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Phytochemical Studies and Antioxidant Properties of Methanolic and Aqueous Extracts of the Leaves of *Mallotus Oppositifolius*

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Abstract

Mallotus oppositifolius is a tropical plant used to treat many medical conditions in Africa. Preliminary phytochemical screening conducted on the crude methanol and aqueous extracts of the plant revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides and terpenoids in both extracts of the plant. However, only the aqueous extract showed the absence of anthraquinones.

The research also used DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical as a substrate to determine the scavenging ability (antioxidant property) of the extracts. Antioxidant activity was further investigated quantitatively for flavonoid and total phenolic contents in the two extracts, using spectrophotometry. Both the methanol and aqueous extracts of the plant were observed to have exhibited antioxidant activity, with the aqueous extract having the higher scavenging ability (% inhibition) of 49.02 ± 0.16 than the methanol extract of 9.04 ± 0.07 .

Similarly, the aqueous extract had a higher flavonoid content of $206.8 \pm 31.06 \mu\text{g/g}$ than the methanol extract $84.00 \pm 33.96 \mu\text{g/g}$. Also, the aqueous extract had its total phenolic content to be $1066.97 \pm 26.42 \mu\text{g/g}$ while the methanol extract had $672.0 \pm 20.07 \mu\text{g/g}$ as its total phenolic content. The percentage alkaloid content in methanol and aqueous extracts were measured to be 8.6 % and 4.3% respectively.

Key Words: Phytoconstituents, antioxidant activity, flavonoid content, alkaloid content

1.0 Introduction

The assertion that the last man dies if the last plant dies is not far from the truth that man, just as all other animals, constantly depends on plants in many regards. Plants, which are the world's chief source of drugs, provide most of the effective and affordable drugs in traditional medicine, as used by over 80% of the world's population (Hunt et al., 1995). Herbal medicines have many advantages over synthetic drugs as, in most cases; synthetic drugs are very expensive and even inaccessible. The extracts of medicinal plants and natural products therefore have become a great source of medicines which are cheap and readily available. Many tend to depend on these medicinal plants due to their high therapeutic values. The quest for plants with medicinal properties therefore continues to receive attention as scientists are in need of plants for a wide range of biological activities such as antibiotic, antiviral, anticancerous and antioxidants. These medicinal properties are linked to the presence of phytoconstituents called secondary metabolites which possess these biological properties. The assessment of the medicinal potency of any plant therefore depends on the phytoconstituents present and the antioxidant capacity. The antioxidant property is the main contributory factor to the therapeutic benefit of many medicinal plants (Nayak et al, 2006). Hence, there has been an increasing interest among scientists in the therapeutic potentials of medicinal plants as antioxidants.

Antioxidants are compounds that detoxify reactive oxygen species and prevent their damage through multi mechanisms (Sathya and Kokilavani, 2013). Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and thus prevent disease. Antioxidants have the ability to prevent, delay or ameliorate many of the effects of free radicals. Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, antiallergic, antithrombotic and vasodilatory activities.

Antioxidant capacity is widely used as a parameter for medicinal bioactive components.

The present study therefore deals with the initial phytochemical screening and evaluation of the antioxidant capacity of the leaf extracts of *Mallotus oppositifolius* to enable us assess the medicinal value of the plant.

Mallotus oppositifolius (Geiseler) Müll. Arg., (Euphorbiaceae), is an endemic shrub from tropical Africa forests and savannas. The plant is widely used in folkloric medicine against infections, intestinal worms and malaria. Different parts of the plant have also been used to demonstrate anti-inflammatory, antioxidant, antidiarrheic, antibacterial, antifungal and antitrypanosomal properties (Kabran et al, 2012).

