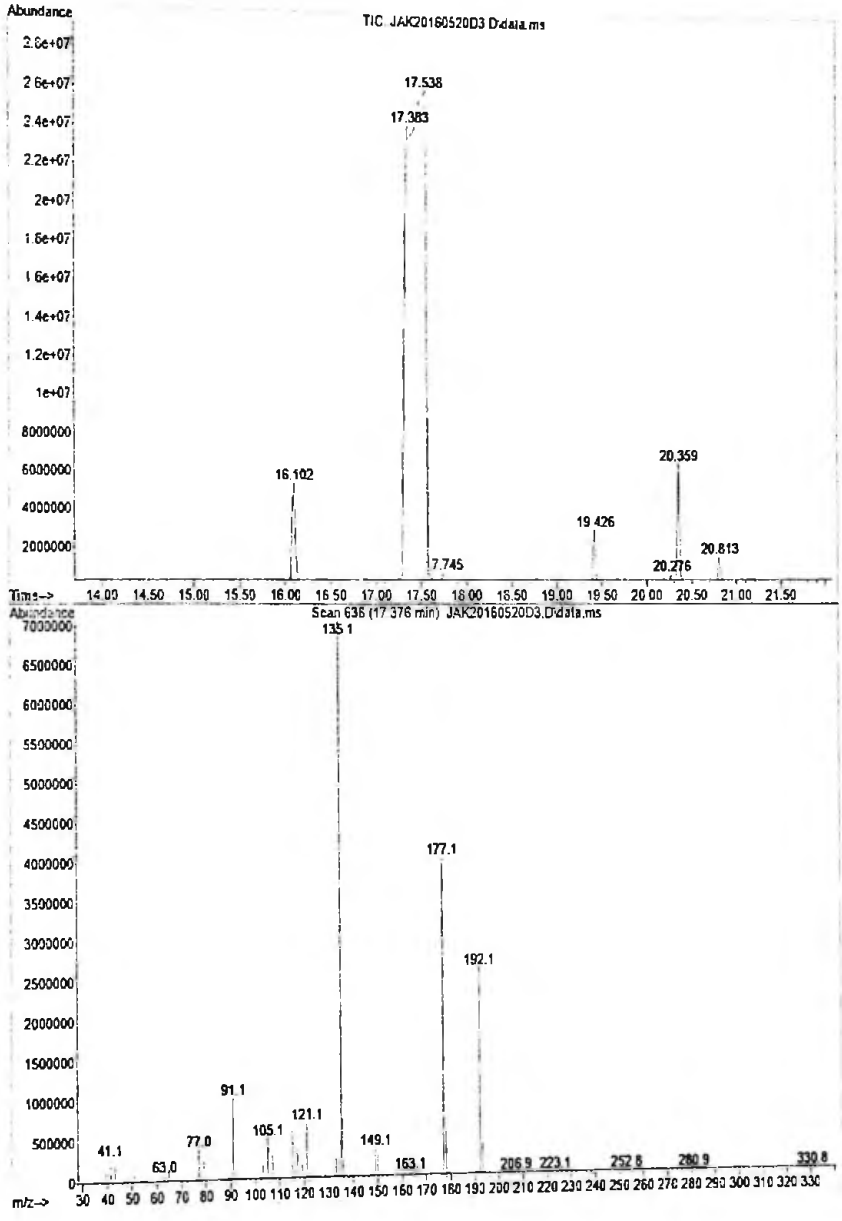
File : C:\gcms\EI_data\JAK20160520C5.D
Operator : JAK
Acquired : 21 May 2016 14:52 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 1U
Misc Info : ESTER DERIVATIVE OF THYMOL
Vial Number: 17



APPENDIX E-1

MASS SPECTRUM (EI) FOR TM 2C

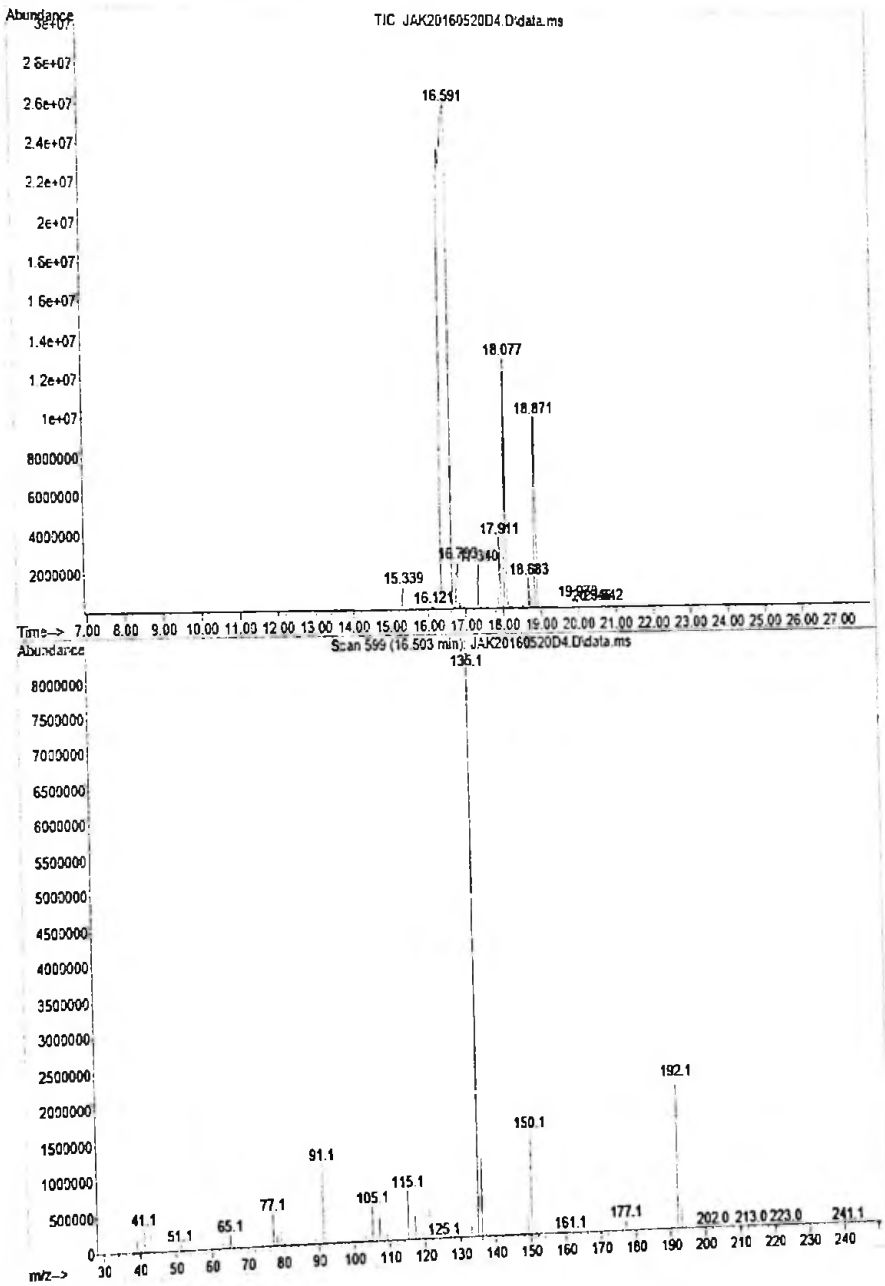
File :C:\gcms\EI_data\JAK20160520D3.D
Operator : JAK
Acquired : 21 May 2016 17:40 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2C
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 20



APPENDIX E-2

MASS SPECTRUM (EI) FOR TM 2D

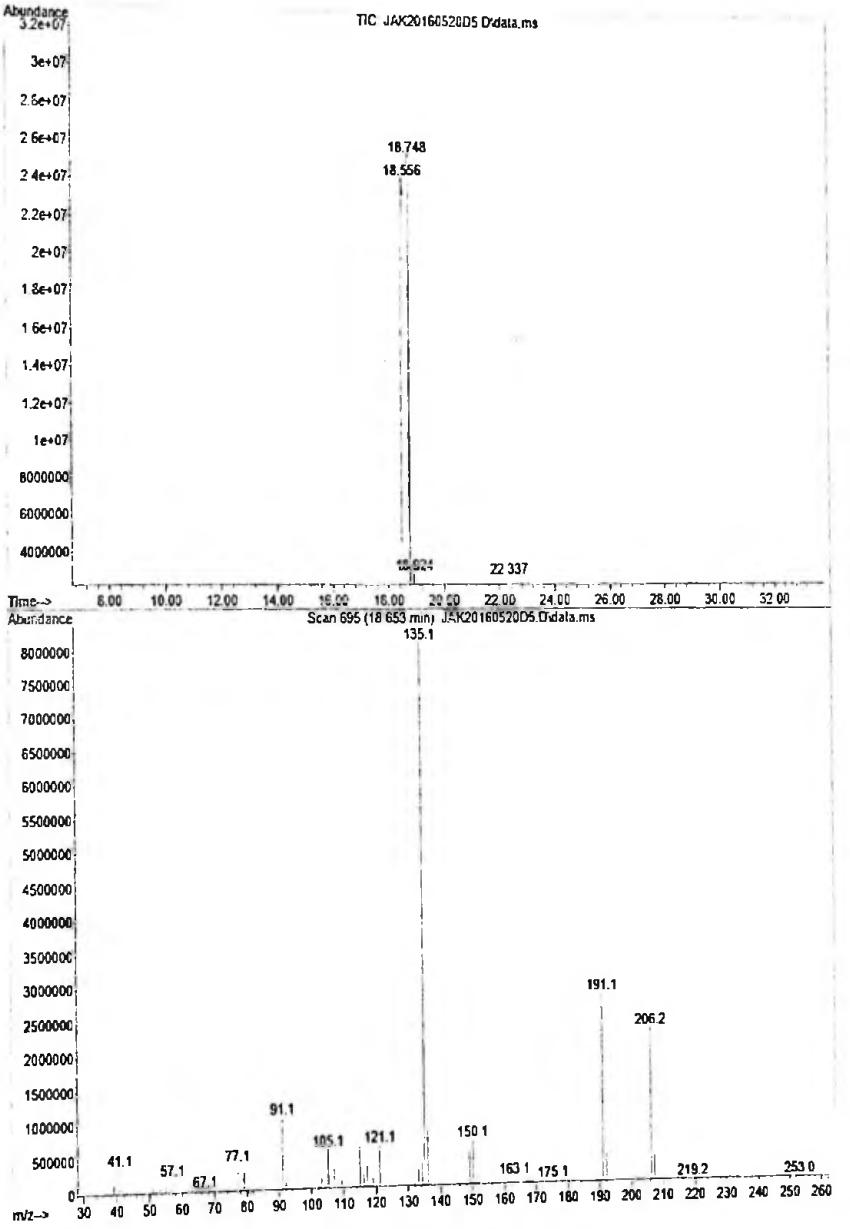
File C:\gcms\EI_data\JAK20160520D4.D
Operator : JAK
Acquired : 21 May 2016 18:24 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2D
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 21



APPENDIX E-3

MASS SPECTRUM (EI) FOR TM 2E

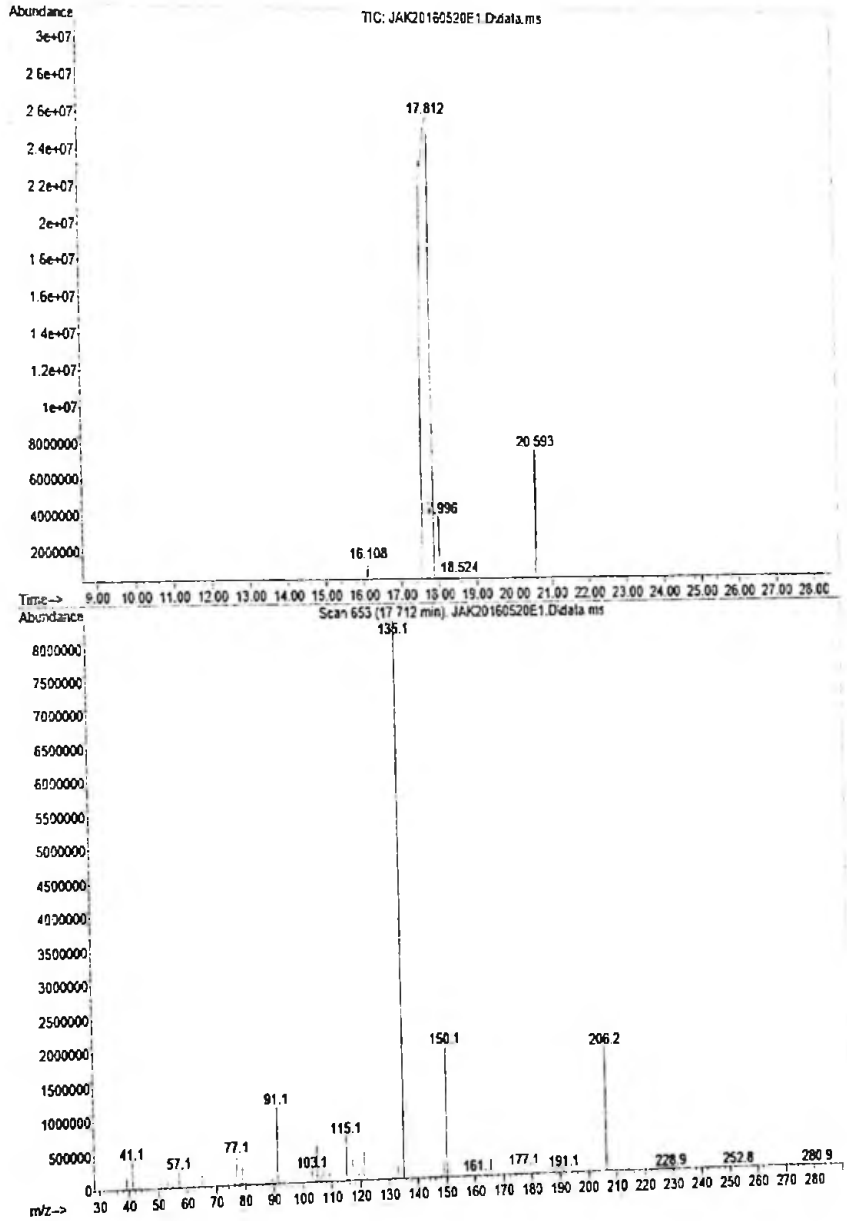
File :C:\gcms\EI_data\JAK20160520D5.D
Operator : JAK
Acquired : 21 May 2016 19:08 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2E
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 22



APPENDIX E-4

MASS SPECTRUM (EI) FOR TM 2F

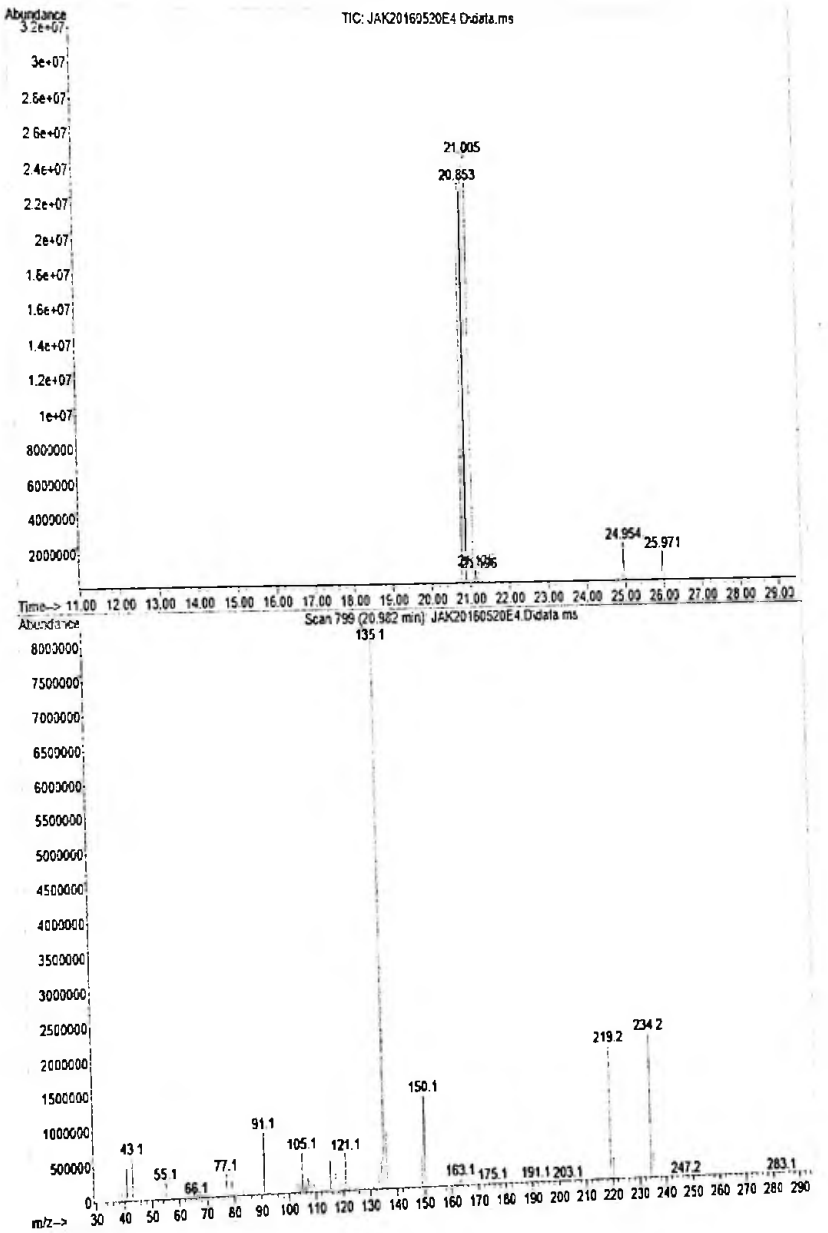
File :C:\gcms\EI_data\JAK20160520E1.D
Operator : JAK
Acquired : 21 May 2016 22:43 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2F
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 23



