

Full Length Research Paper

Phytochemical screening of some indigenous medicinal plant species used in the management of diabetes mellitus in Ghana

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This study was conducted to screen the phytochemical constituents and determine the levels of the major and trace elements of three medicinal plants used for the treatment of diabetes mellitus namely; *Bridelia ferruginea*, *Lagerstroemia speciosa* and *Morus Alba*. The air dried leaves of the plants were subjected to soxhlet extraction using ethanol. The crude extracts obtained were subjected to screening for their phytochemical constituents such as alkaloids, tannins, terpenoids, reducing sugars, flavonoids, saponins, anthraquinones, coumarins, emodols, carotenoids and steroids using various standard methods and reagents. Trace metals in the three medicinal plants were also quantitatively analyzed using Flame Atomic Absorption Spectroscopy. A wet digestion procedure involving the use of 4 ml of perchloric acid and 10 ml of aquaregia was adopted to digest the medicinal plants. Anthraquinones, sterols, tannins, terpenoids, flavonoids, alkaloids, saponins, coumarins and reducing sugars were identified in the leaves of all the three plants. However, emodols were absent in all the plants but carotenoids were absent in only *B. ferruginea*. Elemental concentrations of some of the elements were obtained from the leafy materials in varying quantities. Ten heavy metals (Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Zn, and V), two alkali metals (K and Na) and three alkaline earth metals (Ca, Mg and Al) and two halogens (Cl and Br) were quantitatively analysed. The anti-diabetic properties of the plant extracts could be attributed to the presence of steroids, triterpenes and alkaloids. In general the order of concentration of toxic metals in the medicinal plants was found to follow the order Fe > Mn > Zn > Cu > Ni > V > Pb > Co > Cr > Cd. Sodium content was found to be very high in *L. speciosa* while chlorine content was found to be very high in *B. ferruginea*.

Key words: *Bridelia ferruginea* Benth, *Lagerstroemia speciosa* Linn., *Morus alba* L, diabetes.

INTRODUCTION

Diabetes is a disease of metabolism due to deficiency of insulin. Blood sugar level is maintained constant at a value of 70 to 120 mg of glucose/100 ml. Though several hormones are involved in the maintenance of diabetes, the most important ones are insulin and glucagon. Diabetes is caused as a result of loss balance effect of these hormones, usually due to less insulin production. Sugar starts to accumulate in the blood and blood sugar

level increases and sugar passes into urine along with other minerals. There are two types of diabetes. They are diabetes insipidus and diabetes mellitus.

Diabetes mellitus is a condition in which a person's blood sugar level rises more than normal due to the deficiency of insulin or improper response to the insulin produced by the body cells. This disturbs metabolism of protein and other factors in the body. Diabetes mellitus is made up of two types: Type I and Type II.

Type I diabetes often referred to as juvenile diabetes, is insulin dependent and known to affect only 5% of the diabetic population. The Type II, which is non-insulin dependent, usually develops in adults over the age of 40.

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Figure 1. Violet flowers of *L. speciosa*.

Type II diabetes often show no symptoms. It occurs as a result of the decline in cell membrane insulin sensitivity that can be aggravated by the consumption of high-glycemic carbohydrates, obesity, lack of exercise and aging process.

At the moment a lot of orthodox drugs are in the system for the treatment of diabetes, especially the Type I, which is insulin-dependent. In our local communities, a lot of medicinal plant species are also currently used to manage/treat the Type II diabetes. Some of these medicinal plant species are *Bridelia ferruginea* Benth., *Lagerstroemia speciosa* Linn., *Morus alba* L, among many others.

***Lagerstroemia speciosa* Linn**

L. speciosa Linn belongs to the family Lythraceae. The genus, *Lagerstroemia* is made up of about 50 species of deciduous, evergreen and shrubs native to the Indian subcontinent, Southeast Asia, northern Australia and parts of Oceania. The leaves are prepared and used as natural health supplement, while some research suggests that leaf extract may support blood sugar balance and weight loss.

The leaves of the plant and other parts are used widely by the Philippines, Taiwan and Japan as a Tea preparation. This tea is consumed as a natural means for a variety of reasons involving the kidneys, such as dissolving kidney stones, kidney cleanses, and kidney health in general. Research being conducted in Japan shows much promise for this plant and its potential uses in the medical community. The main primary active ingredient is corosolic acid, and there are also numerous possible synergists including lager-stroemin, flosin B and reginin A (Figures 1 and 2).

