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Studies on the qualitative and quantitative phytochemical constituents of Albizia chevalieri leaf, root, and stem bark

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ABSTRACT

A plant serves as a natural source of treatments and therapies for human beings as from prehistoric times to date; among them, medicinal herbs are the best because of its extensive use and fewer side effects. In the present study, the phytochemical analysis of *Albizia chevalieri* was carried out as this plant may be among one of the imperative medicinal herbs for the treatment of ailments such as malaria, diabetes, diarrhea, and dysentery. The phytochemical analysis was carried out for three aerial 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 aerial parts of the plant. After quantification, it was observed that methanol root extract had the highest phenolic contents (44.83 µg/GAE/g), followed by methanol leaf extract (38.89 µg/GAE/g), methanol stem bark extract (22.00 µg/GAE/g), ethyl acetate root extract (8.19 µg GAE/g), ethyl acetate stem bark extract (13.49 µg GAE/g), and ethyl acetate leaf extract (23.66 µg GAE/g), whereas n-hexane extracts had a very low phenolic content with n- hexane root extract (0.66 µg GAE/g), n-hexane stem bark extract (2.69 µg GAE/g), and n-hexane leaf extract (7.80 µg GAE/g). The presence of high amount of phytoconstituents suggests that *A. chevalieri* may have high medicinal value and it can be carefully studied to extract the natural compounds present, which may be beneficial to human beings and which may be commercialized for higher production than using synthetic drugs with side effects.

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1. Introduction

Phytochemicals are plant chemicals with disease preventive properties. They have nutrients that are non-essential, which are not required by the human body for life sustenance. Plants produce these chemicals to protect themselves, but recent research demonstrates that they can also protect against diseases which are well known (Breslin, 2017). There are thousands of well-known phytochemicals, and some of these phytochemicals are lycopene in tomatoes, isoflavones in soy, and flavonoids in fruits.

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Phytochemicals are present naturally in many foods, but it is discovered that through bioengineering, new plants will be developed naturally, which may contain higher levels of phytochemicals. This would be incorporated to provide enough phytochemicals in our foods Breslin, 2017; Harborne, 1998; Molyneux et al., 2007).

Therapeutic properties have been attributed to natural herbs. Various medicinal plants offer the main basis of new pharmaceutical medicines and healthcare products (Ivanova et al., 2005). Extraction, isolation, and characterization of several active phytochemical compounds from these plant factories have provided to some high activity profile drugs (Mandal et al., 2007) The use of medicinal herb is well known in India (Jeyachandran and Mahesh, 2007). Evidence indicates that secondary plant metabolites play an important role in human health and may be important in nutrition (Hertog et al., 1993). It is believed that the crude extract from medicinal plants is more active in nature than isolated compounds due to their synergistic effects (Jana and Shekhawat, 2010). Preliminary phytoconstituent screening of plants has revealed the presence of numerous chemicals including anthraquinones, alkaloids, tannins, flavonoids, steroids, glycosides, and saponins. The secondary phytoconstituents of plants serve as resistance mechanisms against diseases and predation by many microorganisms' insects and herbivores (Cowan, 1999). Due to this popular principle that green medicine is safe, easily available, and with fewer side effects, customary herbal medicines have become more popular in the treatment of many ailments. Community demand as well as marketplace, today, has been facing either extinction or loss of genetic diversity so that there is a great threat by many medicinal plants (Misra, 2009).

Since time immemorial, plant products have been a part of phytomedicines. These can be derived from any part of the plant such as bark, leaves, flowers, roots, fruits, seeds, and so on (Cragg and Newman), for the synthesis of complex chemical substances. The constituents of plants are beneficial because such information will be of importance. Such phytochemical screening of various plant lives is reported by many workers (Mojab et al., 2003; Parekh and Chanda, 2009).

Plant activities were categorized into "very potent," "good to moderate," "weak," "very weak," and "in active." By principle, the following criteria used by Wilcox et al. (2004), a pure compound may be 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), and 0% (in active) (Rasoanaivo and Oketch-Rabah, 1998).