APPENDIX E-5

MASS SPECTRUM (EI) FOR TM 2I

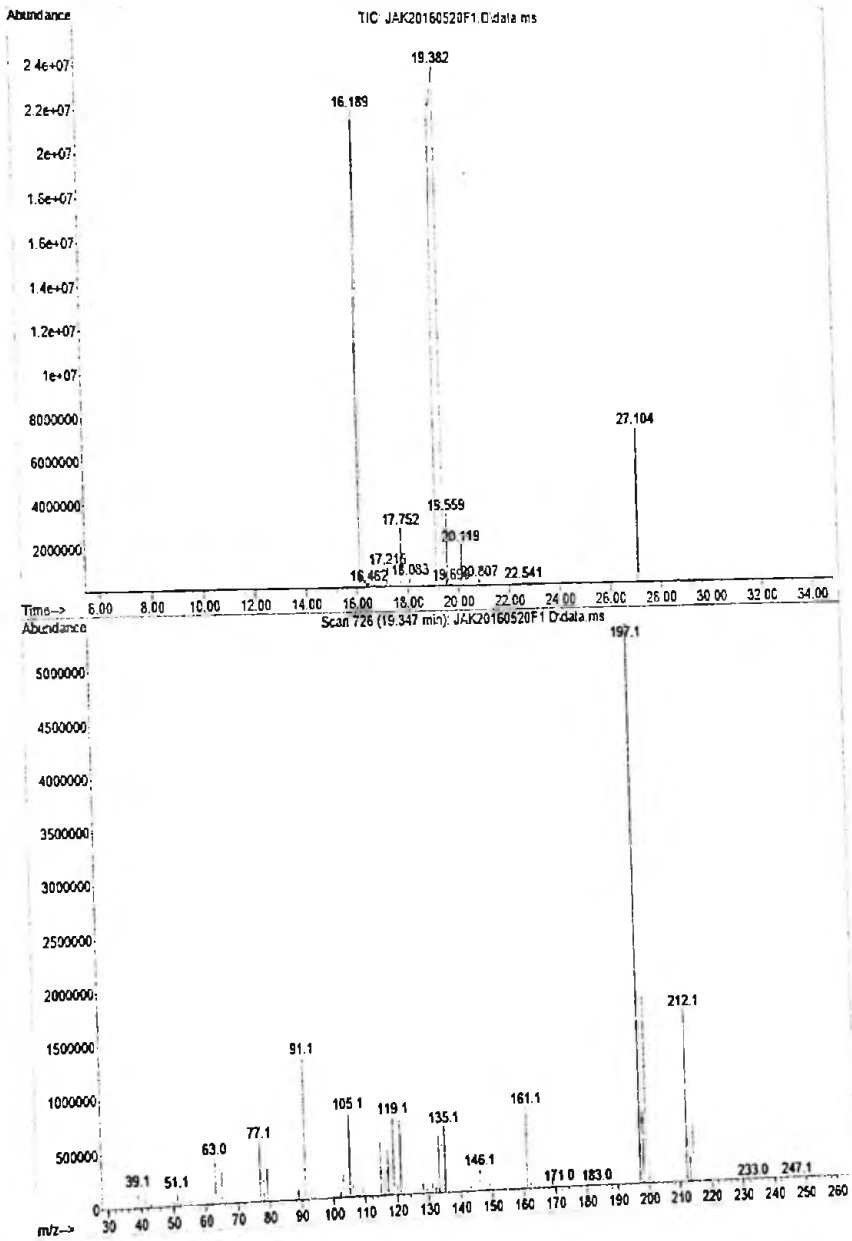
File :C:\gcms\EI_data\JAK20160520E4.D
Operator : JAK
Acquired : 22 May 2016 00:56 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2I
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 26



APPENDIX E-6

MASS SPECTRUM (EI) FOR TM 2K

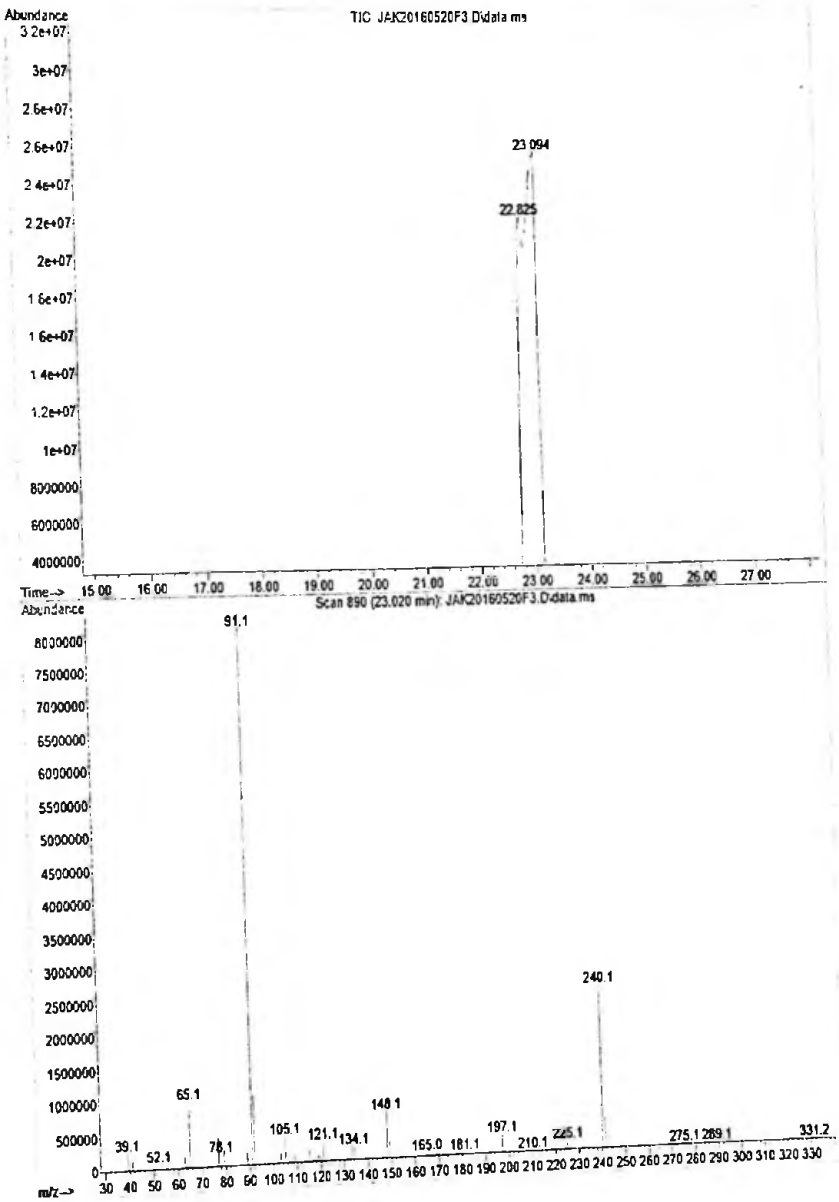
File :C:\gcms\EI_data\JAK20160520F1.D
Operator : JAK
Acquired : 22 May 2016 5:35 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2K
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 28



APPENDIX E-7

MASS SPECTRUM (EI) FOR TM 2N

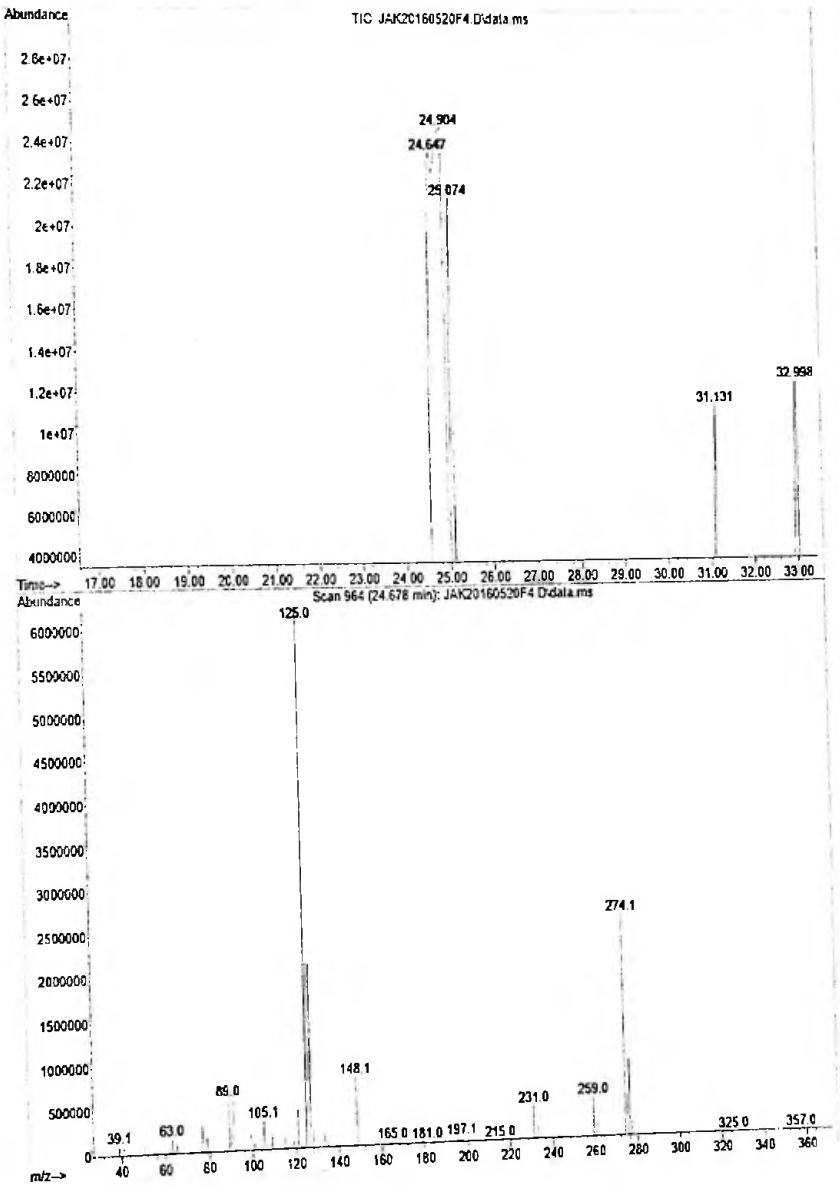
File : C:\gcms\EI_data\JAK20160520F3.D
Operator : JAK
Acquired : 22 May 2016 7:04 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2N
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 30



APPENDIX E-8

MASS SPECTRUM (EI) FOR TM 2O

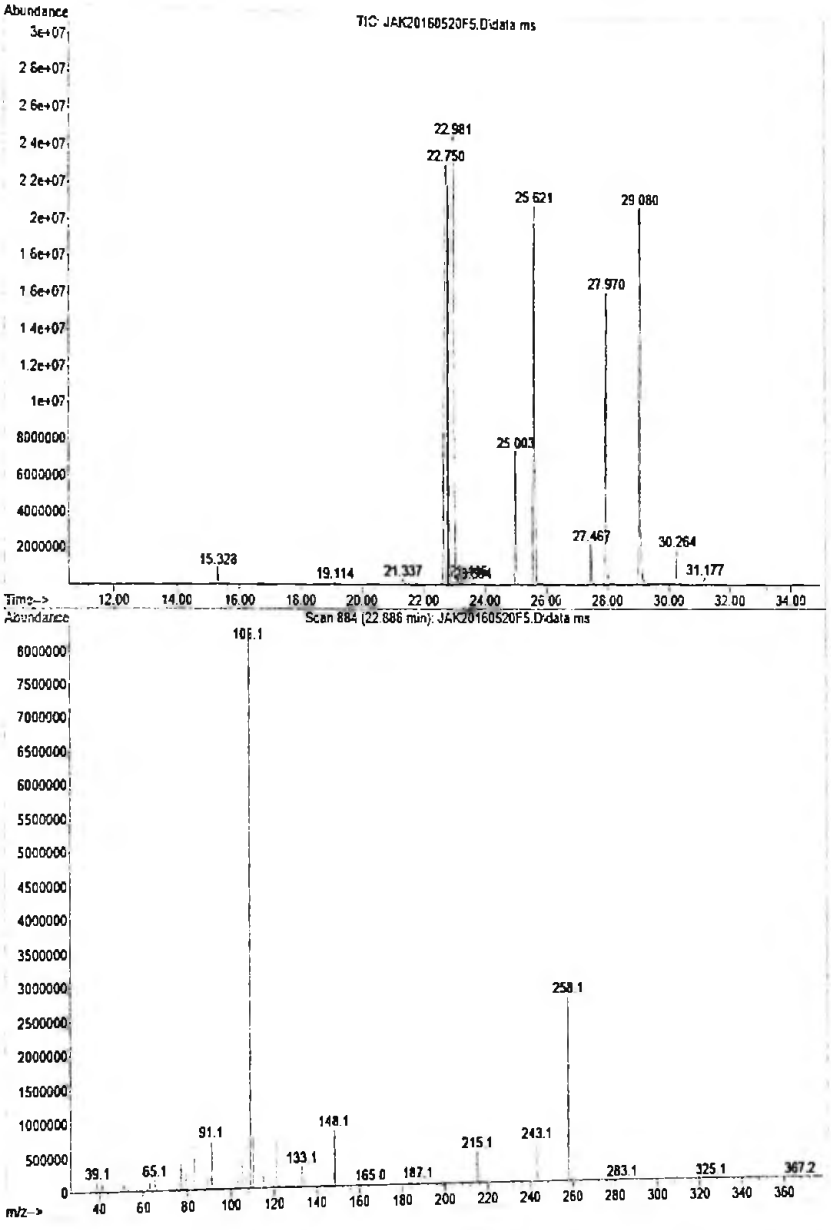
File :C:\gcms\EI_data\JAK20160520F4.D
Operator : JAK
Acquired : 22 May 2016 7:48 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2O
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 31



APPENDIX E-9

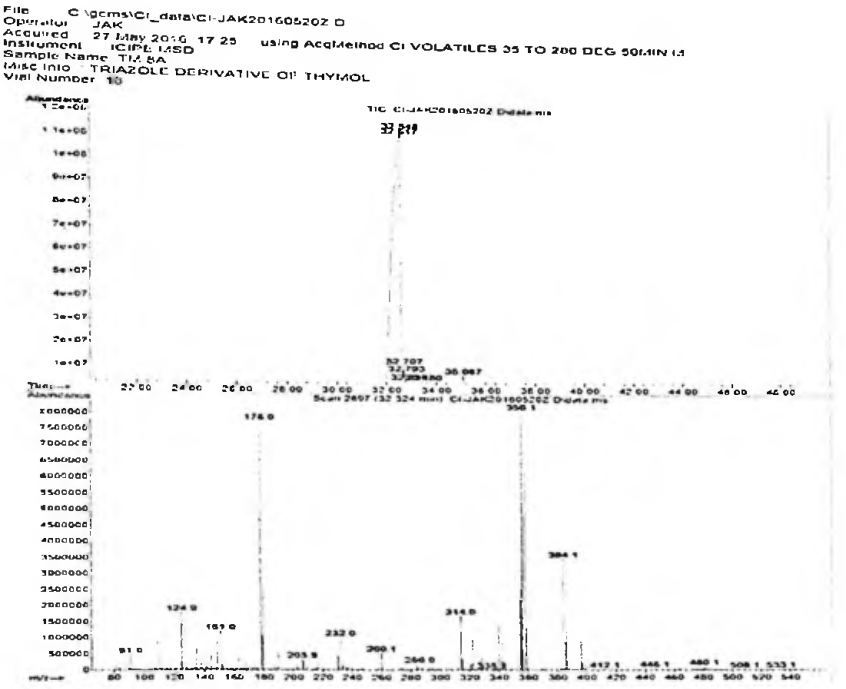
MASS SPECTRUM (EI) FOR TM 2P

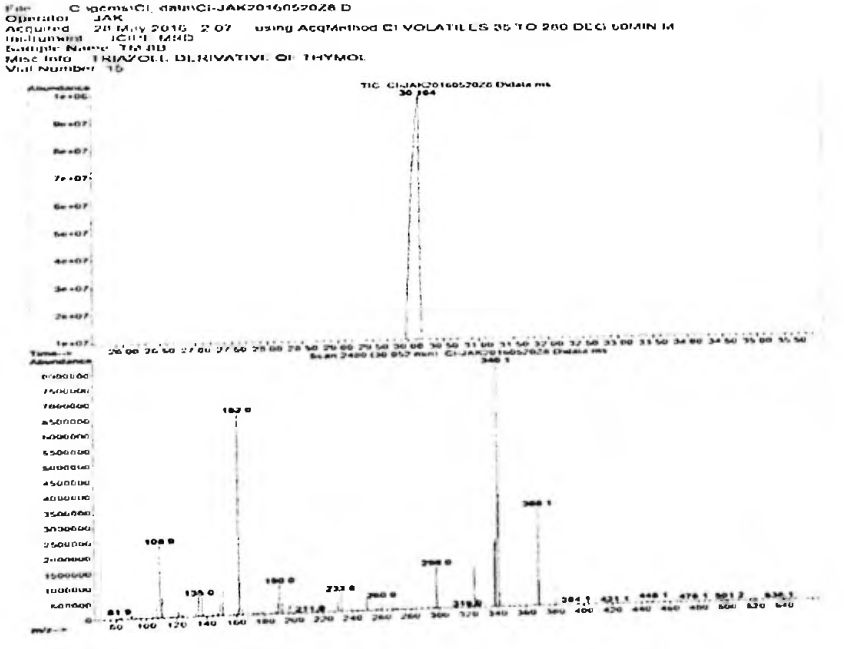
File :C:\gcms\EI_data\JAK20160520F5.D
Operator : JAK
Acquired : 22 May 2016 8:33 using AcqMethod DCM VOLATILES 35-280 XTD 35 MINUTES.M
Instrument : ICIPE MSD
Sample Name: TM 2P
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 32