The effects of the extracts isolated from *L. speciosa* on glucose transport and adipocyte differentiation in 3T3-L1 cells were studied. Glucose uptake-inducing activity of the plant extract was investigated in differentiated adipocytes using a radioactive assay, and the ability of *L. speciosa* induce differentiation in preadipocytes was examined by Northern and Western blot analyses. The results of the study showed that the unique combination of a glucose uptake stimulatory activity and effective inhibition of adipocyte differentiation induced by IS-IBMX-DEX in 3T3-L1 cells suggest the plant extract may be useful for prevention and treatment of hyperglycemia and obesity in Type II diabetes (Enkhmaa et al., 2005).

Three isolated extracts from the leaves of the plant,



Figure 2. Oval nut-like fruits of the plant.

namely; lagerstroemia, flosin B and reginin A increased glucose uptake of rat adipocytes, and could be responsible for lowering the blood sugar level (Kang et al., 2006).

Using bioassay-guided separation, valoneic acid dilactone was isolated from the leaves of the plant as a potent alpha-amylase inhibitor (Grundman et al., 2007). A simple and efficient method for the quantitative determination of valoneic acid and its derivatives in the plant extract was established. Valoneic acid exists as the structural parts of the polyphenols, which like flosin B, reginin A, and lagerstroemin, are characteristic constituents of the plant (Abdel et al., 2005).

L. speciosa has been found to be rich in tannin: fruit, 14 to 17%; leaves, 13%; bark, 10% and corosolic acid is being studied for its glucose lowering effect (Kusano et al., 2002). Corosolic acid has been studied for its activity by using Ehrlich Ascites Tumour Cells, and had been found to activate the mobility of grape sugar (Sonavane et al., 2002).

***Bridelia ferruginea* Benth**

B. ferruginea Benth belongs to the family Euphorbiaceae.

It is mostly found in savanna forest and open coastal plains, as well as, at times on rocky soils. Its distribution stretches from Guinea through Congo to Angola. Again, the plant can be found in Sudan through the corridors of East Africa to South Africa.

Figure 3 shows an aerial part of *B. ferruginea* with brown stem bark. *B. ferruginea* has diverse uses. A decoction of the leaves has been used to treat diabetes. It is also used as purgative and a vermifuge (Adebayo and Ishola, 2009).

The root decoction drunk in the Ivory Coast is copiously diuretic and good for gonorrhoea. The bark sold in the Yoruba markets in Nigerian is used in the preparation of a popular mouth-wash, and as a remedy for thrush in children. The root-bark is used in Togo for intestinal and bladder troubles, and externally for skin diseases and eruptions.

An infusion of the bark or leafy stems is used in a beverage, for bathing and vapour baths, to cure feverish pains, headaches, stiffness, and rheumatic pains. A leaf decoction, either drunk or used in bathing is used for fevers or as a local application for oedema.

Again, the leaves have also been reported to be efficacious in the treatment of ringworm and eczema. The bark has been found to be useful for various skin



Figure 3. An aerial part of *Bridelia ferruginea* with brown stem bark *B. ferruginea*

diseases (Marshall, 1951).

Pharmacological effects of the extract of *Bridelia ferruginea* Benth

Research by Kolawole et al. (2006 a) using leave extract of *B. ferruginea* on female albino rats resulted in a reduction in plasma glucose levels especially in glucose induced hyperglycemic rats. This implies that the methanol extract has anti-diabetic properties, hence, its indigenous application for the treatment or management of Diabetes mellitus.

Again, in another research by Kolawole et al. (2006 b), this time using bark extract of the plant caused 63% reduction of coliform load, and again in a biological oxygen demand (BOD) in a wastewater, it resulted in a 100% coliform load reduction.

Morus alba

Morus or Mulberry is a genus made up of about 10 to 16 species of deciduous trees native to warm, temperate and subtropical regions of Asia, Africa, Europe, and the Americas, with the majority of the species native to Asia. The leaves of the plants are shown on Figure 4.

Ethnobotanical uses

Traditionally, the mulberry fruit has been used as a

medicinal agent to nourish the blood, benefit the kidneys and treat weakness, fatigue, anemia and premature graying of hair. It is also used to treat urinary incontinence, tinnitus, dizziness and constipation in the elderly patient. The plant has other pharmacological properties such as analgesic, anti-asthmatic, anti-rheumatic, anti-tussive, astringent, etc.