Albizia chevalieri is a flowering shrub under harsher conditions of the desiccated savanna from Senegal, Niger, and Nigeria. A plant *A. chevalieri* is a tree that grows up to 12-m tall, which has an open and curved or umbrella-shaped covering, bark pale-grayish, twigs teenage with white lenticels, and 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 as purgative and taenicide in Borno, North-eastern Nigeria, and also cures coughs. A decoction of leaves is used in Northern Nigeria as a cure for dysentery (Burkill, 1995; Le Houerou, 2009). There is also information on the local use of the leaf extract for cancer treatment in Zaria City, Kaduna State.

The preceding studies on the methanol leaf extract of *A. chevalieri* against *Plasmodium berghei* model have indicate the existence of phenol compounds with considerable antiplasmodial activity (Hajara et al., 2017). This research was intended to determine photochemical constituents both qualitative and quantitative in *A. chevalieri.*

Materials and Methods

Plant collection, authentication, and extraction

The fresh leaves, stem bark, and root of *A. chevalieri* were collected in the month of September, 2018, at Kurba-North, Yamaltu-Deba, local government area of Gombe State. It was authenticated by a taxonomist taxonomically at the herbarium unit of the Department of Biological Sciences, Gombe State University. A voucher specimen (649) was deposited in the herbarium for prospect reference. The leaves, stem bark, and roots of *A. chevalieri* were washed under running tap water to get rid of dirt and other foreign particles that may be present. They were air-dried at room temperature (27°C–37°C) away from direct sunlight for 3 weeks and were later crushed into coarse powder using mortar and pestle and into fine powder using an electric grinder. The plant material were then stored in sealed containers until use. Extraction was done via maceration using ethyl acetate, n-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 was

redundant; the filtrate was then concentrated using a rotary evaporator; and the extracts were stored at 4°C.

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 and Turner (1975) and Trease and Evans (1983). These include alkaloids, saponins, tannins, flavonoids, anthraquinones, and steroids.

Test for saponins

Saponins were detected using the froth test method. Exactly, 1 g of the sample was weighed into a conical flask, in which 10 ml of sterile distilled water was added and boiled for 5 minutes. The fusion was filtered, and 2.5 ml of the filtrate was added to 10 ml of the sterile water in a test tube. The test tube was tapered and shaken dynamically for about 30 seconds. It was later boiled for 10 minutes, and the froth was observed for the confirmation of saponins and allowed to set for ½ hour; honey comb froth indicates the presence of saponins (Sofowora, 1993).

Test for tannins

About 0.5 g of the extract was mixed systematically with 10 ml of distilled water and then filtered; about 5 ml of the filtrate was added to 1 ml of 5% ferric chloride solution. The appearance of blue–black, greenish, or blue–green precipitate indicates the presence of tannins.

Test for glycosides

Exactly 25 ml of dilute sulfuric acid was added to 5 ml of the extract in a test tube and boiled for 15 minutes and cooled and neutralized with 10% NaOH; then, 5 ml of Fehling's solution was added, and brick red precipitates indicate the presence of glycosides (Sofowora, 1993).

Test for alkaloids

About 0.5 g of the extract was stirred with 5 ml of 1% hydrochloric acid on a steam bath and filtered. About 1 ml of the filtrate was then treated with few drops of Mayer's reagent. A white or creamy white precipitate considered as an indication of alkaloids (Salehi-Surmaghi et al., 1992).

Test for flavonoids

A few drops of concentrated hydrochloric acid (HCl) were added to a small amount of extract of the plant material; immediate development of red color indicates the presence of flavonoid (Sofowora, 1993).

Test for anthraquinones

Exactly 0.5 g each of the plant extract was shaken with 10 ml of benzene and filtered, and about 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken, and the presence of a pink, red, or violet color in the ammoniacal (lower) phase indicates the presence of anthraquinones (Sofowora).

Quantitative phytochemical analysis

These tests were carried out on the secondary metabolites active on *P. falciparum*, which are flavonoids and alkaloids to determine the total phenolic compounds.