APPENDIX F-1

MASS SPECTRA (CI) FOR TM 8A &TM 8B

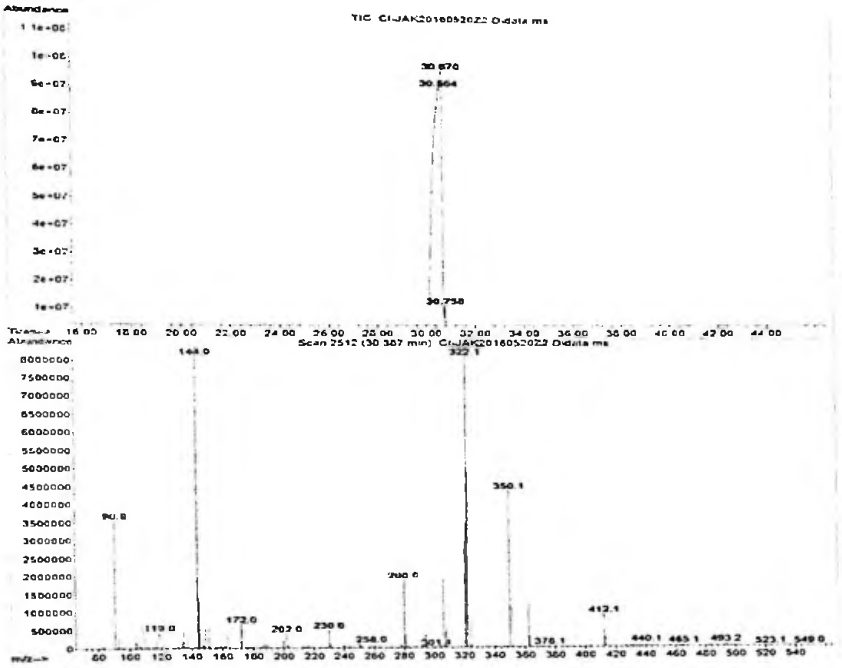




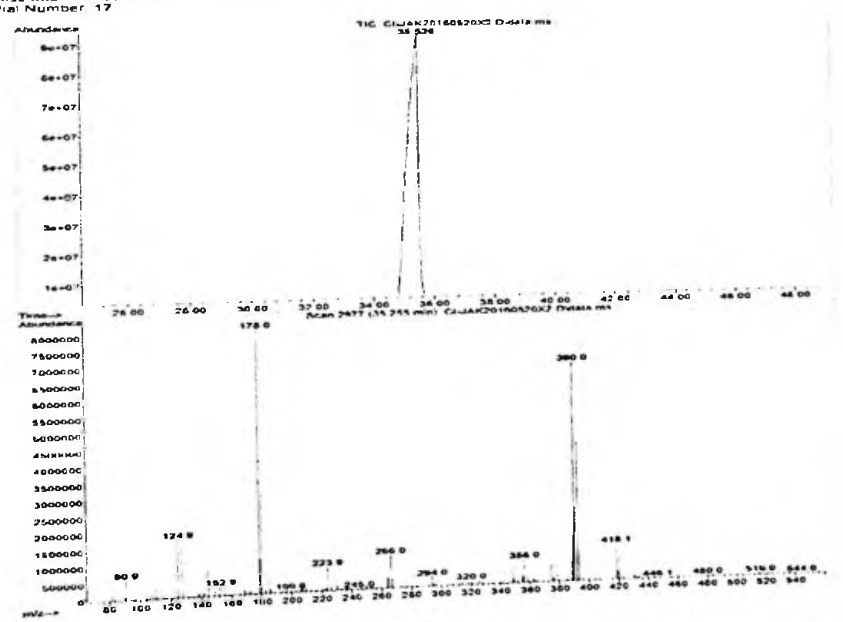
APPENDIX F-2

MASS SPECTRA (CI) FOR TM 8C &TM 8D

File C:\gcms\CI_data\CI-JAK20160520Z2.D
Operator JAK
Acquired 27 May 2016 19:26 using AcqMethod CI VOLATILES 35 TO 280 DEG 60MIN.M
Instrument ICIPE MSD
Sample Name TM 8C
Misc Info TRIAZOLE DERIVATIVE OF THYMOL
Vial Number 20

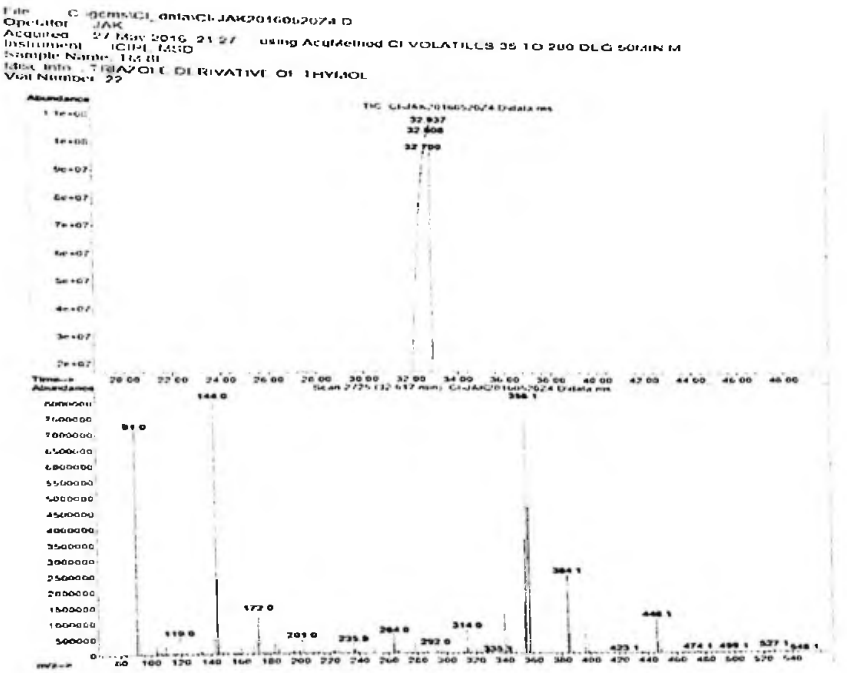


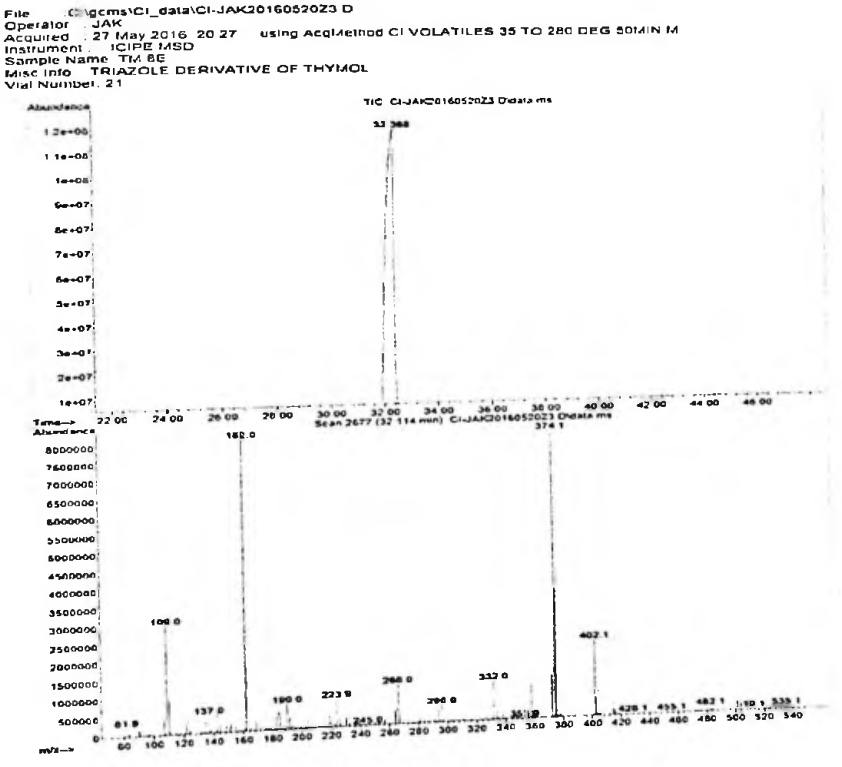
File C:\gcms\CI_data\CI-JAK20160520X2.D
Operator JAK
Acquired 27 May 2016 16:24 using AcqMethod CI VOLATILES 35 TO 280 DEG 60MIN.M
Instrument ICIPE MSD
Sample Name TM 8D
Misc Info TRIAZOLE DERIVATIVE OF THYMOL
Vial Number 17



APPENDIX F-3

MASS SPECTRA (CI) FOR TM 8E &TM 8F

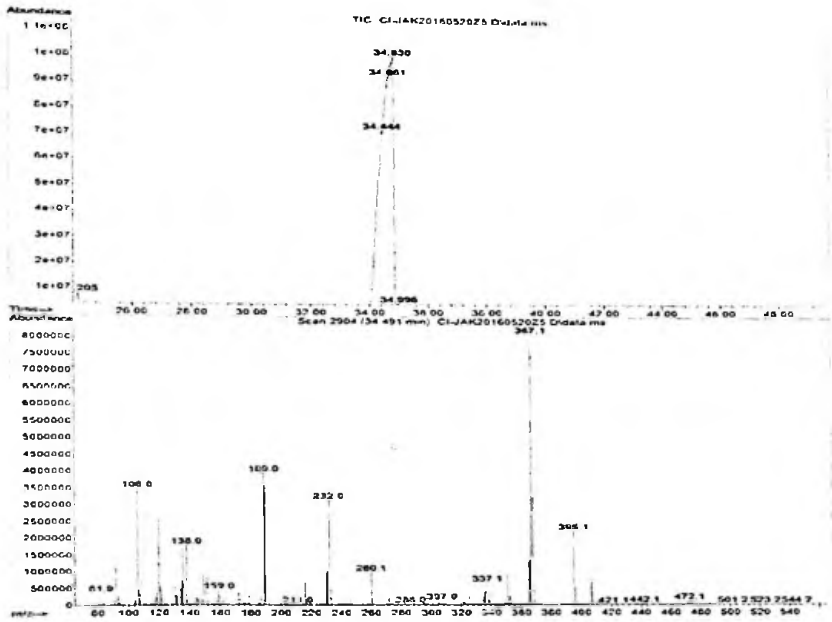




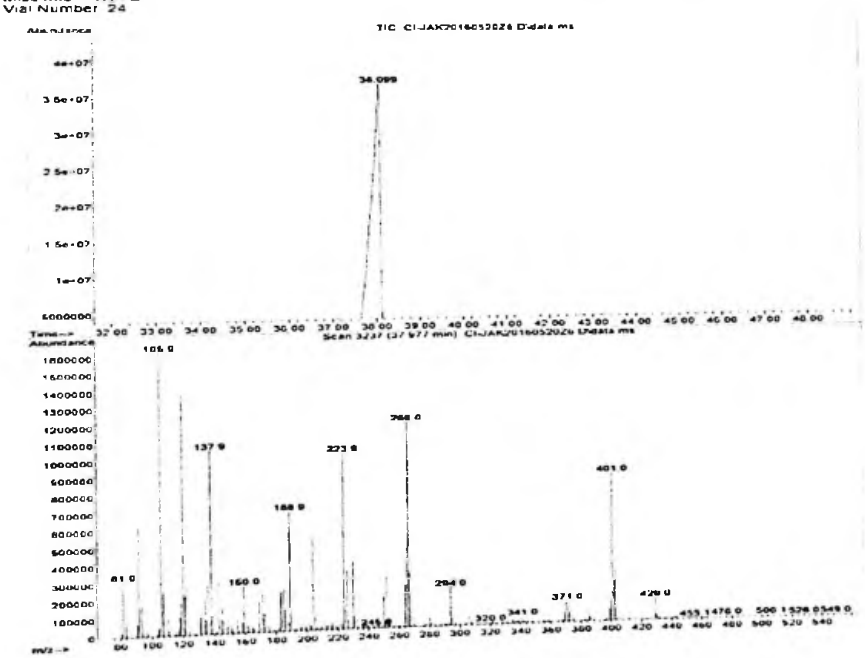
APPENDIX F-4

MASS SPECTRA (CI) FOR TM 8G &TM 8H

File C:\gcms\CI_data\CI-JAK2016052025.D
Operator JAK
Acquired 27 May 2016 22:28 using AcqMethod CI VOLATILES 35 TO 280 DEG 50MIN.M
Instrument ICIPE MSD
Sample Name TM 8G
Misc Info TRIAZOLE DERIVATIVE OF THYMOL
Vial Number 23



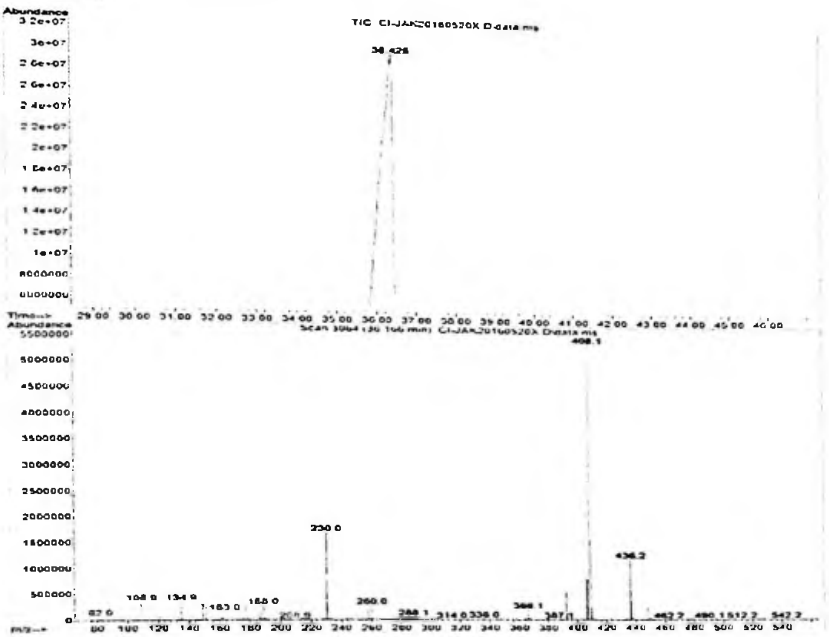
File C:\gcms\CI_data\CI-JAK2016052026.D
Operator JAK
Acquired 27 May 2016 23:29 using AcqMethod CI VOLATILES 35 TO 280 DEG 50MIN.M
Instrument ICIPE MSD
Sample Name TM 8H
Misc Info TRIAZOLE DERIVATIVE OF THYMOL
Vial Number 24



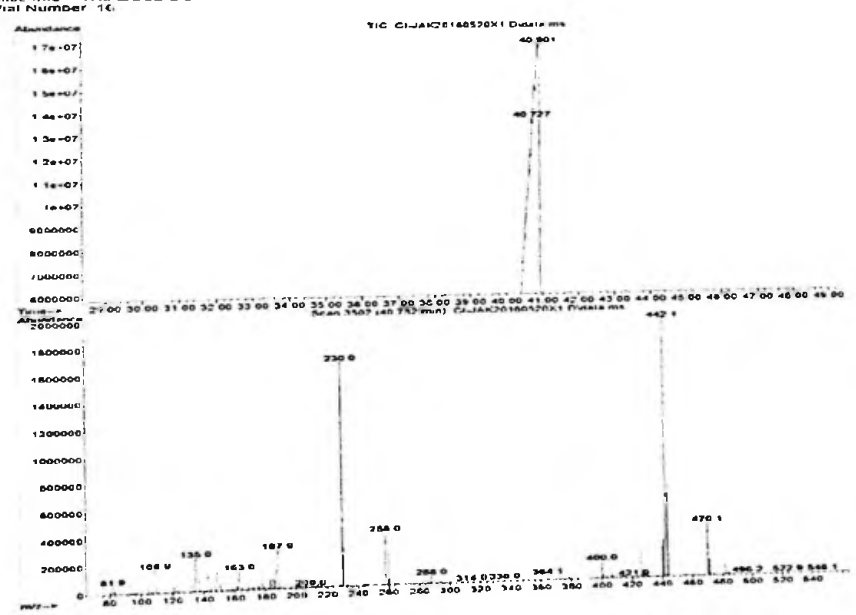
APPENDIX F-5

MASS SPECTRA (CI) FOR TM 8I &TM 8J

File C:\gcms\CI_data\CI-JAK20160520X.D
Operator JAK
Acquired 27 May 2016 14:22 using AcqMethod CI VOLATILES 35 TO 280 DEG 50MIN.M
Instrument ICIPE MSD
Sample Name TM 8I
Misc Info TRIAZOLE DERIVATIVE OF THYMOL
Vial Number 15