The present study is concerned with the assessment of the phyto-constituents and to establish the trace (Zn, Cu, Cr, Ni, Co, Cd, Pb, Mn, V and Fe) and major (K, Na, Ca, Mg and Al) elemental levels of three medicinally important plants. Secondly, this study determines the toxicity of the plants and safety levels for consumption. This research therefore reports on the phyto- and -elemental constituents of the above mentioned medicinal plant specie; *L. speciosa*, *B. ferruginea*, and *M. alba* used in the management of diabetes mellitus.

MATERIALS AND METHODS

Extract preparation

Fresh leaves and fruits of the plant species, *L. speciosa*., *B. ferruginea*, and *M. alba* (1 kg each) were collected from the following locations, namely; North Campus, University of Education, Winneba and by the roadside between Agormeda - Somanya respectively and authenticated at the Herbarium of the Centre for Scientific Research into Plant Medicine, Mampong-Akuapem, Eastern Region of Ghana, where voucher specimens of each of the plant species were also deposited. The leaves were washed and cut into pieces and air dried. The powdered plant materials were defatted using petroleum ether (60 to 80°C) using a Soxhlet extractor. The marc was further extracted by ethanol for 72 h to



Figure 4. Leaves of *Morus alba* showing serrated margins.

obtain the extract. The extract was filtered and evaporated to dryness under reduced pressure on a rotary evaporator. The concentrated extracts were dried by placing them in a dessicator. The weights of the crude extracts after drying were measured.

Phytochemical screening

The crude extracts of the leaves of *B. ferruginea*, *L. speciosa*, and *M. alba* were subjected to phytochemical screening.

Test for alkaloids

To a small amount of the dried extracts (free from ethanol), 5 ml of 10% HCl were added and stirred while heating. From the resulting mixture, 1 ml each of the filtrates pipetted into test tubes. Dragendorff, Mayer and Wagner reagents were added. In each case a sample test tube of each filtrate was reserved as reference.

Saponins

To a small amount of the powdered samples, 2 ml of distilled water were added to each test tube and shaken vigorously. The formation of froth lasting for 15 min suggests the presence of saponins.

Tannins

To 1 ml of dissolved extract, 2 ml of distilled water was added. Few drops of ferric chloride were added. The formation of blue black

colour represents the presence of tannins.

Reducing sugars

To 1 ml of the extract, 2 ml of distilled water was added. 1 ml of Fehling's solution was added and heated. The formation of brick red precipitate indicates the presence of reducing sugar.

Steroids and Triterpenoids

To 1 ml of the extract, 0.5 ml of acetic anhydride and 0.5 ml of chloroform were added, and concentrated sulphuric acid later added. Formation of a brownish green ring at the contact of the two liquids indicates the presence of steroid and triterpenoid.

Coumarins

A small amount of dried extract was dissolved with 2 ml of distilled water. It was then divided into two. To one, 10% ammonia solution was added and observed for fluorescence under UV light. The other was used as a control. Intense fluorescence under UV light indicates the presence of coumarins.

Sample preparation for Atomic absorption spectrophotometry

Plant samples were washed with deionized water and oven dried at 80°C for 2 days and then subjected to grinding for powder

formation.

Digestion

Two gram powder of each plant sample (leaves) was dissolved in 10 ml of nitric acid for 12 h and then heated until the reddish brown fumes disappear. 4 ml of perchloric acid was added to the above solution and heated for 5 min then 10 ml of aquaregia was added and heated to small volume and up to marked 250 ml by adding deionized water.

Atomic absorption spectrophotometry

Major and trace elemental contents were determined using flame atomic absorption spectroscopy using Perkin Elmer A Analysit 700.

Determination of elements

The dried alcoholic extracts were again subjected to flame atomic absorption spectrometry (AAS) to determine and quantify some basic elements in the leaves of the three plant species under investigation.

RESULTS AND DISCUSSION

The present studies carried out on the three medicinal plant species, revealed the presence of bio-active constituents of medicinal value. The phytochemical compounds of these three plants were qualitatively analysed and the results of the ethanol extracts of all the three plants were almost the same (Table 1). The phytochemical analysis showed good result for all major phytoconstituents except emodols. The qualitative analysis revealed the presence of the biomolecules such as alkaloids, anthraquinones, steroids, reducing sugars, coumarins, flavonoids, terpenoids, tannins and saponins in all the three medicinal plant species. The presence of these phytoconstituents may contribute to the pharmacological actions of these plants.