2.0 Materials and Methods

2.1 Plant Material

Leaves of *Mallotus oppositifolius* were obtained from a garden in Kwaprow, near the University of Cape Coast, and also at the backyard of Our Lady Seat of Wisdom Catholic Church, UCC. These leaves were duly identified by Mr. Mayking Sogbey, a horticulturalist, and Mr. Otoo the curator at the School of Biological Sciences, University of Cape Coast herbarium. They were thoroughly rinsed with clean water and evenly spread on a mosquito net-size mesh, and then air-dried at room temperature for two weeks before being ground to an even powder with a Heavy Duty blender.

2.2 Preparation of extracts

About 185g of the ground sample was cold macerated in 1145ml of methanol. The contents were intermittently shaken vigorously for three days. The extracts were then filtered using vacuum pump, and concentrated using rotary evaporator. The concentrates were then dried

in desiccators and re-weighed to calculate the percentage yield. The same procedure was followed to extract 123g of the powdered plant sample in 1150ml of distilled water. Phytochemical tests were conducted on both the methanol and aqueous extracts of the plant. Fractions of the crude extracts obtained from both solvents were separately dispersed in distilled water, filtered and successively extracted with CHCl₃, EtOAc and n-ButOH using separatory funnel. The yields from the n-ButOH were used to investigate for antioxidant activity.

2.3 Scavenging activity against 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH)

The extracts were screened for DPPH radical scavenging activity. DPPH radical scavenging activity was evaluated as described by Stankovic et al., 2010. The stock solution of the plant extract was prepared in methanol to achieve a concentration of 100µg/ml. Dilutions were made to obtain concentrations of 80µg/ml, 40µg/ml, and 20µg/ml. 1ml of each of the diluted solutions was mixed with 1ml of DPPH methanolic solution (40µg/ml). After 30 minutes in the darkness at room temperature (23°C), the absorbance was recorded at 517nm. The control sample contained all the reagents except the extract. Ascorbic acid was used as the standard antioxidant. Percent inhibition was calculated using the following expression:

$$\% \text{ inhibition} = \frac{(A_{blank} - A_{sample}) \times 100}{A_{blank}}$$

Where A_{blank} and A_{sample} stand for absorption of the blank sample and absorption of tested extract solution respectively.

2.4 Phytochemical Screening

Phytochemical screening for major bioactive constituents like alkaloids, phenolics, flavonoids, tannins, steroids and terpenoids were determined by using standard phytochemical methods (Harborne, 1998; Trease & Evans, 1978; Sofowora 1993).

2.5 Determination of phenolic content

Total phenolic content was estimated using Folin-Ciocalteu reagent based assay as previously described (McDonald et al, 2000) with a little modification. To 1ml of each extract (100 μ g/ml) in methanol, 5ml of Folin-Ciocalteu reagent and 4ml (75g/l) of Na₂CO₃ were added. The mixture was allowed to stand at room temperature for 30mins and the absorbance of the developed colour was recorded at 765nm using UV-Vis spectrophotometer. 1ml aliquots of 20, 40, 60, 80 and 100 μ g/ml methanolic solutions of gallic acid were used as standard for the calibration curve. All determinations were done in triplicate. Total phenolic content was obtained from the regression equation $y = 0.0055c - 0.0068$, $R^2 = 0.9944$ and expressed as μ g/g gallic acid equivalent using the formula $C = cV/M$ where C = total phenolic compounds in μ g/g GAE. C = the concentration of gallic acid (μ g/ml) established from the calibration curve, V = volume of extract (1ml) and M = the weight of the pure plant extract (0.05g).

2.6 Determination of Flavonoid Content

The aluminium chloride colorimetric method was used to measure the flavonoid content of the two plant extracts (Nguyen & Eun 2011). Each extract solution (1ml, 1mg/ml) was added to 1ml of distilled water. Sodium nitrite solution (0.5ml, 50%) was then added to the mixture followed by incubation for 5mins after which 0.3ml 10% aluminium chloride solution was added. The mixture was allowed to stand for 6min at room temperature before 0.5ml of 1M sodium hydroxide was finally added. The absorbance of the reaction mixture was measured at 510nm with a UV-Vis spectrophotometer immediately. Quercetin was used as the standard for the calibration curve (of the equation, $y = 0.005c - 0.0028$, $R^2 = 0.9945$). Flavonoid contents were expressed as μ g quercetin equivalent (QE)/ dry weight.