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 hours. Fresh methanolic ammonia was added. The procedure was repeated three times, and the extract was then pooled and evaporated using a flash evaporator. The residue was then treated with 1N 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. The basic fraction basified the acidic layer with concentrated NaOH to pH 12 and was extracted with $CHC1_3$ (20 ml) thrice. The $CHC1_3$ layer was pooled, dried over absorbent cotton, and evaporated to dryness. The fraction that contains ajmalicine was weighed, and serpentine was expressed as mg/100 g.

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

Determination of flavonoids

The determination of flavonoids was done 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 beaker, covered, and allowed to stand for 24 hours at room temperature. After the removal of supernatant, the deposit was re-extracted (three times) with the same volume of ethanol. The Whatman filter paper number 42 (125 mm) was used to filter the 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 follows:

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

Estimation of total phenols

The amount of total phenols in the tissues was estimated by the method proposed by Mallick and Singh (1980).

Procedure

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

Results

The fresh leaves, stem bark, and root of *A. chevalieri* were collected in the month of December, 2018 at Kurba-North, Yamaltu-Deba, local government area of Gombe State. The qualitative phytochemical screening for secondary metabolites showed the presence of a variety of phytochemicals in different extracts for each plant part. Table 1 shows the existence 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 were absent in the leaves of ethyl acetate extract, tannins were absent in the stem bark of ethyl acetate extract, and saponins were present only in the root of ethyl acetate extract, whereas saponins were absent in leaves and stem bark of ethyl acetate extract. Flavonoids were present in N-hexane extract of all the plant parts, alkaloids and cardiac glycosides were only present in the n-hexane leaf extract, and steroids and anthraquinones were only present in n-hexane stem bark extract.

Discussion

The *A. chevalieri* is a plant whose qualitative and quantitative determination in this study was shown to contain considerable amount of phenolic compounds; these phenolic compounds are secondary metabolites in plant which are concerned in a number of metabolic pathways and are vital for plant maturity and replica as well as defensive 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 property and have been shown to hinder the beginning, support, and progression of tumors (Kim et al., 2005). Lessening of coronary heart disease has been reported

Table 1. Qualitative phytochemical screening in three aerial parts of *Albizia chevalieri.*

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	+	+	+	-	-	-	+	-	-

+ Positive
- Negative
S/bark Stem bark

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 valuable effects on human health. Flavonoids have shown to possess many pharmacological properties such as antioxidant activities, anti-inflammatory activities, anticancer activities, and antimicrobial effects; hence, flavonoids may have a causal effect to its fertility properties and other pharmacological effects that the plant possesses (Harborne, 1998; Verma et al., 2011). Flavonoids are rich plant phenolic compounds. More than 6,000 have been identified to date, and some have shown to own antiparasitic activity (Saxena et al., 2003).). This research has shown that the leaf, stem bark, and root of *A. chevalieri* contain a considerable amount of flavonoids. Table 2 shows that flavonoid contents were 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), whereas methanol leaf extract, ethyl acetate l stem bark extract, n-hexane stem bark extract, n-hexane leaf extract, and ethyl acetate leaf extract were with a very little contents of 22.00, 19.05, 16.67, 7.14, and 4.17 g/100 g, respectively.

Tannins are formed by polymerization of quinone units, which is one of the main 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 making of inks. Other uses of tannin are used for wine, fruit juice, and beer clearing up in food industries (Wurdig et al., 1989). Tannin has been reported to hinder HIV replication (Kashiwada et al., 1992). Therefore, *A. chevalieri* is possible in the provision of tannin.

Table 2. Phytochemical composition in three parts of *A. chevalieri*.

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

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

Alkaloids are generally fatal to other organisms. They often have pharmacological sound effects and are used as medications, antimicrobial, antihypertensive agent, local anesthetic and stimulant, antibacteria, anticancer, antiasthma, and antimalarial agents (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 plant effects as antimalarial, anti-diarrheal, 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 considerable antimalarial activity in studies published from 1990 to 2000; some of these were more efficient than chloroquine (Saxena et al., 2003). It was observed after quantifying alkaloids as shown in Table 2; that methanol root extract have the highest alkaloid contents (55.08%), followed by methanol leaf extract, ethyl acetate stem bark extract, ethyl acetate root extract, ethyl acetate leaf extract, and methanol stem bark extract having 46.94%, 25.72%, 21.34 %, 16.30%, and 10.78%, respectively.

