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STUDIES ON THE QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL CONSTITUENTS OF ALBIZIA CHEVALIERI'S LEAVES, ROOT AND STEM BARK

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ABSTRACT Plants have served human beings as natural source for treatments and therapies from ancient times to date; amongst them medicinal herbs which have gain attention because of its wide use and less side effects. In the present study, phytochemical analysis of A. chevalieri was carried out as this plant have been proved to be one of the important medicine for the treatment of ailments like malaria, diabetes, diarrhea, and dysentery. Phytochemical analysis was carried out for the three Arial parts of the plant extracted with three different solvents (methanol, ethyl acetate and n-hexane). Qualitative analysis showed that methanol extracted almost all the secondary metabolites in all the three Arial parts of the plant. After quantification, it was observed that methanol stem bark extract was having the highest phenolic contents (44.83µg/GAE/g), followed by methanol Root extract (38.89 µg/GAE/g), and methanol leave extract (22.00 µg/GAE/g). Root ethyl acetate extract (8.19 µg GAE/g), stem bark ethyl acetate extract (13.49 µg GAE/g), and leaves ethyl acetate extract (23.66 µg GAE/g), while n-hexane extracts were having a very low phenolic content with; n- hexane root (0.66 µg GAE/g), n-hexane stem bark extract (2.69 µg GAE/g), and n-hexane leaves extract (7.80 µg GAE/g). The presence of high amount of phytochemical compounds suggest that A. chevalieri plant has high medicinal value and can be carefully studied to extract the natural compounds which are beneficial to human beings and that could be commercialized for higher production than using synthetic drugs with side effects.

KEY WORDS : phytochemical, qualitative, quantitative, Albizia chevalieri, phyto- constituents

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect against diseases (Breslin, 2017). There are more than thousand known phytochemicals, some of the well-known phytochemical are lycopene in tomatoes, isoflavones in soy and flavonoids in fruits.

Phytochemicals are naturally present in many foods but it is expected that through bio-engineering new plants will be developed, which will contain higher levels of phytochemicals. This would make it easier to incorporate enough phytochemicals with our food ((Breslin, 2017) (Molyneux *et al*, 2007) and (Harborne *et al.,* 1998)).

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and health care products (Ivanova, *et al,* 2005). Extraction and characterization of several active phyto-compounds from these green factories has given birth to some high activity profile drugs (Mandal, Mohan , & Hemalatha, 2007) The use of traditional medicine is widespread in India (Jeyachandran and Mahesh, 2007). A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (Hertog *et al.,* 1993). It is believed that crude extract from crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana & Shekhawat, 2010). Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides and saponins etc. secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms' insects and herbivores (Cowan, 1999). Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with less side effects. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity (Misra, 2009).

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Cragg and Newman, constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers (Mojab *et al.,* 2003; Parekh and Chanda 2009).

By convention, plant activities were categorized into "very potent," "good to moderate" "weak", "very weak" and "in active" following criteria used by Wilcox *et al* 2004, a pure compound was considered highly active if IC50 < 0.06 µM, being active with 0.06 µM <10 µM and compounds with IC50 >10 µM were considered inactive. The following inhibition percentages were proposed in vivo activity of antimalarial extracts at a fixed dose of 250 mg kg^{-1} day^{-1} 100-90% (very good activity), 90-50 % (good to moderate) 50-10 (moderate to weak), 0 % (in active) (Rasoanaivo *et al.,* 1998).

The plant *Albizia chevalieri* is a tree that grows up to 12 m high or a

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shrub under harsher conditions of the dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella shaped canopy, bark pale-greyish, twigs pubescent with white lenticels, leaves with 8-12 pairs of pinnate and 20-40 pairs of leaflets each. The bark was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal. It is used in Borno-North eastern Nigeria as purgative, taenicide and also remedy for coughs. A decoction of leaves is used in Northern Nigeria as remedy for dysentery (Burkill, 1995; Le Houerou, 2009). There are also reports on the local use of the leaves extract for cancer treatment in Zaria city, Kaduna State.