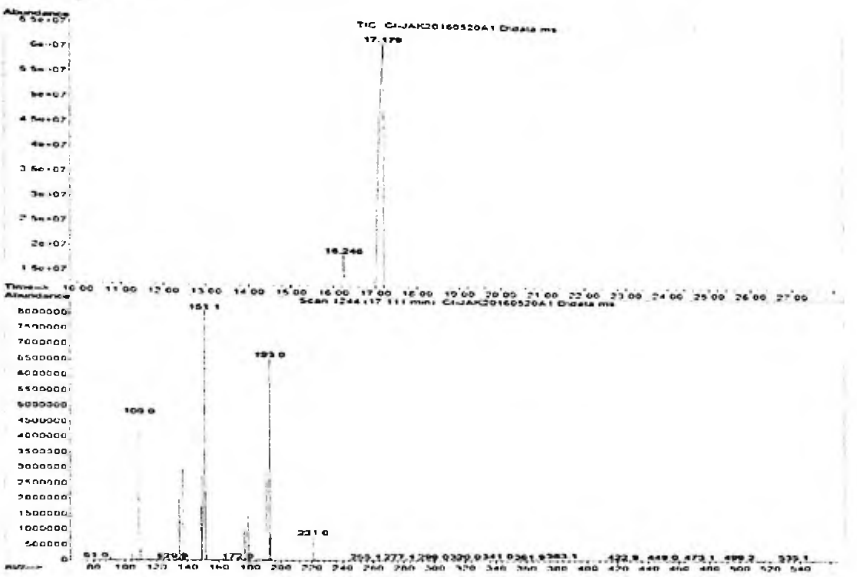
File C:\gcms\CI_data\CI-JAK20160520X1.D
Operator JAK
Acquired 27 May 2016 15:23 using AcqMethod CI VOLATILES 35 TO 280 DEG 50MIN.M
Instrument ICIPE MSD
Sample Name TM 8J
Misc Info TRIAZOLE DERIVATIVE OF THYMOL
Vial Number 16



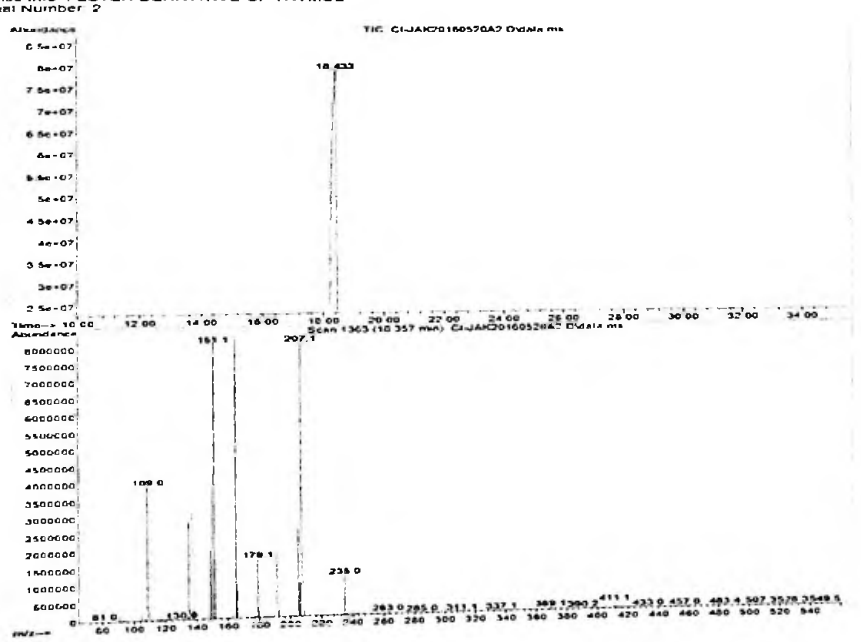
APPENDIX G-1

MASS SPECTRA (CI) FOR TM 1A & TM 1B

File C:\gcms\CI_data\CI-JAK20160520A1.D
Operator JAK
Acquired 25 May 2016 2:06 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 1A
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 1



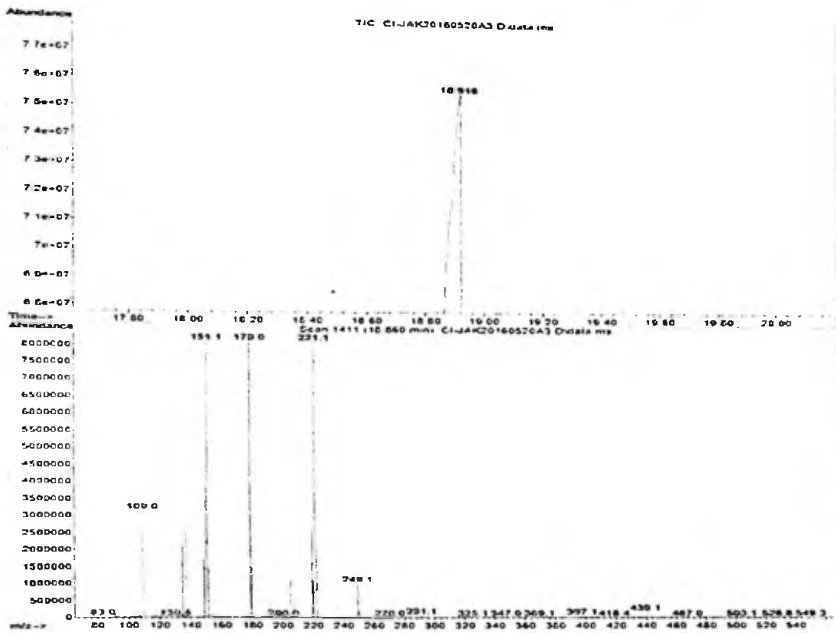
File C:\gcms\CI_data\CI-JAK20160520A2.D
Operator JAK
Acquired 25 May 2016 2:54 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 1B
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 2



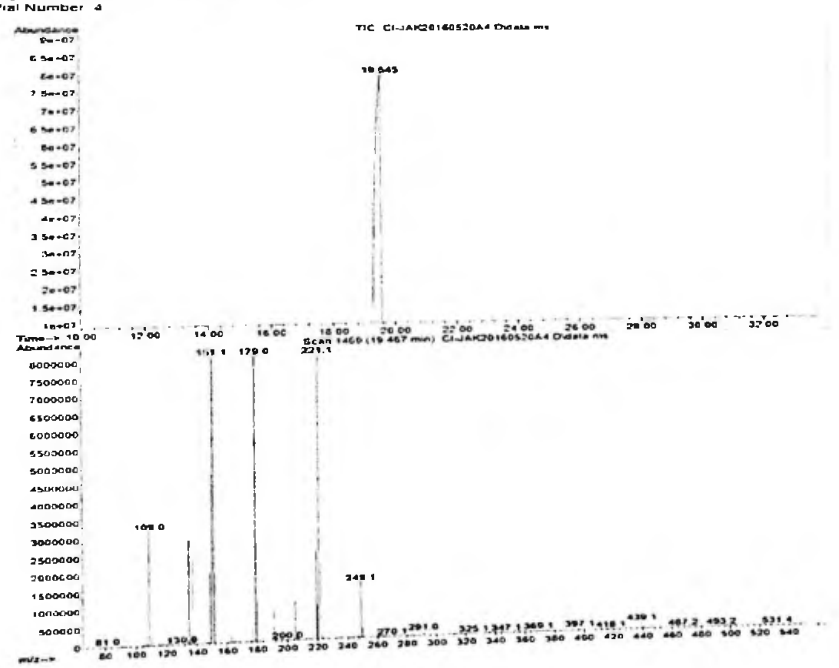
APPENDIX G-2

MASS SPECTRA (CI) FOR TM 1C & TM 1D

File C:\gcms\CI_data\CI-JAK20160520A3.D
Operator JAK
Acquired 25 May 2016 3:46 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN M
Instrument ICIPE MSD
Sample Name TM 1C
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 3

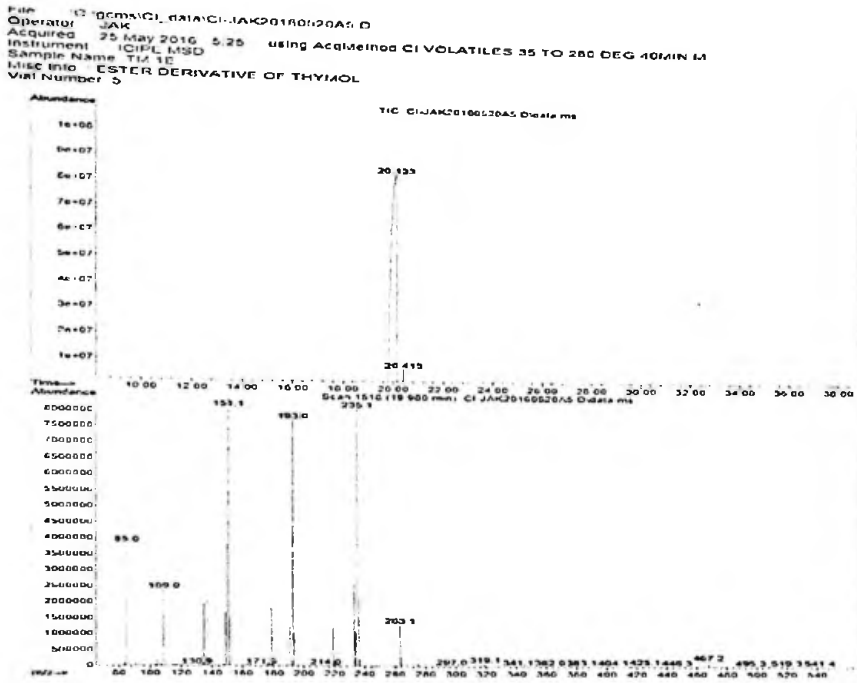


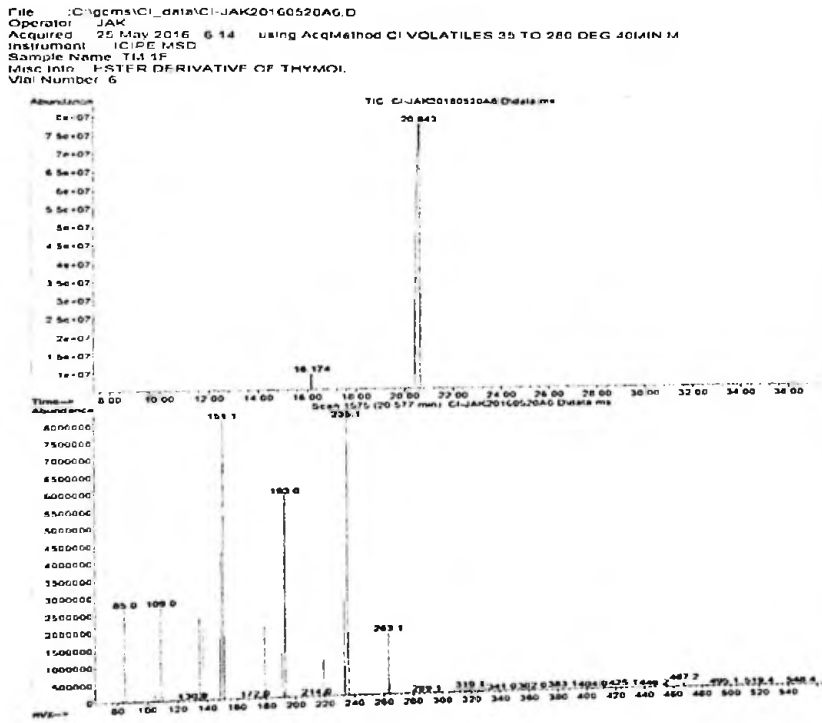
File C:\gcms\CI_data\CI-JAK20160520A4.D
Operator JAK
Acquired 25 May 2016 4:35 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN M
Instrument ICIPE MSD
Sample Name TM 1D
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 4



APPENDIX G-3

MASS SPECTRA (CI) FOR TM 1E & TM 1F

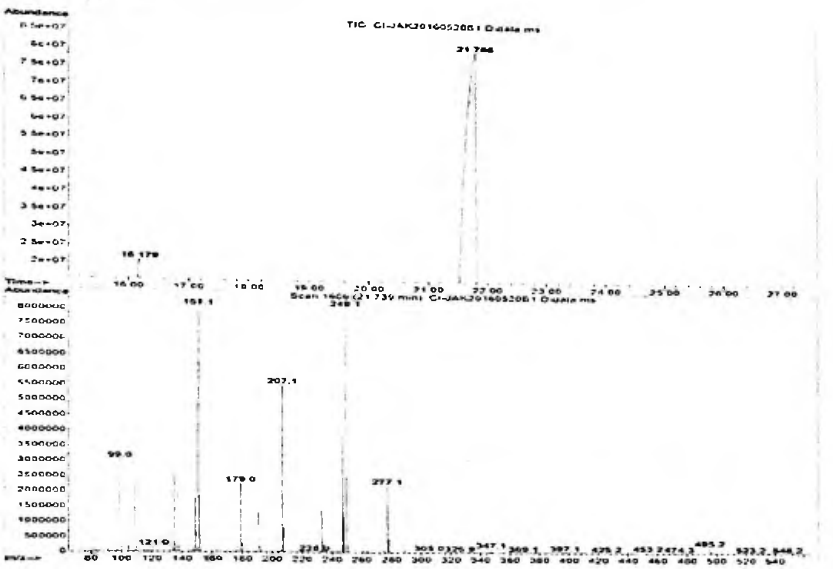




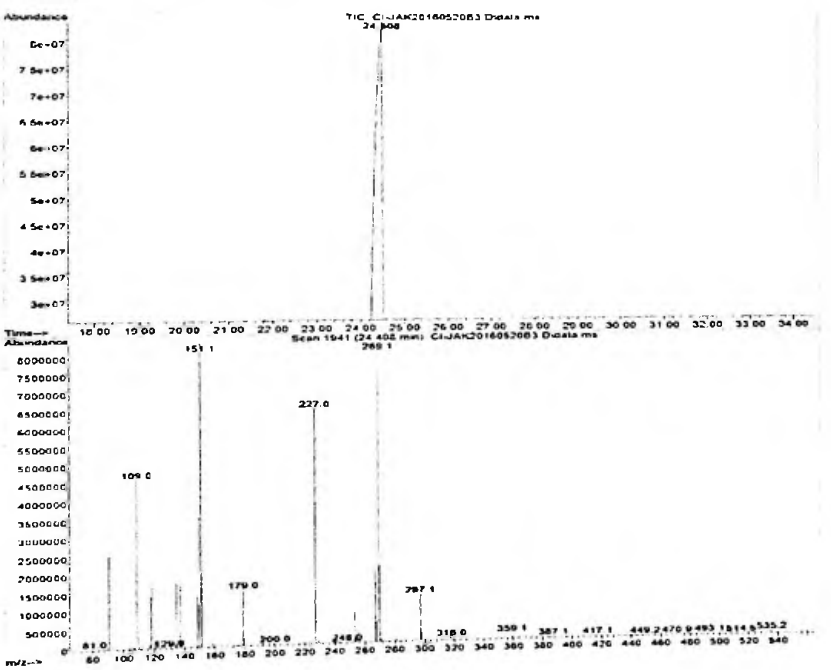
APPENDIX G-4

MASS SPECTRA (CI) FOR TM 1G & TM 1I

File C:\gcms\CI_data\CI-JAK20160520B1.D
Operator JAK
Acquired 25 May 2016 7:40 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE 5975D
Sample Name TM 1G
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 7



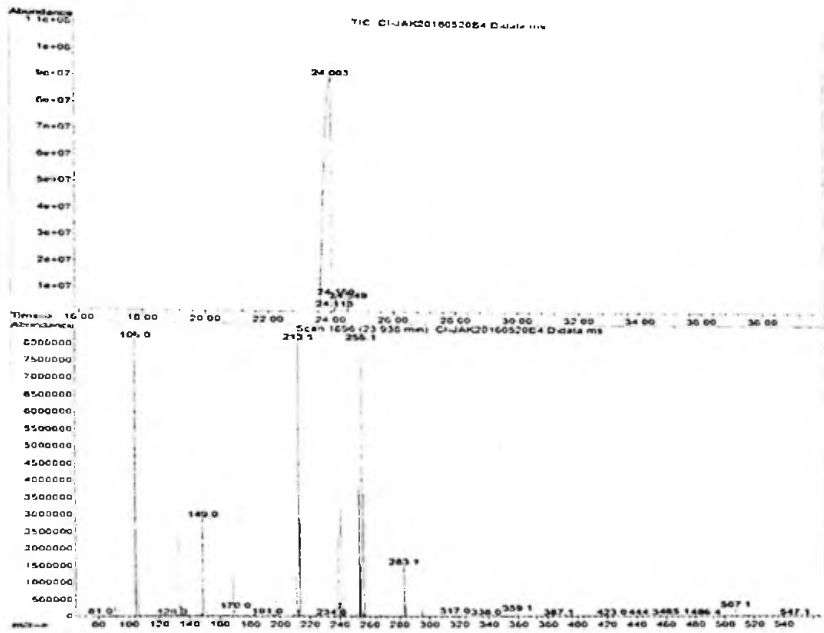
File C:\gcms\CI_data\CI-JAK20160520B3.D
Operator JAK
Acquired 25 May 2016 9:20 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 1I
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 9



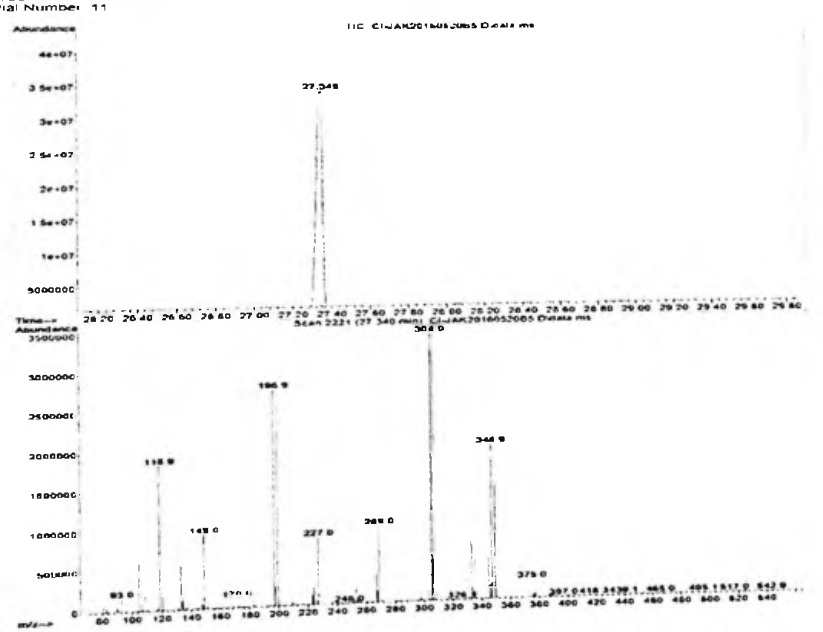
APPENDIX G-5

MASS SPECTRA (CI) FOR TM 1K & TM 1L

File C:\gcms\CI_data\CI-JAK20160520B4.D
Operator JAK
Acquired 25 May 2016 10:10 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 1K
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 10