Terpenoids are a large group of naturally occurring organic compounds and are key constituents of plant resin essential oil extracted from plants. Terpenoids with the most potential antimalarial property are summarized with their chemical structures. *A. chevalieri* methanol leaf extract was found to own high-quality amount of terpenoids, which agrees with the study of Hajara et al. (2017) and is also in line with the present research.

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 linked with the intact molecule (Verma et al., 2010). Appreciable quantities of saponins are found in the leaf, stem bark, and root of *A. chevalieri* as shown in Table 1.

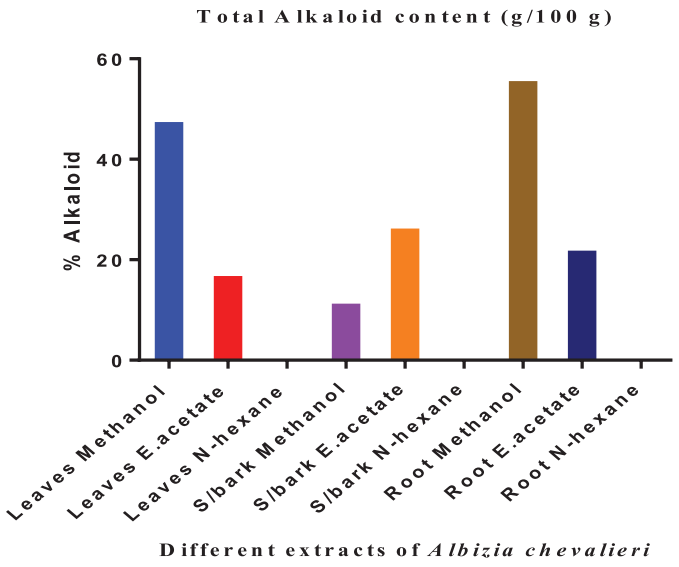

Figure 1. Total alkaloid content of different extracts of *A. chevalieri.*

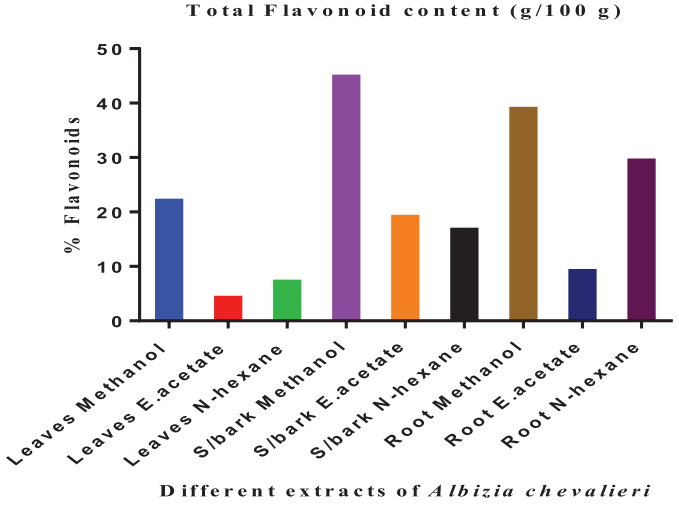

Figure 2. Total flavonoid content of different extracts of *A. chevalieri.*

Pharmacologically imperative phytochemicals in different plant parts of *A. chevalieri* were quantified, and the total phenolic content was found to be in good amounts. The methanol root extract showed maximum phenolic content as 44.48 µg GAE/g, followed by methanol leaf extract (33.69 µg GAE/g), methanol stem bark extract (33.69 µg GAE/g), ethyl acetate root extract (8.19 µg GAE/g), ethyl acetate stem bark extract (13.49 µg GAE/g), and ethyl acetate leaf extract (23.66 µg GAE/g), whereas n-hexane extracts had a very low phenolic content with n- hexane root