Previous studies on methanol leaf extract of *Albizia chevalieri* against *Plasmodium berghei* model have indicated the presence of phenolic compounds with significant antiplasmodial activity (Hajara *et al.,* 2017). This research was designed to determine phytochemical constituents both qualitative and quantitative in *A. chevalieri.*

MATERIALS AND METHODS

Plant collection, authentication and extraction

The fresh leaves, stem bark and root of *Albizia chevalieri* was collected in the month of September, 2018 at Kurba -North, in Yamaltu-Deba local government area of Gombe State. It was taxonomically authenticated by a taxonomist at the herbarium unit of the Department of Biological Sciences, Gombe State University. A voucher specimen (649) was deposited there for future reference. The leaves, stem bark and the roots of *A. chevalieri* were washed under running tap water to eliminate dirt and other foreign particles that may be present, they were air-dried at room temperature (27 - 37 ^{o}C) away from direct sun light for three weeks and were later pulverized into coarse powder using mortar and pestle, and into fine powder using an electric grinder, the plant material were then stored in an air-tight containers until use. Extraction was done via maceration using ethyl acetate, hexane and methanol, the macerates after the extraction process were filtered twice through cotton wool and through Whatman No.1 filter paper, the residue after the filtration process were discarded, the filtrate was then concentrated using a rotary evaporator, the extracts was stored at 4 ^{o}C according to (Adoum, 2009).

Qualitative Phytochemical Analysis

The extracts were subjected to phytochemical screening to determine the classes of secondary metabolites present in the plant materials according to Brain & Turner, 1975 Trease and Evans 1983). These include; Alkaloids, Saponins, Tannins, Flavonoids, Anthraquinones and Steroids.

Quantitative phytochemical analysis

These tests were carried out on the secondary metabolites active on *P. falciparum* which are: Flavonoids, Alkaloids and phenols.

ESTIMATION OF TOTAL ALKALOIDS

Procedure:

Exactly 10 mg of plant extract was homogenized and 20 ml of methanol was added: ammonia (68:2), the ammonium solution was decanted after 24 hrs. Fresh methanolic ammonia was added, the procedure was repeated three times, the extract was then pooled and evaporated using a flash evaporator, the residue was then treated with 1 N HCl and was kept overnight, the acidic solution was extracted with 20 ml of $CHC1_3$ thrice, the organic layer was pooled and evaporated to dryness, and basic fraction basified the acidic layer with conc. NaOH to pH 12 and was extracted with $CHC1_3$ (20 ml) thrice, the $CHC1_3$ layer was pooled, and dried over absorbent cotton, evaporated to dryness, the fraction that contains ajmalicine was weight and serpentine was expressed as mg/100g. (Harborne, 1973).

$$Alkaloids\ (\%) = \frac{weight\ of\ Alkaloids}{weight\ of\ sample} \times 100$$

Determination of flavonoids

Flavonoids determination was by the method reported by Ejikeme *et al.,* 2014 and Boham and Kocipai (1994).

Procedure:

Exactly 50 ml of 80 % aqueous methanol was added to 2.50 g of sample in a 250 ml of sample in a 250 ml beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each extract. Each filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. (Okwu, 2004). The percentage of flavonoid was calculated as:

$$\%\ flavonoids = \frac{weight\ of\ flavonoid}{weight\ of\ sample} \times 100$$

Estimation of Total phenols

The amount of total phenols in the tissues will be estimated by the method proposed by Mallick & Singh, 1980.

Procedure:

The extract (0.5 g) was homogenized in 10 X volume of 80 % ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated with 80 % ethanol. The supernatants was pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipette out and the volume in each tube was made up to 3.0 ml with distilled water. Folin-Ciocalteau reagent (0.5 ml) was added and equal volume of Na_2CO_3 and the tubes were placed in a boiling water bath for exactly one minute. The tubes were allowed to cool and the absorbance of each tube was read at 650 nm in a spectropho tometer against a reagent blank. Standard Gallic acid solutions (0.2-1 ml) corresponding to 2.0 – 10 µg concentrations was also treated as above. The concentration of phenols was expressed as µg/g.

RESULTS

The fresh leaves, stem bark and root of *Albizia chevalieri* were collected in the month of September, 2018 at Kurba -North, in Yamaltu-Deba local government area of Gombe State. The qualitative phytochemical screening for secondary metabolites showed the presence of various phytochemicals in different extracts for each plant parts. **Table 1**, shows the presence of all the secondary metabolites in good amounts in the three parts of methanol extracts. In ethyl acetate extracts, Flavonoids, Alkaloids, steroids and Anthraquinones were present, cardiac glycosides was absent in the leaves of ethyl acetate extract, tannins was absent in the stem bark of ethyl acetate extract and saponins was present only in root of ethyl acetate extract, while absent in leaves and stem bark of ethyl acetate extract. N-hexane extracted flavonoids in all the plant parts, Alkaloids and cardiac glycosides were only present in the n-hexane leaves extract, while steroids and Anthraquinones were only present in stem bark of n-hexane extract.