According to the literature, the pharmacological activity and the phytochemical compositions confirm the traditional use of some antidiabetic plants. It is noted that the antidiabetic effect results from several chemical elements: alkaloids, sterols, essential oils and triterpenes. The results of the phytochemical investigations on the plants under study confirmed the traditional use of these antidiabetic plants. The pharmacology of these three plants can therefore be linked to the presence of alkaloids, triterpenoids and sterols phytoconstituents in the plants (Table 3).

The study also focused to estimate the concentrations of some toxic and essential metal ions in the plants. Ten heavy metals (Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Zn, and V), two alkali metals (K and Na) and three alkaline earth metals (Ca, Mg and Al) and two halogens (Cl and Br) were chosen on the basis of their importance to health.

The investigation revealed the presence of these

elements in varying concentrations in the extracts as presented in Table 2. The accumulation of the elements varied from one plant organ to the other.

Although, a lot of toxic/trace elements were recorded in the plants, these levels or concentrations were far below the World's Permissible Levels as indicated by Bowen (1979); Kloke (1979); Kabata-Pendais and Pendais (1984), thus, making the plant species, *L. speciosa*, *B. ferruginea* and *M. alba* safe for the production of herbal medicines, and in the management of *Type II* diabetes. Again, the high levels of iron (Fe) in the plant organs may probably make it a very powerful tonic to boost anaemic conditions in humans. In this regard *M. alba* with the highest Fe concentration presents itself as the best choice. It is worth mentioning that almost all the essential elements required for the natural remedies for diabetes are present in all the three plant species even though in lower quantities compared to the permissible levels. These are zinc (15 to 25 mg), which lowers blood sugar; chromium (200 to 1,000 mcg), improves glucose tolerance and magnesium (1,000 mg), that leads to improved insulin production in elderly people and reduces eye damage. The presence of these nutrients and the phytoconstituents in all the three plants makes these plants unique and significant good sources of natural remedies for diabetes.

There are no guidelines to establish a permissible level of metals in herbs. By monitoring the level of metals in medicinal plants one is able to indicate the level of environmental pollution in that area. Even though the plant samples were collected from locations exposed to some level of vehicular activities, the results does not point at any serious pollution concern in the area as at the time of collection.

The efficacy of medicinal plants for curative purposes is often accounted for in terms of their organic constituents like essential oils, vitamins, glycosides, etc. However, it has been established that over dose or prolonged ingestion of medicinal plants leads to the chronic accumulation of different elements which causes various health problems. This is because these essential metals can also produce toxic effects when the metal intake is in high concentrations, whereas non-essential metals are toxic even in very low concentrations for human health (Sharma et al., 2009). Elemental contents of the medicinal plants are therefore very important and need to be screened for their quality control (Liang et al., 2004; Arceusz et al., 2010).

The permissible limits set by FAO/WHO (1984) in edible plants for iron (Fe) and zinc (Zn) were 27.4 ppm and 3.00 ppm respectively. The permissible limits for Cu set by China and Singapore for medicinal plants were 20 ppm and 150 ppm respectively (WHO, 2005). According to Jabeen et al. (2010) and Allaway (1968), the range of Cu in agricultural products should be between 4 to 15 ppm. Reddy and Reddy (1997) reported that the range of Cu contents in the 50 medicinally important leafy material

Table 1. Identification of some basic compounds obtained from the three plant species.

Source of extract	Compound	Test	Observation	Inference
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Alkaloid	Dragendorff + test solution Mayer + test solution Wagner + test solution	Turbidity brown colour Pale yellow colour Brown precipitate	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Anthraquinones	10 ml CCl ₄ extract + 5 ml H ₂ O + 5 ml dil. NH ₃	Pink to cherry-red coloration	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Coumarins	0.5 ml of 10% NH ₃ sol. + 2 ml of test solution	Intense fluorescence under UV light	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Reducing sugar	1ml of dilute extract + Fehling's solution A and B	Formation of brick-red precipitation	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Steroids	Liebermann-Burchards reaction	Reddish-brown ring formed; chloroform layer turns green	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Flavonoids	2 ml of ethanol extract + 3 pieces of Mg ribbon + 3 Drops of conc. HCl	Orange pink colour	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Terpenoids	1 ml of test solution + 2 ml of extract	Yellow orange colour	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Saponins	1 g of pulverised material + 2 ml of H ₂ O; shake vigorously	Persistence of froth, Lasting for 10 to 15 min	Present
<i>L. speciosa</i> <i>B. ferruginea</i> <i>M. alba</i>	Tannins	1 ml of extract + Distilled water + 3 drops of 3% FeCl ₃	Greenish black solution formed	Present
<i>L. speciosa</i>	Emodols (anthracenoside aglycones)	3 ml extract solution + 1 ml of 25 ml NH ₃ + shaking	Red coloration	Absent