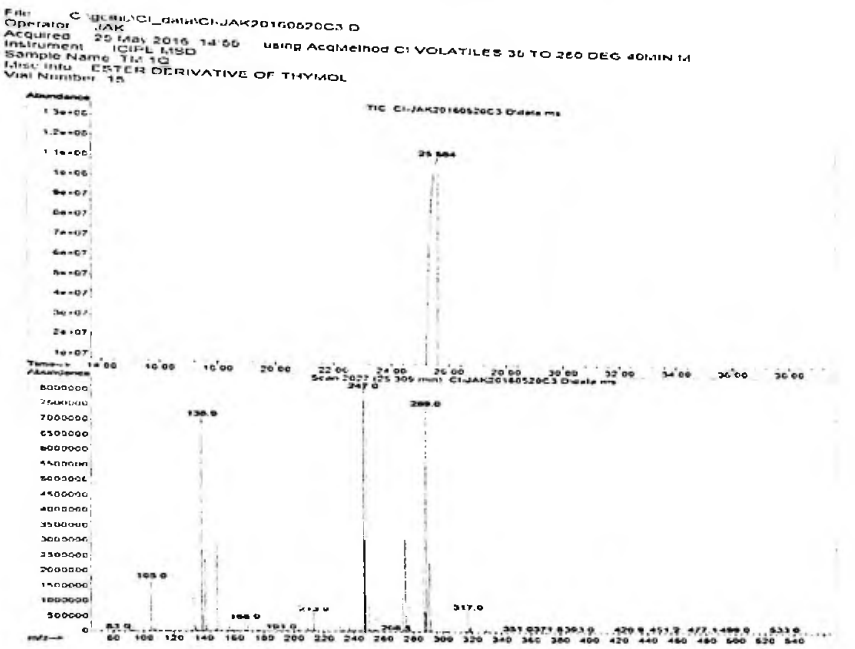


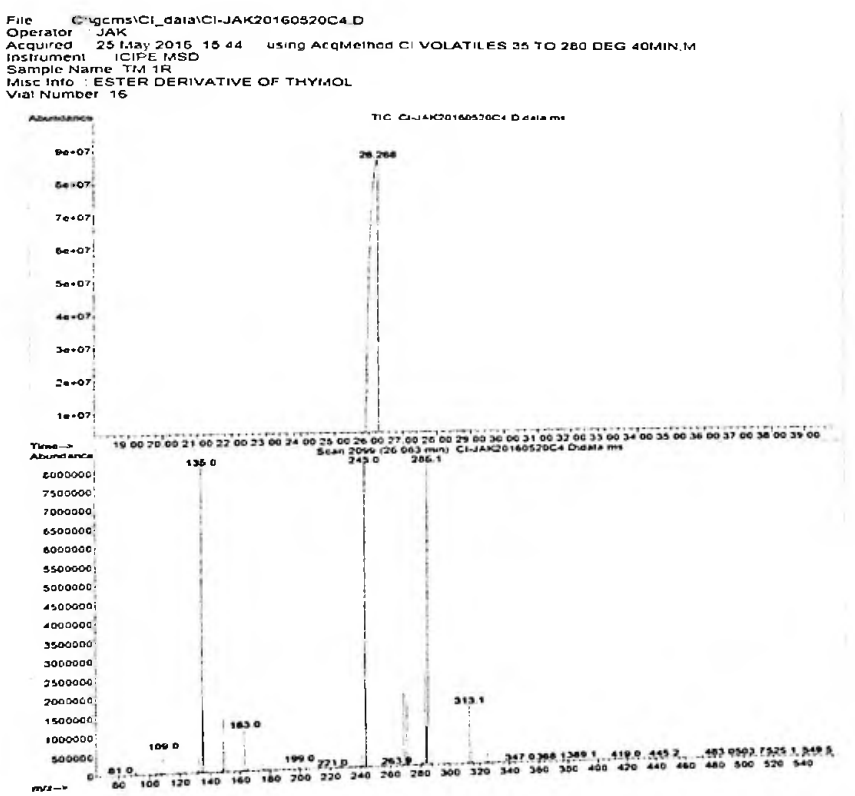
File C:\gcms\CI_data\CI-JAK20160520B5.D
Operator JAK
Acquired 25 May 2016 11:00 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 1L
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number 11



APPENDIX G-7

MASS SPECTRA (CI) FOR TM 1Q & TM 1R

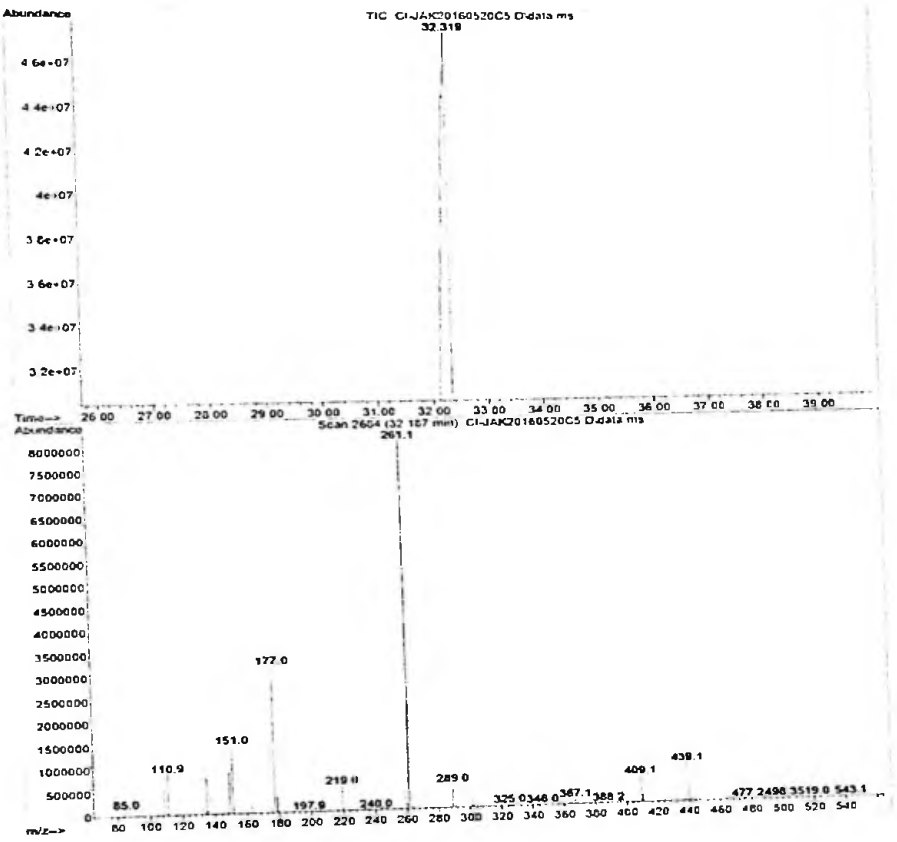




APPENDIX G-8

MASS SPECTRUM (CI) FOR TM 1U

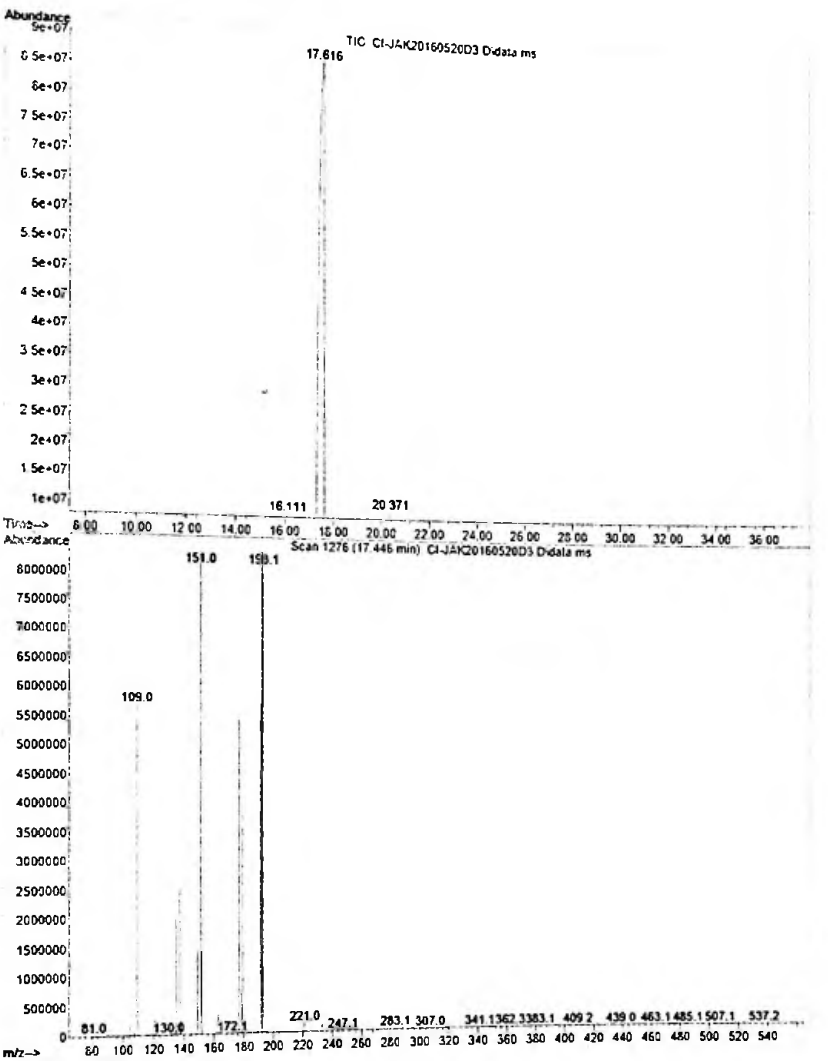
File C:\gcms\CI_data\CI-JAK20160520C5.D
Operator JAK
Acquired 25 May 2016 16:35 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN M
Instrument ICIPE MSD
Sample Name: TM 1U
Misc Info ESTER DERIVATIVE OF THYMOL
Vial Number: 17



APPENDIX H-1

MASS SPECTRUM (CI) FOR TM 2C

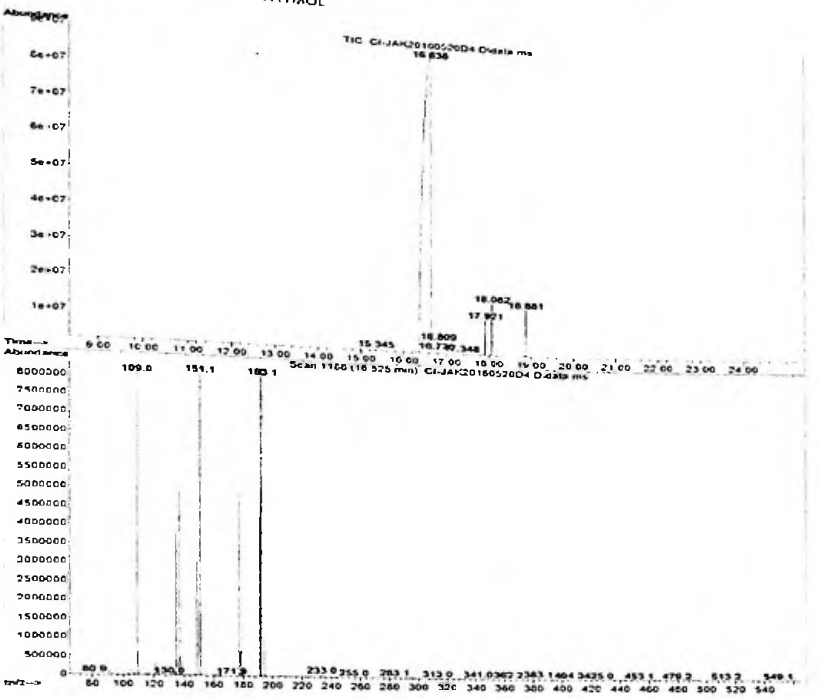
File : C:\gcms\CI_data\CI-JAK20160520D3.D
Operator : JAK
Acquired : 25 May 2016 19:40 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument : ICIPE MSD
Sample Name: TM 2C
Misc Info : ETHER DERIVATIVE OF THYMOL
Vial Number: 20



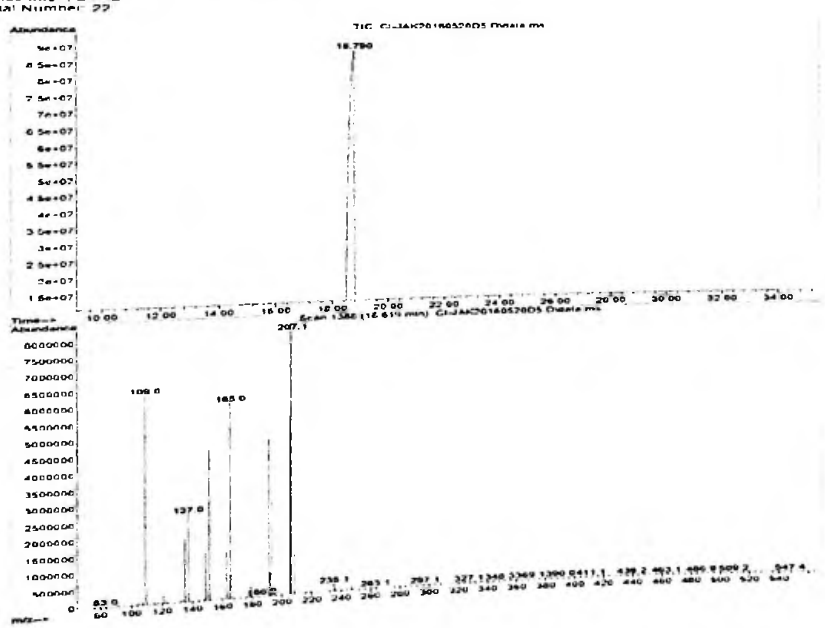
APPENDIX H-2

MASS SPECTRA (CI) FOR TM 2D & TM 2E

File C:\gcms\CI_data\CI-JAK20160520D4.D
Operator JAK
Acquired 25 May 2016, 20:30 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 2D
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number: 21



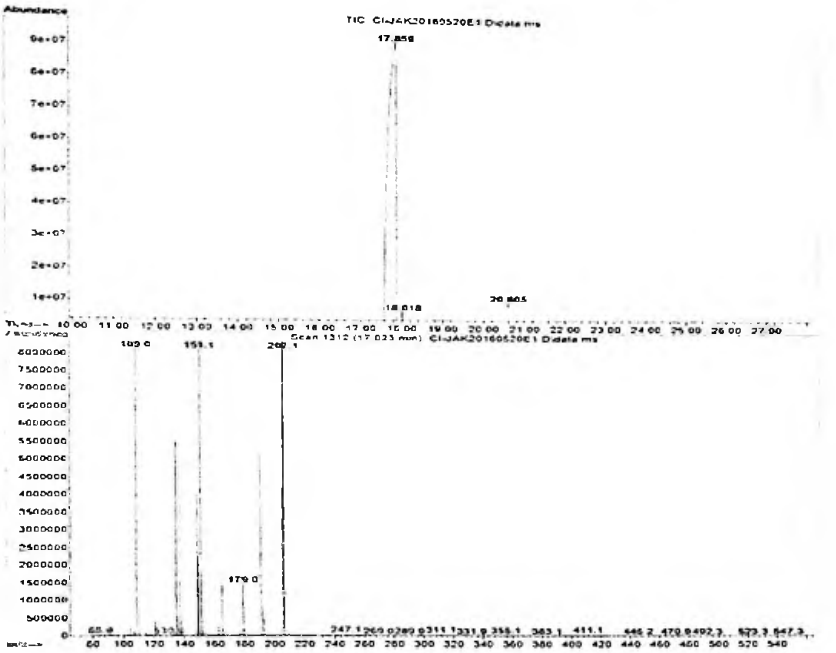
File C:\gcms\CI_data\CI-JAK20160520D5.D
Operator JAK
Acquired 25 May 2016, 21:20 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 2E
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number: 22



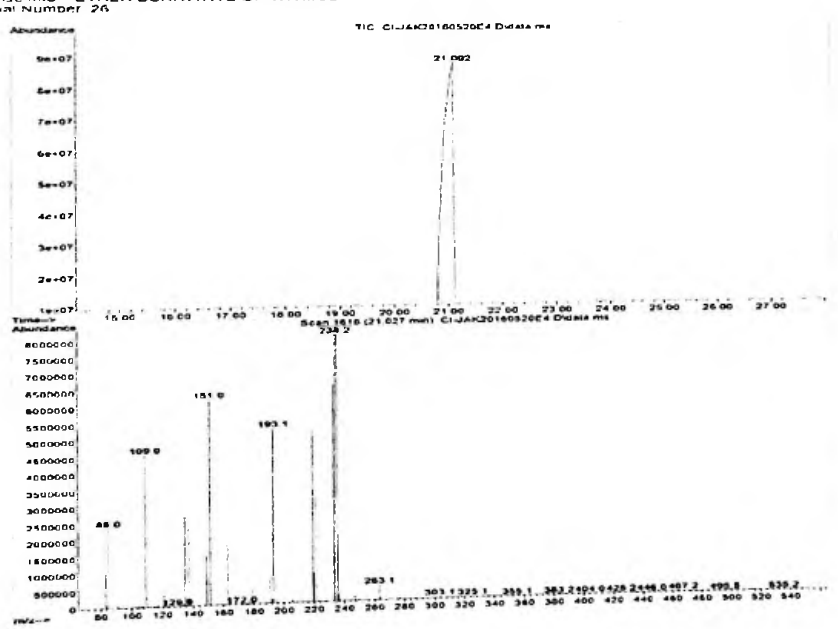
APPENDIX H-3

MASS SPECTRA (CI) FOR TM 2F & TM 2I

File C:\gcms\CI_data\CI-JAK20160520E1.D
Operator JAK
Acquired 20 May 2016 2:12 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 2F
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number 23