TABLE 1: Qualitative Phytochemical Screening in Three Parts of *Albizia chevalieri* Extracted Using Methanol, Ethyl acetate and N-hexane

Phytochemicals	Methanol			Ethyl acetate			N-hexane		
	Leaves	S/bark	Root	Leaves	S/bark	Root	Leaves	S/bark	Root
Flavonoids	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	-	-
Glycosides	+	+	+	-	+	+	+	-	-
Tannins	+	+	+	+	-	-	-	-	-
Steroids	+	+	+	+	+	+	-	+	-
Anthraquinones	+	+	+	+	+	+	-	+	-
Saponins	+	+	+	-	-	-	+	-	-

Key:
+ Positive
- Negative

S/bark Stem bark

GALLIC ACID STANDARD GRAPH- (PHENOLICS)

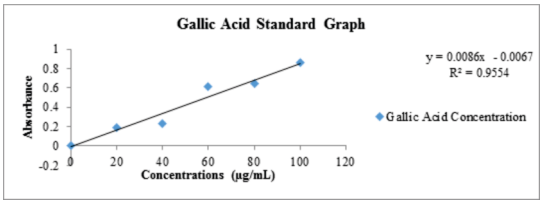

Figure 1: Shows Gallic Acid Graph (Phenolics)

Table 2: Phytochemical Composition in Three Parts of *Albizia chevalieri* Extracted Using Methanol, Ethyl acetate and N-hexane

Samples	Total Alkaloids (g/100 g)	Total Phenols (µg GAE/ g)	Flavonoids (g/100 g)
Leaves Methanol	46.94	33.69	22.00
Leaves E.acetate	16.30	23.66	4.17
Leaves N-hexane	-	7.80	7.14
S/bark Methanol	10.78	33.69	44.83
S/bark E.acetate	25.72	13.49	19.05
S/bark N-hexane	-	2.96	16.67
Root Methanol	55.08	44.48	38.89
Root E.acetate	21.34	8.19	9.09
Root N-hexane	-	0.66	29.41

DISCUSSION OF RESULTS

The plant *Albizia chevalieri* is a tree that grows up to 12 m high or a shrub under harsher conditions of the dry savannah from Senegal, Niger and Nigeria. *A. chevaleri* is a plant whose qualitative and quantitative determination in this study were shown to contain significant amount of phenolic compounds; Phenolic compounds are secondary metabolites in plant that are involved in a number of metabolic pathways and are essential for plant growth and reproduction and as protecting agents against pathogens. Phenolic compounds may play an important role in preventing chronic illnesses such as cardiovascular diseases, certain type of cancers, neurodegenerative disease, and diabetes (Verma *et al.,* 2010). In plants, these metabolites and their derivatives play an important role in cell wall integrity and defense against pathogens (Verma *et al.,* 2010).

Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion, and progression of tumors (Kim *et al.,* 1994). Reduction of coronary heart disease has been reported to be associated with intake of flavonoid (Hertog *et al.,* 1993) Flavonoids and other polyphenols belong to the recently popular phytochemicals, chemicals derived from plant material with potentially beneficial effects on human health. Flavonoids have shown to possess many pharmacological properties such as: anti-oxidant, activities, anti-inflammatory activities, anti-cancer activities, and anti-microbial effects, hence, flavonoids may have a contributory effect to its fertility properties and other pharmacological effects the plant possesses (Verma *et al.*, 2011; Harborne (1998)). Flavonoids are abundant plant phenolic compounds. More than 6000 have been identified to date, and some have shown to possess anti-parasitic activity (saxena *et al*., 2003).). This research has shown that the leaves stem bark and the root of *A. chevalieri* contain significant amount of flavonoids. It can be seen in **Table 2** that flavonoids contents was observed to be high in methanol stem bark extract with 44.83(g/100 g), followed by methanol root extract (38.89 g/100 g) while methanol leaves extract, ethyl acetate leaves extract, n-hexane leaves extract, ethyl acetate and stem bark extracts were having a very low contents; 22.00 g/100 g, 19.05 g/100 g, 16.67 g/100 g, 7.14 and 4.17 respectively.

Tannins are formed by polymerization of quinone units, which is one of the major active ingredients found in plant based medicines (Haslam, 1996). It serves as caustics for cationic dyes used in the dyestuff industry as well as in production of inks. Other uses of tannin are for wine, fruit juice, and beer clarification in food industries (Wurdig *et al.,* 1989). Tannin has been reported to inhibit HIV replication (Kashiwada *et al.,* 1992). Therefore *A. chevalieri* has potential in the provision of tannin.