2.7 Determination of Alkaloid Content

2.5g of the powdered plant sample was weighed and 20% of acetic acid in methanol (or water in the case of aqueous extraction). The mixture was left for 4 hrs, after which it was filtered and concentrated to about 25ml. Concentrated ammonium chloride solution was added to the filtrate and this was left for the precipitates to settle. The precipitate was then washed with dilute ammonium hydroxide. It was then filtered, dried and weighed to ascertain the mass of the alkaloid extracted (Yousuf et al, 2012).

3.0 Results and Discussion

Preliminary phytochemical screening of *Mallotus oppositifolius* extract revealed the presence of different phytoconstituents as shown in Table 1. Different varieties of phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and cardiac glycosides have been found to be present in both the methanolic and aqueous extracts of the plant. However, both extracts indicated the absence of carbonyls in the plant sample while only the aqueous extract showed the absence of anthraquinones. The result from the phytochemistry partly agreed with what pertains in literature (Adekunle and Ikumapayi, 2006), which revealed the presence of flavonoids, saponins, steroids and tannins in the aqueous extract of the plant. The medicinal properties of *Mallotus oppositifolius* may be attributed to the presence of these phytoconstituents. According to Selvam et al 2013, the secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example saponins are glycosides of both triterpenes and steroids having hypotensive and cardio depressant properties (Olaleye, 2007), while anthraquinones possess astringent, purgative, anti-inflammatory, moderate antitumor and bactericidal effects (Muzychkina, 1998). Cardiac glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia. This means that the presence of these

phytochemicals in *Mallotus oppositifolius* presents the plant as a potential source of drugs for treating many medical conditions.

Table 1: Preliminary phytochemical screening of *Mallotus oppositifolius* extracts

Phytoconstituents	Test Performed	Result	
		Methanol	Aqueous
Alkaloids	Dragendorf, Mayer, Wagner test	+	+
Flavonoids	Alkaline reagent and Shinoda test	+	+
Steroid	Liebermann Burchard and Salkowski test	+	+
Terpenoid	Liebermann Burchard and Salkowski test	+	+
Tannins	FeCl ₂ test	+	+
Saponins	Foam test	+	+
Cardiac glycosides	Killer-Killiani test	+	+
Anthraquinones		+	-

(+): Indicates the presence of chemical constituents, (-): Indicates the absence of chemical constituents.

Table 2: Quantitative estimation of phytoconstituents present in methanol and aqueous extracts of *Mallotus oppositifolius*

Phytoconstituents	Methanol Extract	Aqueous Extract
Phenolic content/ $\mu\text{g/g}$	672.00 ± 20.07	1066.97 ± 26.42
Flavonoid content/ $\mu\text{g/g}$	84.00 ± 33.96	206.8 ± 31.06
Alkaloid content %	8.60 ± 1.41	4.30 ± 0.87

Table 3: TLC results for butanol layer obtained from the crude extracts (Spots viewed under u.v lamp)

Chloroform to methanol ratio	R _f for methanol extract	R _f for aqueous extract
1 : 1	0.64	0.64
2: 1	0.76	0.56
3 : 1	0.53	0.47
CH ₃ OH: CHCl ₃ =2:1	0.76	0.76

Table 4: Antioxidant activities of the standard ascorbic acid and the butanol-soluble substances obtained from the crude extracts.