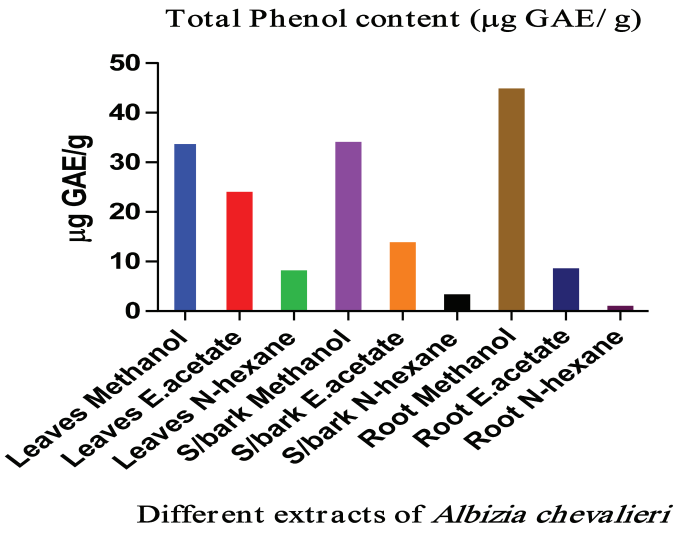

Figure 3. Total phenolic content of different extracts of *A. chevalieri.*

extract (0.66 µg GAE/g), n-hexane stem bark extract (2.69 µg GAE/g), and n-hexane leaf extract (7.80 µg GAE/g) as shown in Table 2. Appreciable amount of phenolic compounds was found in the methanol leaf extract of *A. chevalieri*, which agrees with the study of Hajara et al. (2017).

Conclusions

The resultant metabolites are present in considerable amounts in the various crude extracts of the three different plant parts; hence, the methanolic extracts were found to contain elevated amounts of these secondary metabolites than the rest of the extracts of ethyl acetate and n-hexane. Their qualitative analysis revealed their presence, whereas their quantitative analysis gives almost approximate idea for their quantity present. Pharmacologically vital phytochemicals in different aerial parts of *A. chevalieri* were quantified, and total phenolic content was found to be in good amounts. The methanol root extract showed maximum phenolic content as 44.48 µg GAE/g, followed by methanol leaf extract (33.69 µg GAE/g), methanol stem bark extract (33.69 µg GAE/g), ethyl acetate root extract (8.19 µg GAE/g), ethyl acetate stem bark extract (13.49 µg GAE/g), and ethyl acetate leaf extract (23.66 µg GAE/g), whereas n-hexane extracts had a very low phenolic content with n-hexane root extract (0.66 µg GAE/g), n-hexane stem bark extract (2.69 µg GAE/g), and n-hexane leaf extract

(7.80 µg GAE/g) as shown in Table 2. This research result has acknowledged through the study 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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Conflict of interest

There is no divergence of interest among the authors in whatever structure.

References

Boham B, Kocipai A. Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pac Science 2004; 48:458–63.

Brain KR, Turner T. Practical evaluation of phytopharmaceuticals. 1st edition, Bristol, Bristol, UK, 1975.

Braunwald E, Bloodwell R, Goldberg L, Morrow A. Studies on digitals IV observations in man on the effects of digitalis preservations on the contractility non-failing heart and on total vascular resistance. J Clin Investig 1993; 40:1007–11.

Breslin A. The chemical composition of green plants. Sciencing, Leaf Group Ltd, Santa Monica, CA, 2017.

Burkill H. The useful plants of west tropical Africa. Families J'L, Royal Botanical Gardens 1995; 3:207–8.

Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4):564–82.

Cragg G, Newman D. Natural product drug discovery in the next millennium. Pharm Biol 2001; 39, 8–17.

Ejikeme C, Ezeonu C, Eboatu N. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger delta area of nigeria. Eur Sci J 2014; 10:247–70.