According to Braunwald *et al.,* 1961, cardiac glycoside has been used in treatment of congestive heart failure due to its direct action which increases the force of myocardial contraction. They also explained that in the vascular system cardiac glycoside acts directly on smooth muscles. Their effects on neutral tissues and indirect effect on electrical activities of the heart and vascular resistance as well as capacitance are equally reported (Braunwald et al., 1961). The leaves, stem bark and root of *A.chevaleri* in this study were shown to contain glycosides which could be exploited for their medicinal properties.

Alkaloids are generally toxic to other organisms. They often have pharmacological effects and are used as medications, antimicrobial, antihypertension agent, local anesthetic and stimulant, anti-bacteria, anticancer, antiasthma, and antimalarial (Verma *et al.,*2010). The presence of alkaloids in *A. chevalieri* confirms its uses as antimalarial drug (Hajara *et al.,* 2017). Alkaloids and Tannins may also contribute to the plants effects as antimalarial, anti-diarrhea and analgesic agent. Alkaloids are one of the major classes of natural products that exhibit antimalarial activity. Indeed, quinine, the first antimalarial drug, belongs to this class. Over 1000 alkaloids from higher plants were reported to demonstrate significant antimalarial activity in studies published from 1990-2000; some of these were more potent than chloroquine (Saxena *et al.,* 2003). It was observed after quantifying Alkaloids as seen in **Table 2;** that methanol root extract is having the highest Alkaloids contents (55.08 %), followed by leaves methanol extract (46.94 %), stem bark ethyl acetate extract, root ethyl acetate extract, leaves ethyl acetate extract, and stem bark methanol extract were having 25.72 %, 21.34 %, 16.30 %, and 10.78 % respectively.

Terpenoids are large class of naturally occurring organic compounds and are major constituents of plant resin essential oil extracted from plants. Terpenoids with most promising antimalarial properties are summarized with their chemical structures. *A. chevalieri* methanol leaf extract was found to possess good amount of terpenoids which agrees with the work of (Hajara *et al.,* 2017).

Saponins are being used commercially as dietary supplements and nutraceuticals. They are expected to lead to hydrolysis of glycoside from terpenoids and hence reduce the toxicity associated with the intact molecule (Verma *et al.,* 2010). Appreciable quantities of saponins are found in the leaves, stem bark and root and *A. chevalieri* as shown in Table 1.

Pharmacologically important phytochemicals in different plant parts of *A. chevalieri* were quantified, total phenolic content was found to be in good amounts. The root methanol extract showed maximum phenolic content as **44.48 µg GAE/g**, followed by leaves methanol extract (**33.69 µg GAE/g**) and stem bark methanol extract (**33.69 µg GAE/g**), Root ethyl acetate extract (**8.19 µg GAE/g**), stem bark ethyl acetate extract (**13.49 µg GAE/g**), and leaves ethyl acetate extract (**23.66 µg GAE/g**), while n-hexane extracts were having a very low phenolic content with; n- hexane root (**0.66 µg GAE/g**), n-hexane stem bark extract (**2.69 µg GAE/g**), and n-hexane leaves extract (**7.80 µg GAE/g**) **Table 2.** Appreciable amount of phenolic compounds were found in the methanolic leave extract of *A. chevalieri (*Hajara *et al., 2017).*

CONCLUSION

The secondary metabolites are present in significant amounts in the various organic extracts of the 3 different plant parts, hence the methanolic extracts were found to contain high amounts of these secondary metabolites than the rest of the extracts of ethyl acetate

and n-hexane. Their qualitative analysis revealed their presence where as their quantitative analysis give almost approximate idea for their quantity present. Pharmacologically important phytochemicals in different plant parts of *A. chevalieri* were quantified, total phenolic content was found to be in good amounts. The Root methanol extract showed maximum phenolic content as **44.48 µg GAE/g**, followed by leaves methanol extract (**33.69 µg GAE/g**) and stem bark methanol extract (**33.69 µg GAE/g**), Root ethyl acetate extract (**8.19 µg GAE/g**), stem bark ethyl acetate extract (**13.49 µg GAE/g**), and leaves ethyl acetate extract (**23.66 µg GAE/g**), while n-hexane extracts were having a very low phenolic content with; n- hexane root (**0.66 µg GAE/g**), n-hexane stem bark extract (**2.69 µg GAE/g**), and n-hexane leaves extract (**7.80 µg GAE/g**) **Table 2.** This research result has established through the investigation of their phytochemistry that they have potential to be used as substituent of antimalarial as well as antibiotic drugs; the industries particularly pharmaceutical, may advance this study and bring out the best out of this plant.

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