Drug	Percentage inhibition (I %)			
	20 µg/ml	40 µg/ml	80 µg/ml	100 µg/ml
Methanol Extract	9.04 ± 0.07	12.48 ± 0.06	20.40 ± 0.12	39.21 ± 0.90
Aqueous Extract	22.10 ± 0.35	31.41 ± 0.13	47.05 ± 0.18	49.02 ± 0.16
Ascorbic Acid	94.61 ± 2.52	99.10 ± 0.65	99.64 ± 0.08	99.94 ± 0.03

The phenolic content of the butanol fraction of the aqueous extract was 1066.97 ± 26.42 µg/g of gallic acid equivalent while that of the methanol extract was 672.00 ± 20.07 µg/g. The flavonoid content was 84 ± 33.96 µg/g for the methanol extract and 206.8 ± 31.06 µg/g for the aqueous extract. This shows that more of phenolic substances and flavonoids were in the aqueous extract than the methanol extract.

The DPPH radical scavenging activity assay assesses the capacity of the extract to donate hydrogen i.e. to reduce or to scavenge such radicals as superoxide (O₂⁻), hydroxyl (OH[·]), peroxy (ROO[·]), nitrogen dioxide (NO₂[·]) and nitric oxide (NO[·]) radicals. These radicals when produced in excessive amounts may be dangerous and harmful to the body since they have the ability to attack numerous molecules, including proteins and lipids (Murat Tosun et al, 2009). This may result in significant

damage to cell structure, contributing to various diseases, such as cancer, stroke, diabetes, arthritis, haemorrhagic shock, coronary artery diseases, cataract and cancer as well as age-related degenerative brain diseases (Parr and Bolwell, 2000; Ames, 1983; Wiseman and Halliwell, 1996) Both the methanol and aqueous extracts of the plant exhibited DPPH scavenging ability. The aqueous extract exhibited the higher scavenging ability (49.02 ± 0.16) while the methanol extract showed the lower scavenging ability (9.02 ± 0.07). Also in the quantitative analysis, the aqueous extract was observed to have exhibited a higher average phenolic content of $1066.97 \pm 26.42\mu\text{g/g}$ than the methanol extract, which showed a phenolic content of $672.00 \pm 20.07\mu\text{g/g}$. The flavonoid content of the two extracts in terms of quercetin equivalent (with a standard calibration curve of equation: $y = 0.0055x - 0.0028$; $R^2 = 0.9945$) were $206.80 \pm 31.06\mu\text{g/g}$ for aqueous extract and $84.00 \pm 33.96\mu\text{g/g}$ for the methanol extract (Table 2).

From the results, it was observed that the extract with the highest phenolic content and flavonoid content exhibited the highest percentage DPPH inhibition. The antioxidant activity of the plant could be attributed directly to the presence of these polyphenolic compounds (Chaman et al, 2011). However, even though the observed percentage inhibition of the plant extracts were significantly lower than that of the standard ascorbic acid (Table 4), the plant can be said to be a considerably good source of antioxidants to reduce the effects of reactive oxygen species.

TLC analysis on the butanol fractions of the crude methanol and aqueous extracts showed the presence of one component in each of the fractions in methanol-chloroform solvent system of different proportions, when viewed under UV lamp (Table 3). When the spots developed in the chloroform-methanol (ratio 1:1) system and viewed in an iodine chamber, two components were, however, observed in the methanol extract while only one component was seen in the aqueous extract. These methanol-extract components had R_f values of 0.71 and 0.65 while the

only component observed in the aqueous extract had an Rf value of 0.67. This pointed out that there may be more than one component in the extracts, though the extraction method used might have assisted in eliminating interfering compounds of varying significance.

4.0 Conclusion

The result from the study showed that the leaves of *Anogeissus leiocarpus* is rich in secondary metabolites and thus possesses antioxidant properties. The presence of the bioactive compounds in the plant indicates the medicinal values such as antioxidant and antimicrobial properties that are of great interest in both research and in food industry for their possible use not only as natural additives to replace synthetic antioxidants and antimicrobial products but may lead to drug discovery and development.

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