growing in India were 17.6 ppm to 57.3 ppm. After comparison, metal limit in the studied medicinal plants with those proposed by FAO/WHO (1984) the result indicates that metal contents in all the plants are within this limit except for zinc and iron in terms of edible plants. Although all plants are found to accumulate good quantity of Fe, Zn, K, Na, Cu, Na and Mg, however, their trace heavy metal contents are not high according to the international safety standards for the consumption of human beings except Fe which exceeds the limit level in

only *Morus alba* and Zn which exceeds the limit in all the three plants in comparison with permissible limits set by FAO/WHO (1984) in edible plants. However, these levels are, for instance, Zn (15-25 mg) within the acceptable range for lowering blood sugar.

Conclusion

The results of this investigation are indicative of possible

Table 2. Basic elements obtained from the leaves of the plant species using Atomic Absorption spectrometry.

Element	LS	BF	MA
Br	93.94 ± 3.28	95.91 ± 4.83	1.24 ± 0.02
Na	1658.90 ± 3.71	237.61 ± 1.40	160.65 ± 1.45
K	1.08 ± 0.03	1.38 ± 0.03	0.94 ± 0.02
Mg	0.07 ± 0.002	0.39 ± 0.01	0.04 ± 0.001
Cu	2.58 ± 0.03	4.57 ± 0.04	1.61 ± 0.01
V	ND	ND	0.40 ± 0.02
Cl	231.53 ± 2.54	1514.03 ± 10.81	ND
Al	7.61 ± 0.56	8.83 ± 0.55	169.40 ± 0.51
Mn	6.58 ± 0.45	21.50 ± 0.55	3.95 ± 0.07
Ca	0.15 ± 0.003	0.11 ± 0.001	0.02 ± 0.001
Fe	22.14 ± 0.55	21.54 ± 0.55	47.90 ± 2.23
Zn	6.66 ± 0.47	4.10 ± 0.14	20.08 ± 0.52
Pb	< 0.01 ± 0.001	< 0.01 ± 0.001	< 0.01 ± 0.001
Cr	< 0.006 ± 0.001	< 0.006 ± 0.001	< 0.006 ± 0.001
Ni	1.34 ± 0.03	1.96 ± 0.04	0.34 ± 0.01
Cd	< 0.002 ± 0.001	< 0.002 ± 0.001	< 0.002 ± 0.001
Co	0.015 ± 0.001	< 0.005 ± 10.81	0.007 ± 0.001

Table 3. Phytochemistry/Pharmacology of some antidiabetic plant species found in literature N'guessan et al (2009).

Plants species	Phytochemistry/Pharmacology
<i>Annona muricata</i>	Sterols: Stimulate the release of insulin
<i>Anchomanes difformis</i>	Alkaloids: Stimulates the hepatic glycogenogenesis
<i>Cassia occidentalis</i>	Triterpenes: Stimulates the release of insulin
<i>Combretum paniculatum</i>	Sterols: Stimulates the release of insulin
<i>Picralima nitida</i>	Alkaloid (Picraline): Hypoglycaemic effect
<i>Psidium guajava</i>	Sterols: Decrease the rate of blood glucose
<i>Ricinus communis</i>	Essential oil (nérolidol): Hypoglycaemic effect
<i>Spathodea campanulata</i>	Triterpenes: Hypoglycaemic effect

pure active principle of natural origin from the extract with possible high potency which could serve as a lead to the isolation of chemotherapeutic agents. Some of these active principles are alkaloids, sterols and triterpenes whose presence in some plant species are noted to have antidiabetic effects.

The phytochemical information indicated the rightfulness of the traditional use of the studied plants as antidiabetics.

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