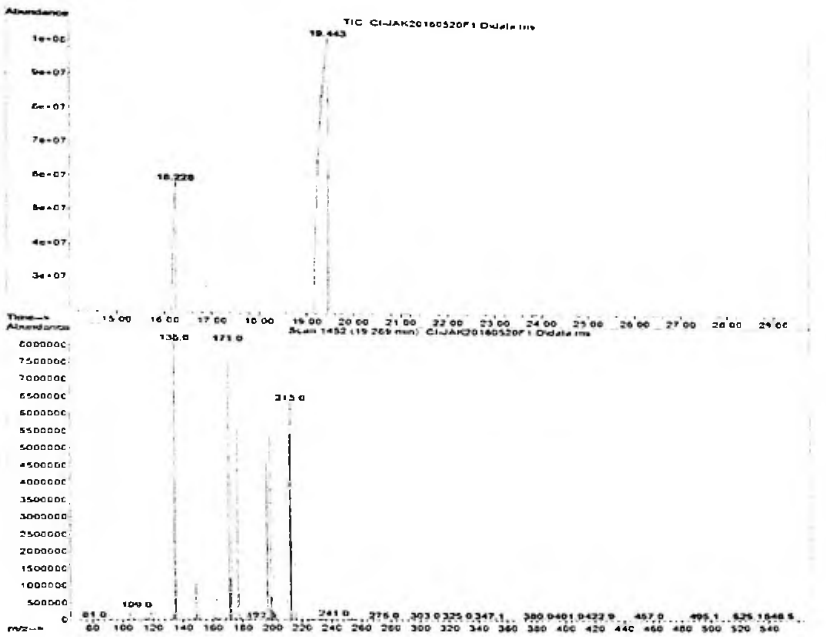
File C:\gcms\CI_data\CI-JAK20160520E4.D
Operator JAK
Acquired 20 May 2016 4:42 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 2I
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number 26



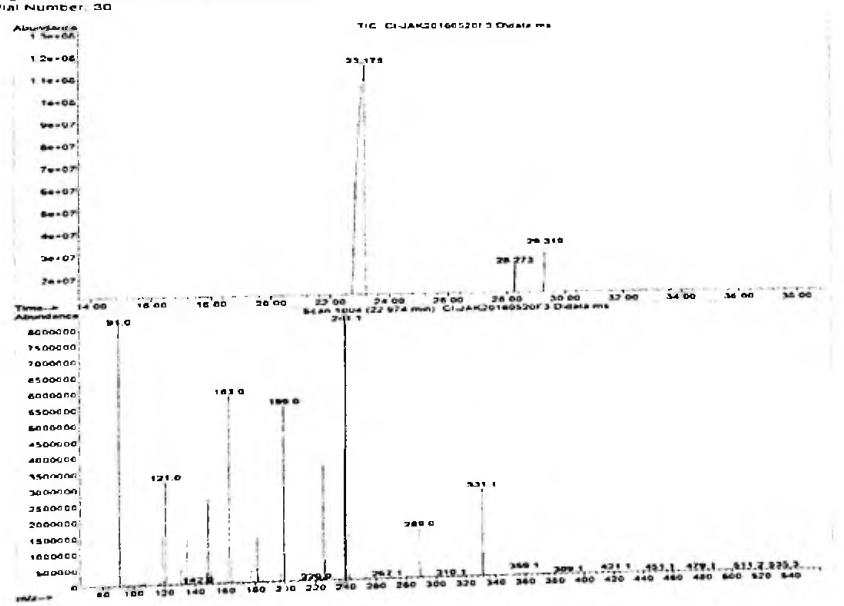
APPENDIX H-4

MASS SPECTRA (CI) FOR TM 2K & TM 2N

File C:\gcms\CI_data\CI-JAK20160520F1.D
Operator JAK
Acquired 26 May 2016 6:57 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 2K
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number 28



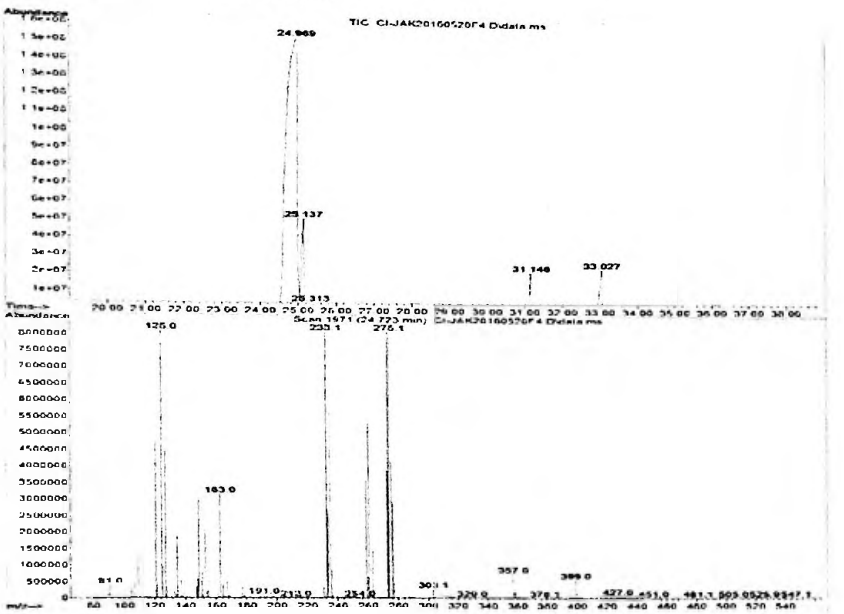
File C:\gcms\CI_data\CI-JAK20160520F3.D
Operator JAK
Acquired 26 May 2016 8:37 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name TM 2N
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number: 30



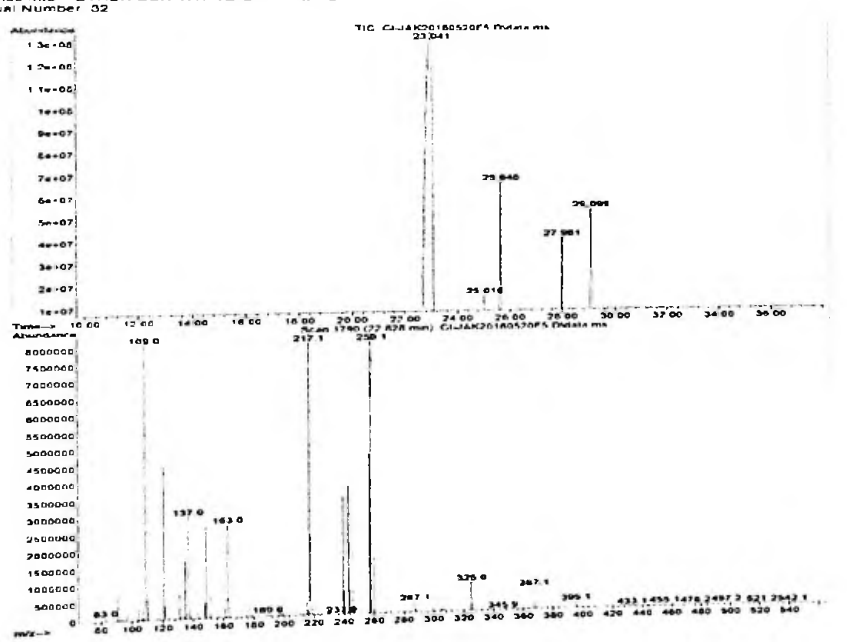
APPENDIX H-5

MASS SPECTRA (CI) FOR TM 2O & TM 2P

File C:\gcms\CI_data\CI-JAK20160520F4.D
Operator JAK
Acquired 26 May 2016 9:27 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name: TM 2O
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number: 31

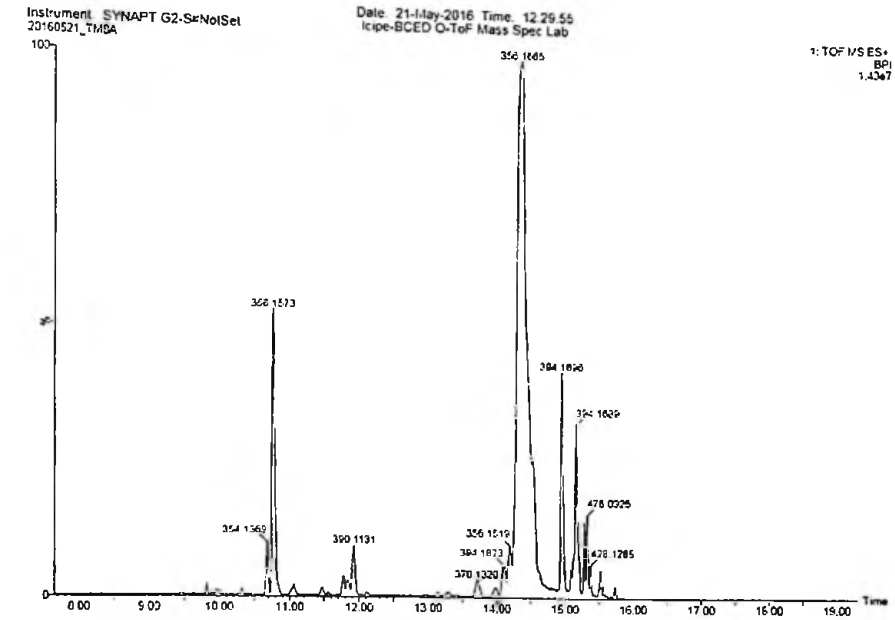


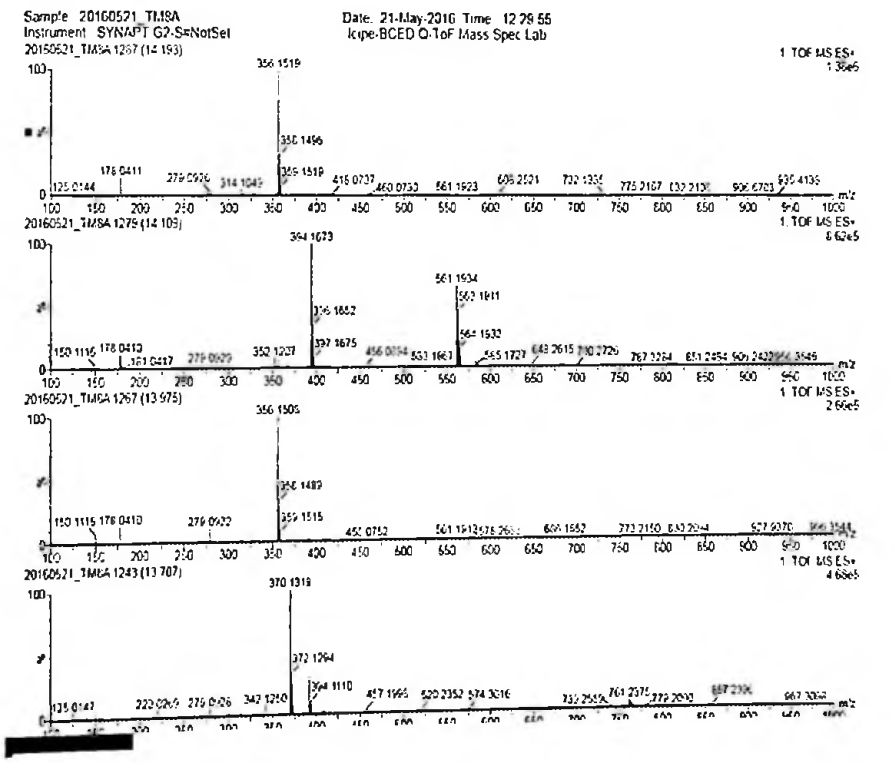
File C:\gcms\CI_data\CI-JAK20160520F5.D
Operator JAK
Acquired 26 May 2016 10:17 using AcqMethod CI VOLATILES 35 TO 280 DEG 40MIN.M
Instrument ICIPE MSD
Sample Name: TM 2P
Misc Info ETHER DERIVATIVE OF THYMOL
Vial Number: 32



APPENDIX I-1:

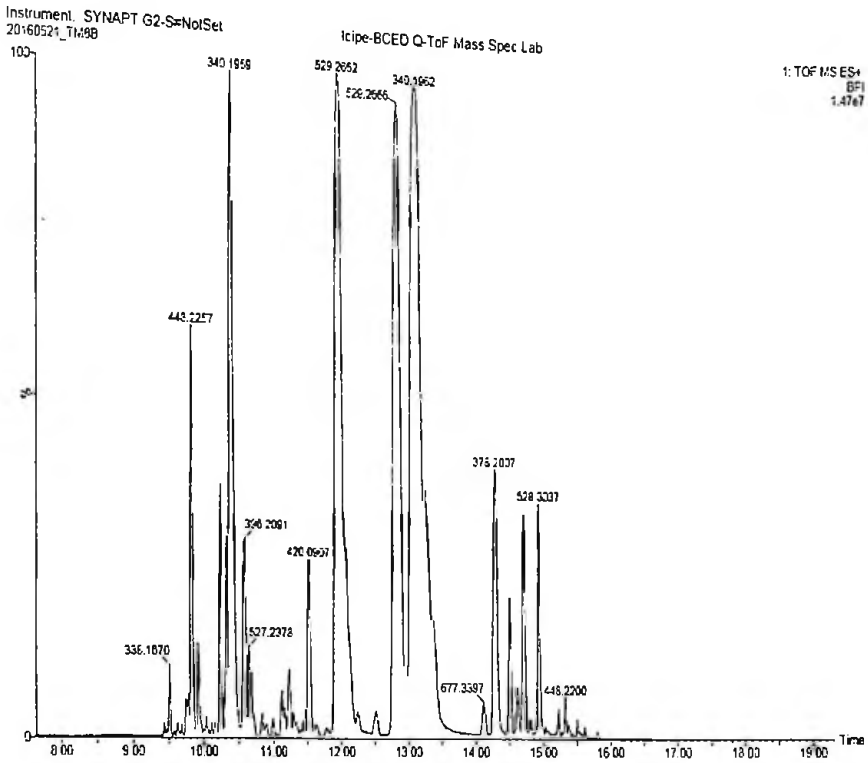
MASS SPECTRUM (HRMS ES^{+}) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8A)

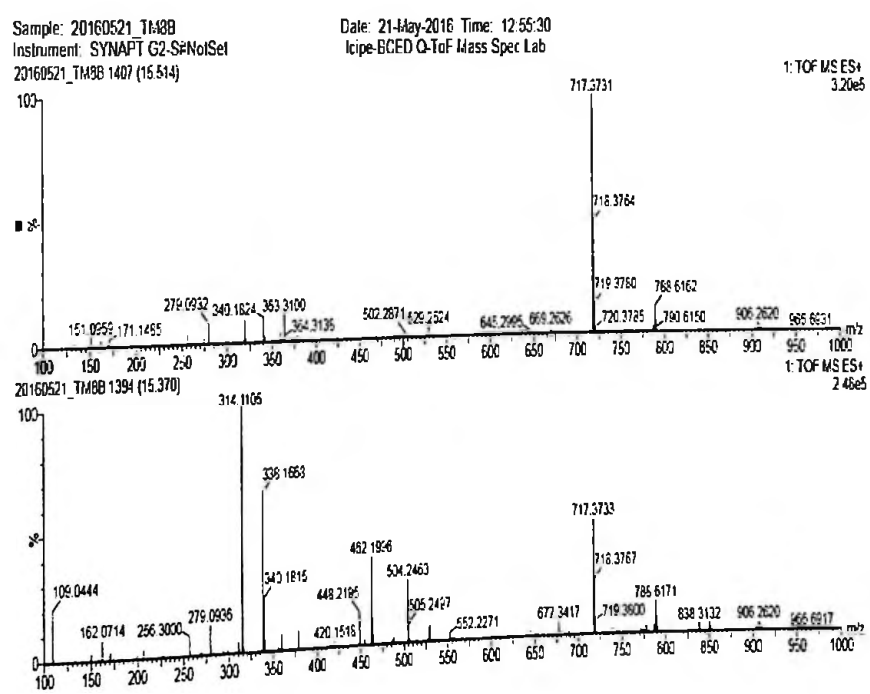




APPENDIX I-2:

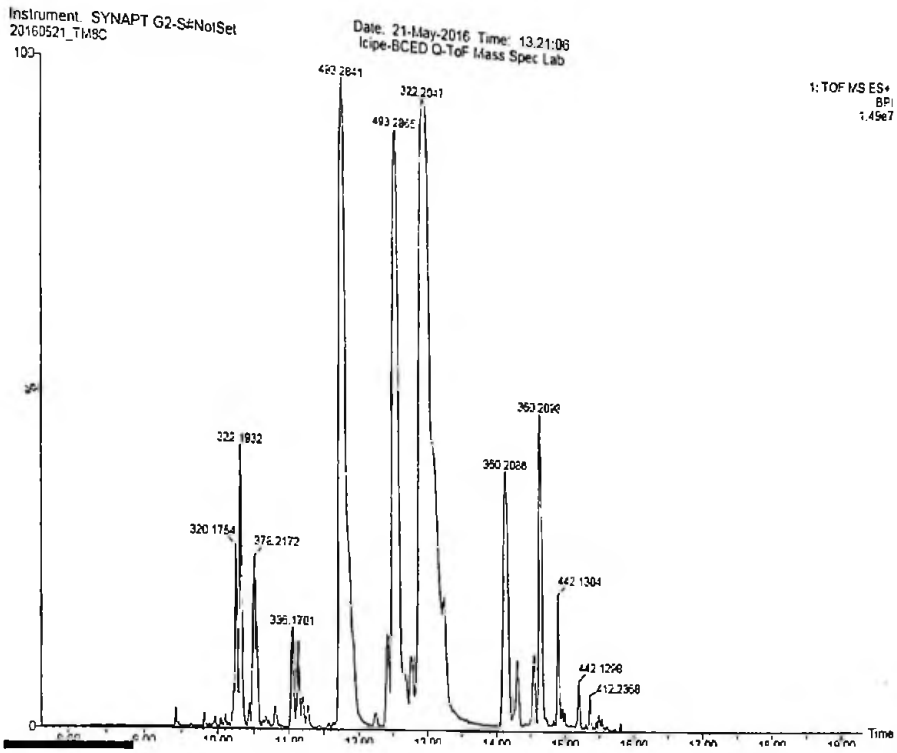
MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8B)

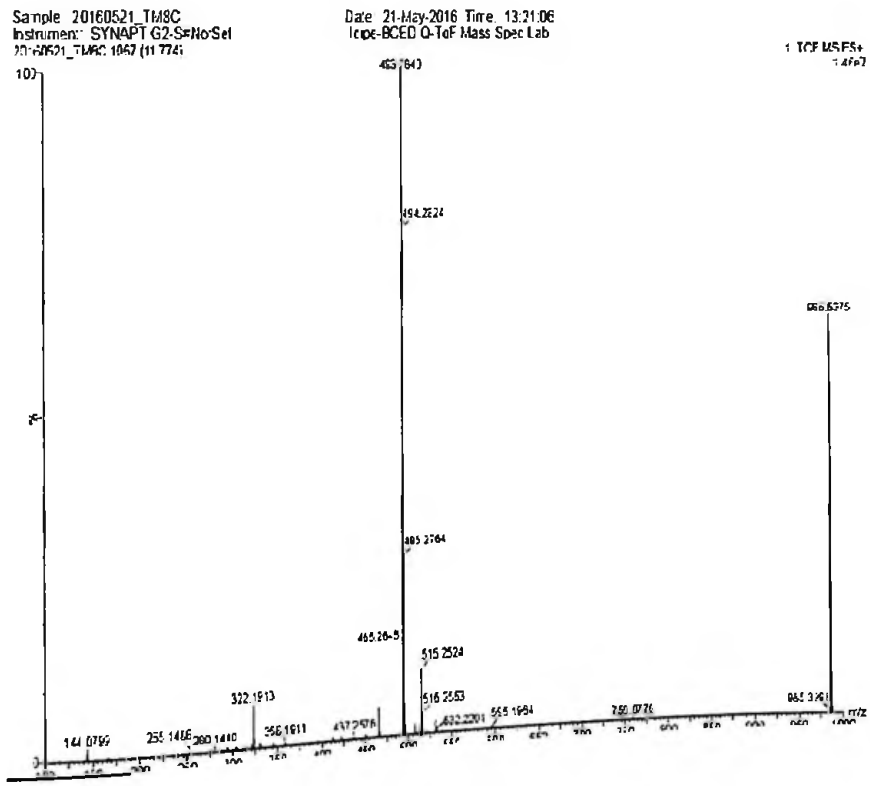




APPENDIX I-3:

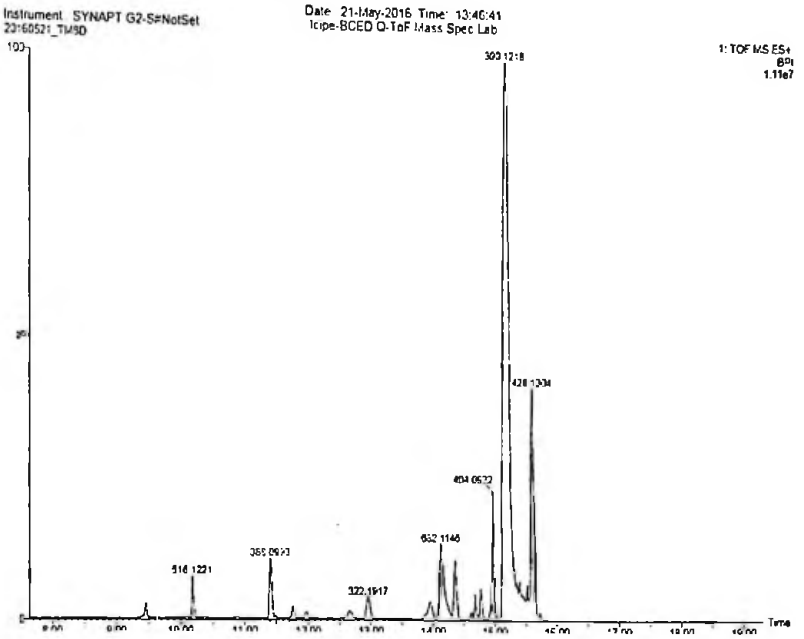
MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8C)

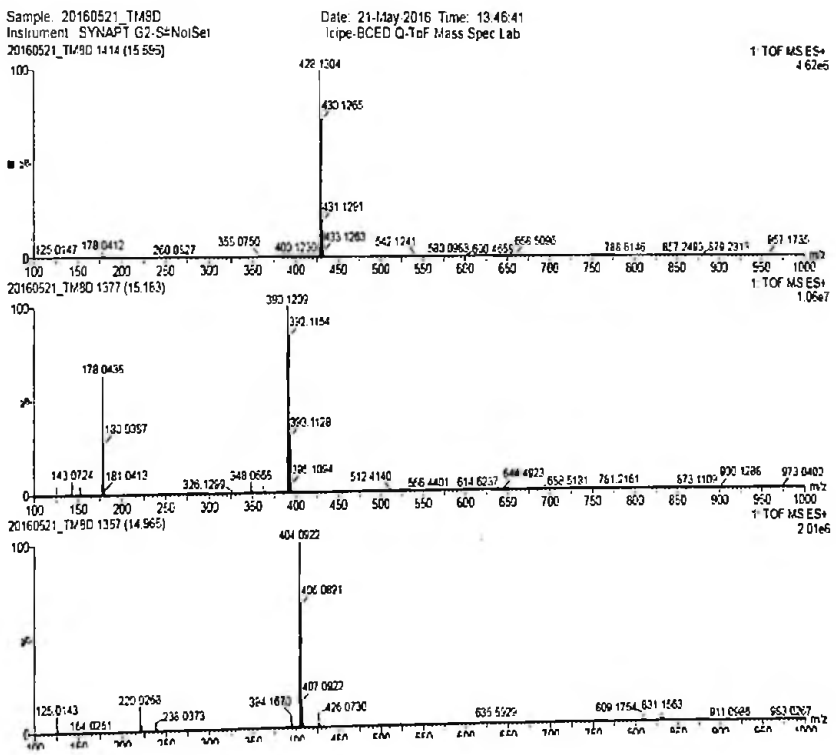




APPENDIX I-4:

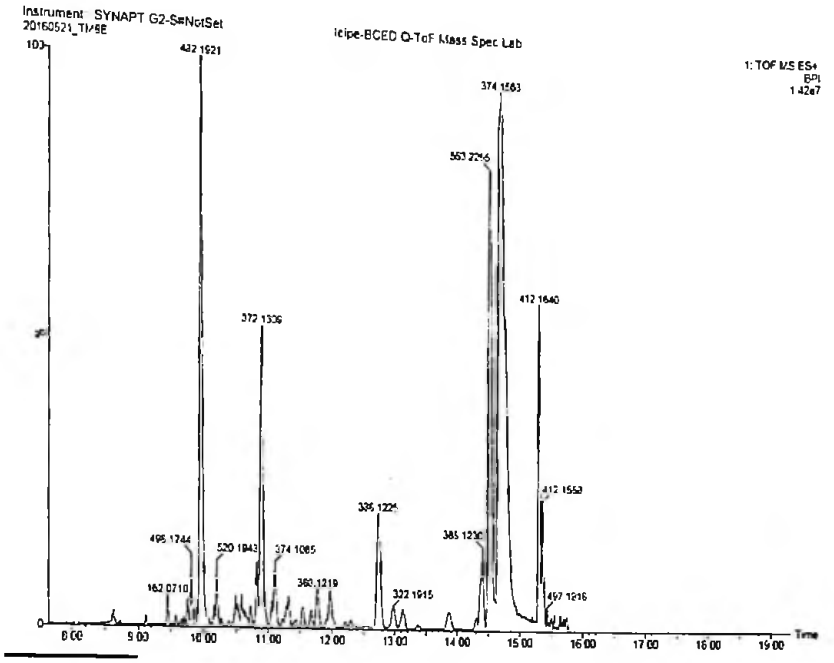
MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8D)

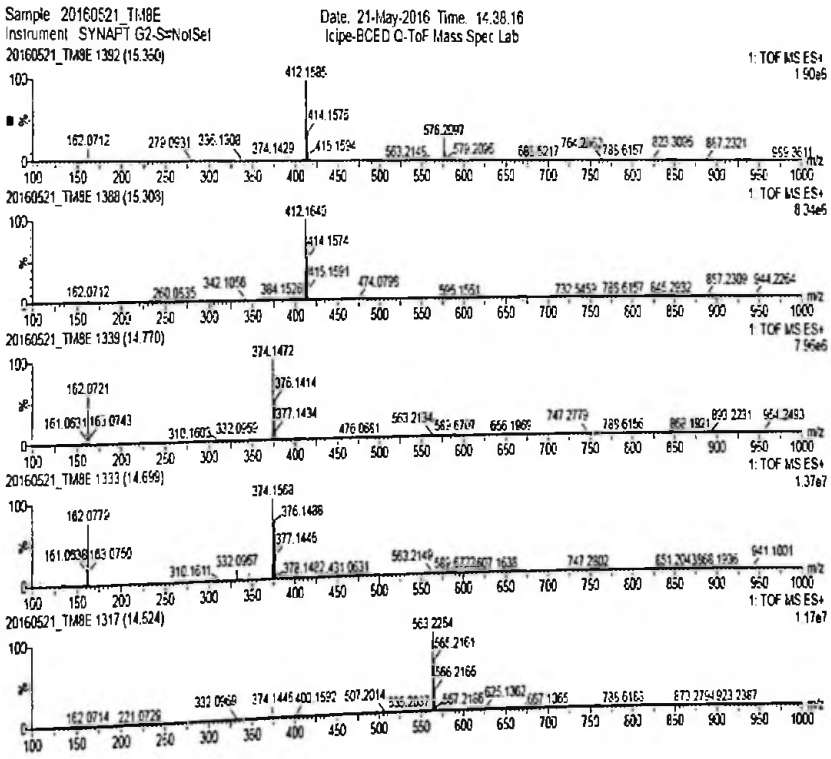




APPENDIX I-5

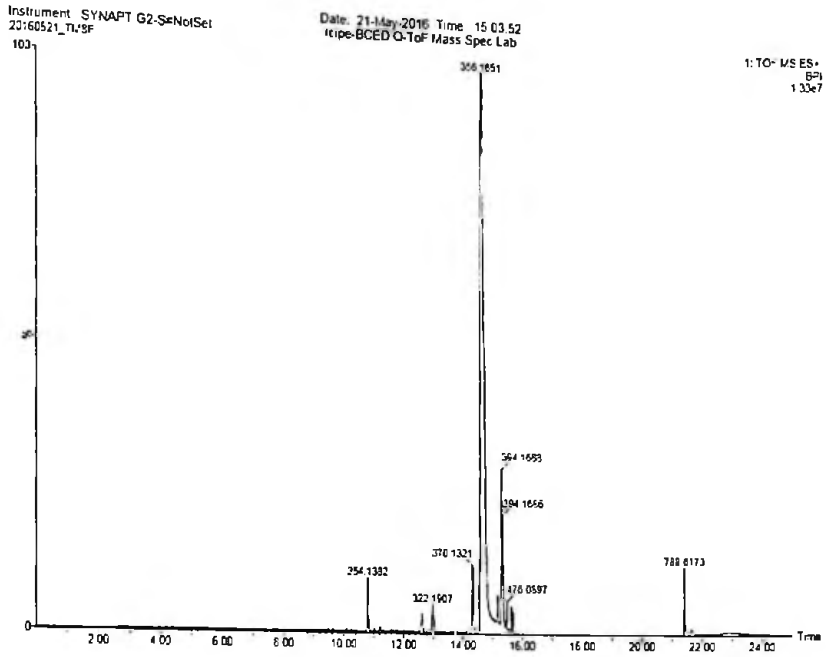
MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8E)

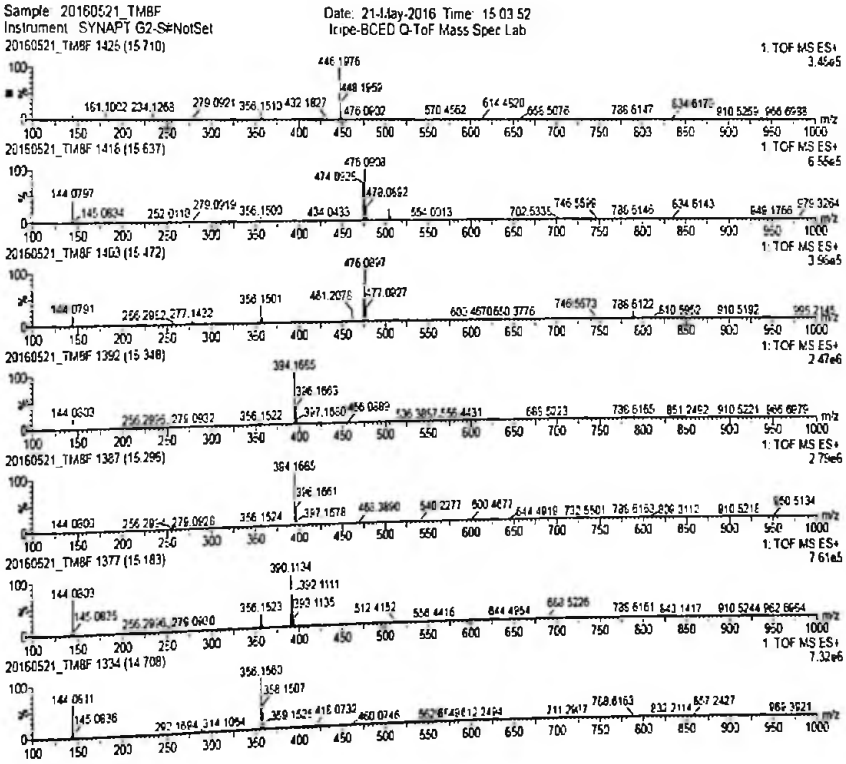




APPENDIX I-6

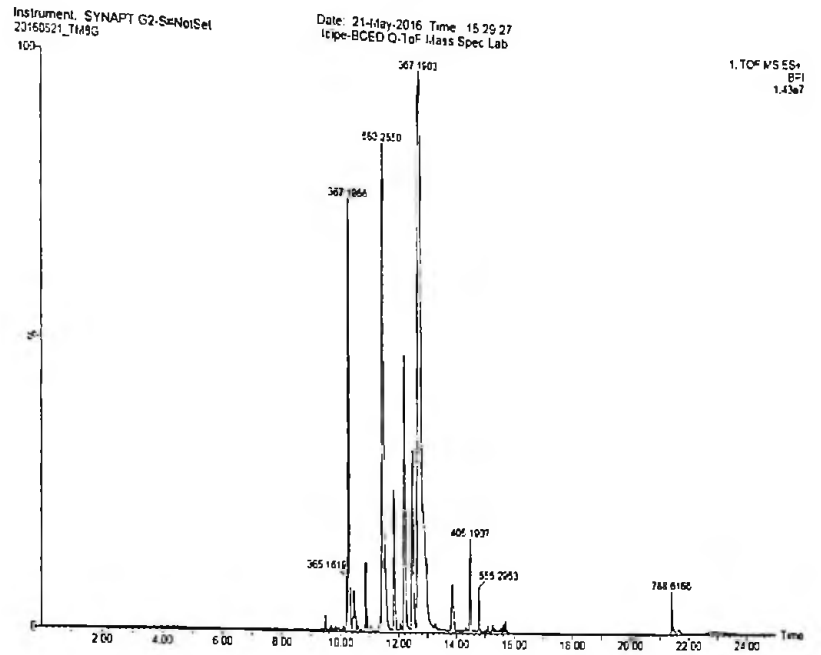
MASS SPECTRUM (HRMS ES^{+}) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8F)

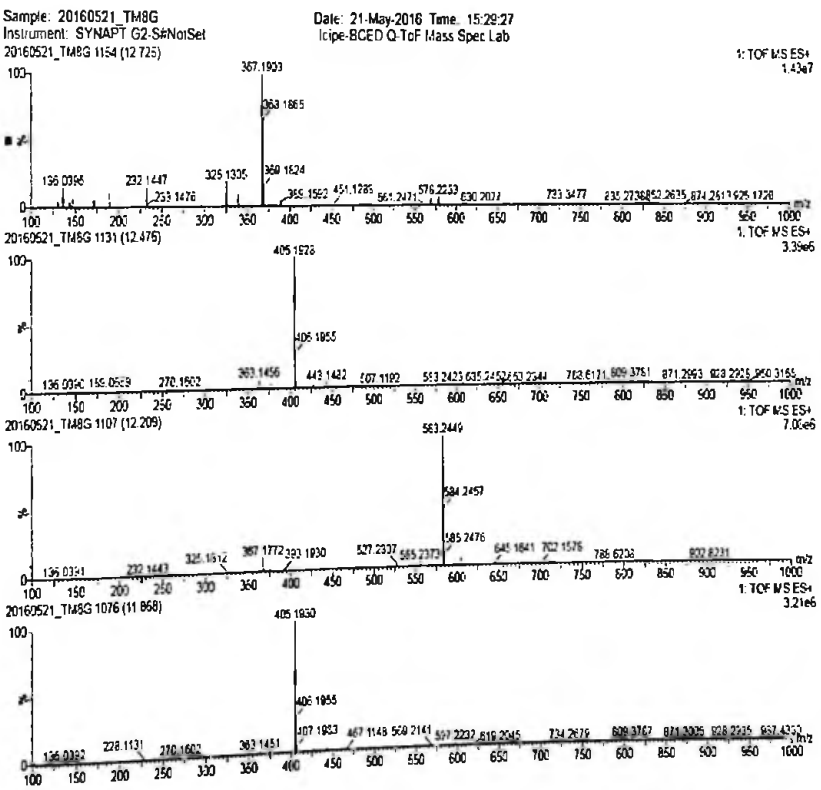




APPENDIX I-7

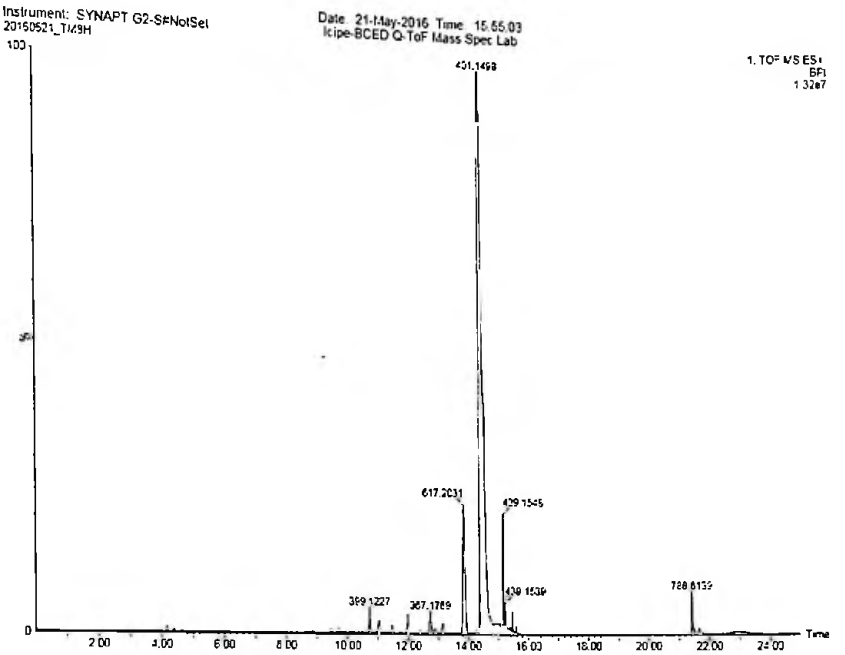
MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8G)

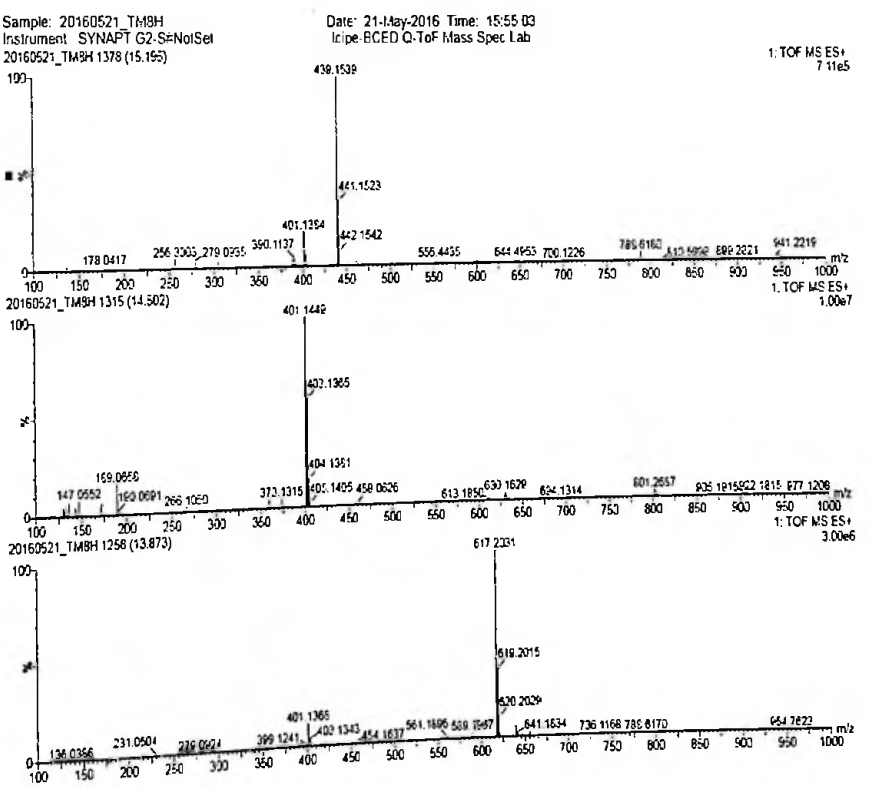




APPENDIX I-8

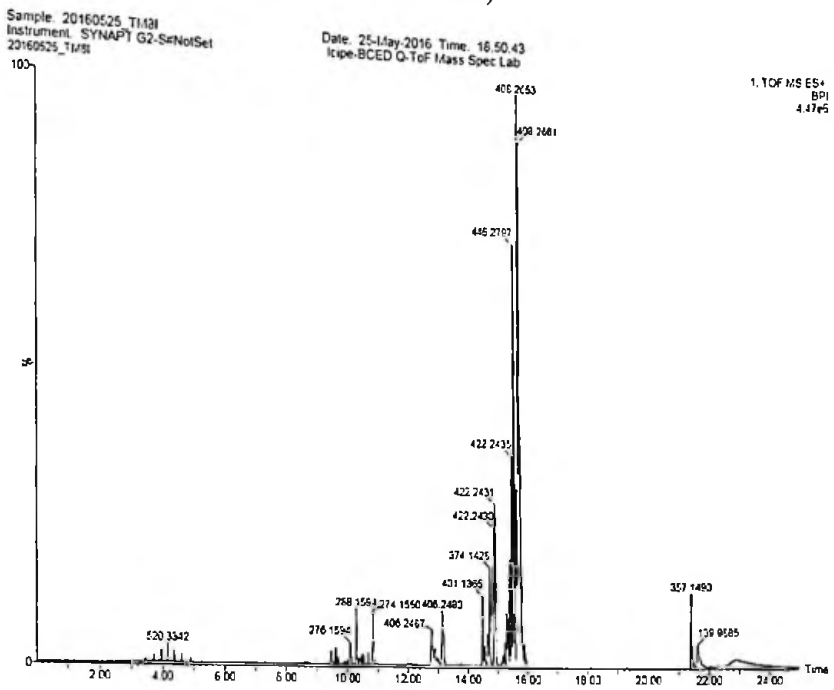
MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8H)

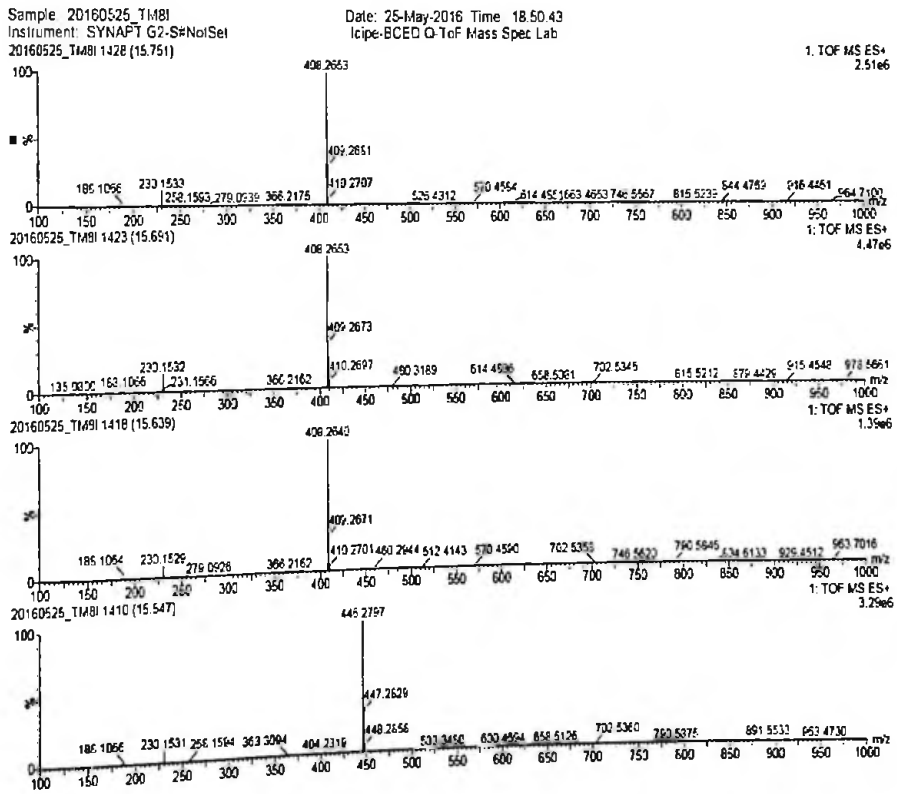




APPENDIX I-9

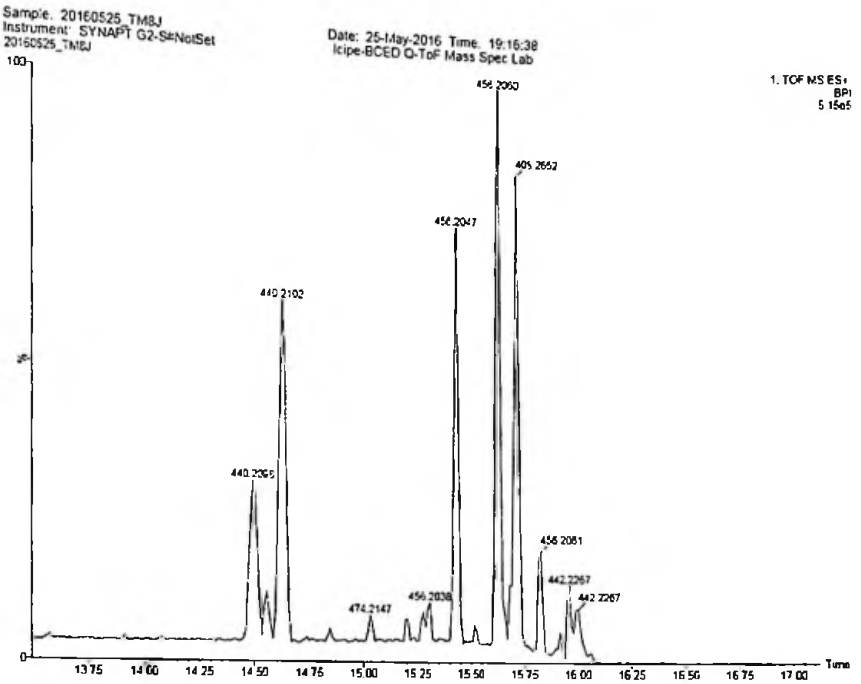
MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8I)

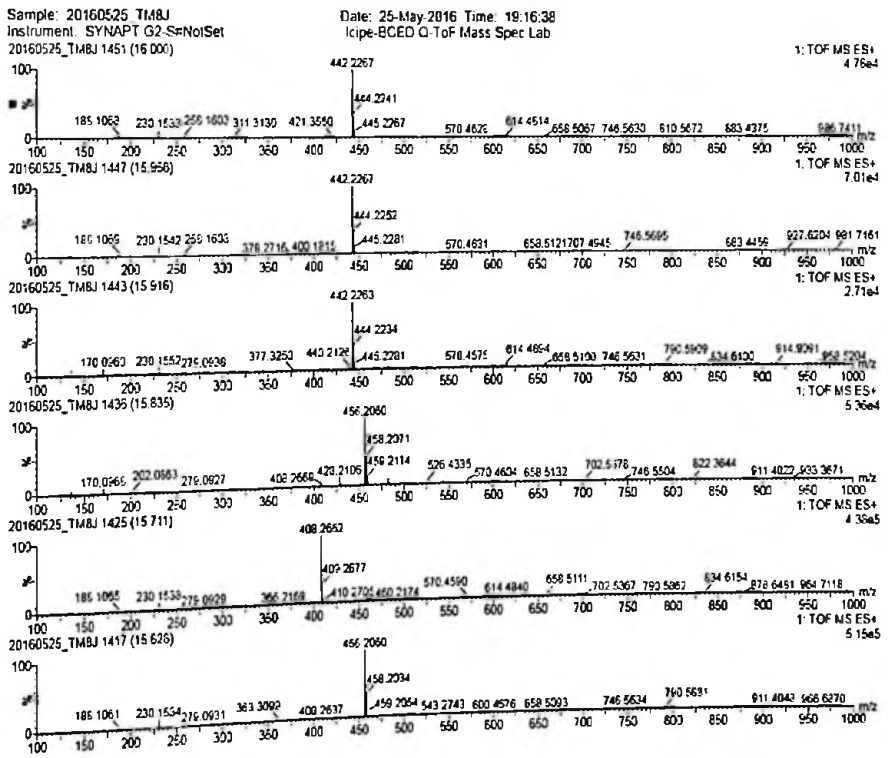




APPENDIX I-10

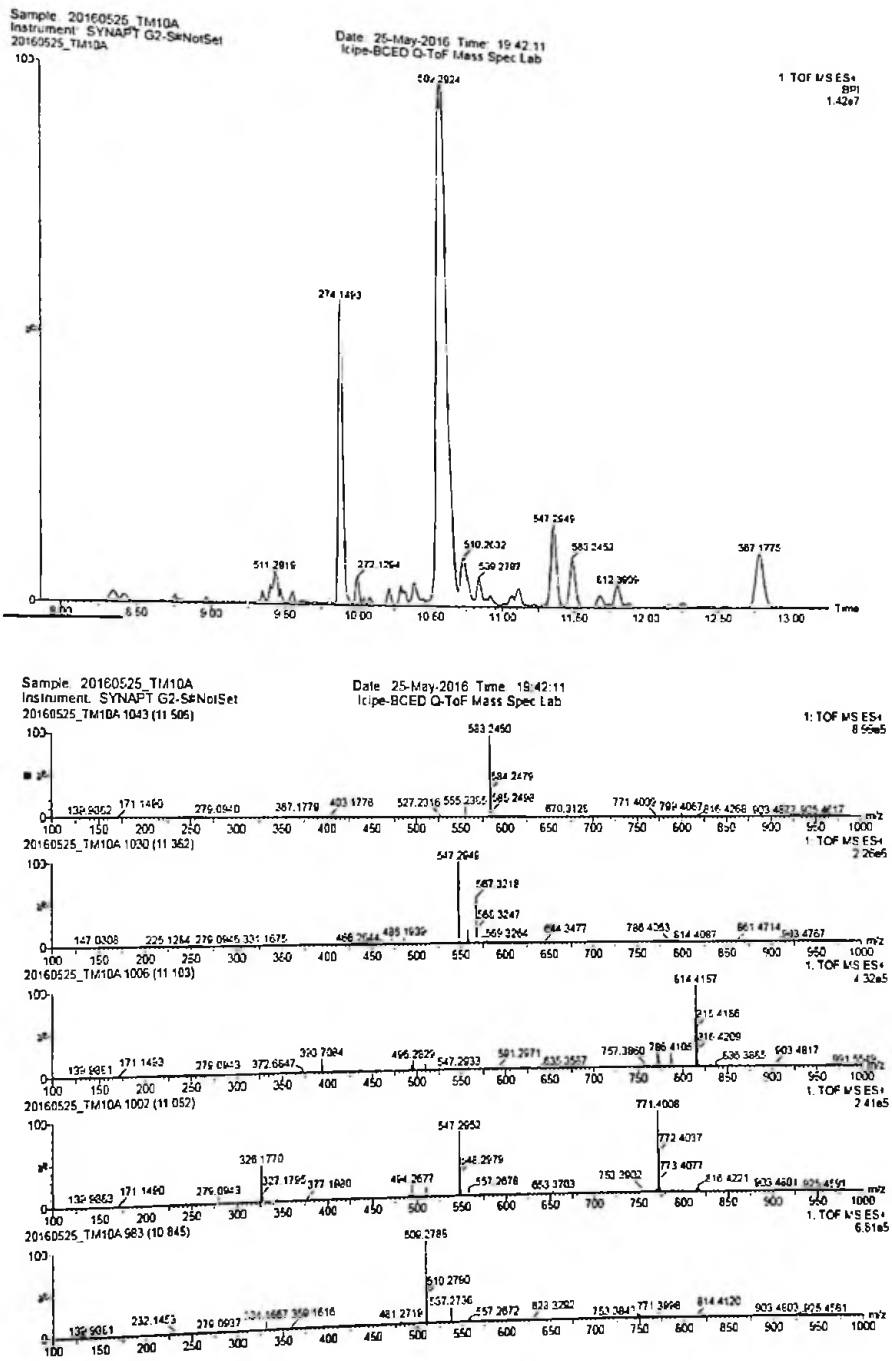
MASS SPECTRUM (HRMS ES^{+}) FOR 1, 2, 3-TRIAZOLE DERIVATIVES (TM 8J)





APPENDIX J-1

MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES OF THYMOL-PARTHENIN COUPLING PRODUCT (TM 10A)



APPENDIX J-2

MASS SPECTRUM (HRMS ES$^+$) FOR 1, 2, 3-TRIAZOLE DERIVATIVES OF THYMOL-PARTHENIN COUPLING PRODUCT (TM 10B)