Hajara S, Samaila A, Nayaya A, Danladi M, Musa M, Abdulwahab A, Nazeef I. In vivo screening of antiplasmodial activity of methanolic leaf extract of Albizia chevalieri against Plasmodium berghei Model. Greener J Biol Sci 2017; 7(4):034–44.

Harbourne J. Phytochemical methods. Chapman and Hall, New York, 1998.

Haslam E. Natural polyphenols (vegetable tannins) as drugs. J Nat Prod 1996; 59:205–15.

Hertog M, Feskens E, Hollman P, Katan J, Kromhout D. Dietry antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet 1993; 342:1007–11.

Ivanova D, Gerova D, Chervenkov T, Yankova T. Ploy phenols and antioxidant capacity of Bulgarian medicinal plants. J. Ethnopharmacol 2005; 96:145–50.

Jana S, Shekhawat GS. Phytochemical analysis and antibacterial screening of in vivo and in vitro extracts of Indian medicinal herb: Anethum graveolens. Res J Med Plant 2010; 4:206–12.

Jeyachandran R, Mahesh A. Enumeration of antidiabetic herbal Flora of Tamil Nadu. Res J Med Plant 2007; 1:144–8.

Kashiwada Y, Huang L, Kikuskie R, Bodner A, Lee K-H. New hexahydroxydiphenyl derivatives as potent inhibitors of HIV replication in H9 lymphocytes. Biorgan Med Chem Lett 1992; 2:235–8.

Kim H, Yokozawa T, Kim H, Tohda C, Juneja L. Influence of Amla (Emblica offinalis Gaertn) o hypercholesterolemia and lipid peroxidation in cholesterol-fed-rats. J Nutri Sci Vitaminol 2005; 51:413–28.

Le Houerou H. Albizia chevalieri Harms, 2009. Available via http://www.fao.org/ag/AGP/AGPC/doc/GBASE/Data/pfooo366.HTM

Mallick EP, Singh MB. Plant enzymology and histoenzymology. 1st edition, Kalyani Publishers, New Delhi, India, 1980.

Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction: an innovative and promising extraction tool for medicinal plant research. Pharmacogn Rev 2007; 1:7–18.

Misra A. Studies on biochemical and physiological aspects in relation to phyto-medicinal qualities and efficacy of the active ingredients during the handling, cultivation and harvesting of medicinal plants. J Med Plants Res 2009; 3:1140–6.

Mojab F, Kamalinejad M, Ghaderi N, Vahidpour H. Phytochemical screening of some species of Iranian plants. Iran J Pharm Res 2003; 2:77–82.

Molyneux RJ, Lee ST, Gardner DR, Panter KE, James LF. Phytochemicals: the good, the bad, and the ugly. Phytochemistry 2007; 2973–85.

Okwu D. Phtochemicals and vitamins content of indigenous spices of South Eastern Nigeria. J Susutain Agric Environ 2004; 6:30–4.

Parekh J, Chanda S. In vitro antimicrobial activity and photochemical analysis of some Indian medicinal plants. Turk J Biol 2009; 3:53–8.

Rasoanaivo P, Oketch-Rabah H. Preclinical considerations on antimalarial phytomedicines: efficacy evaluations. Antannarivo: inst., Malgache de Recherches Appliquees, 1998.

Salehi-Surmaghi M, Aynehchi G., Amin G, Mahhmoodi Z. Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins. DARU 1992; 2:1–11.

Saxena S, Pant N, Jain D, Bhakunl S. (2003). Antimalarial agents from plant sources. Curr Sci 1992; 12:1341–29.

Sofowora A. Medicinal plants and traditional medicine in Africa. (2nd edition, Spectrum Books Ltd., Nigeria, 1993.

Trease G, Evans W. Textbook of pharmacognosy. 12th edition, Balliere, Tindall, London, UK, 1989.

Verma K, Dixit V, Khare D, Awasthi A. Phytochemical screening and antimicrobial activity of Buchanania lanzan sreng. J Phytol Res 2010; 23(2):339–43.

Wilcox CB. Genetic interactions with C-terminal domain (CTD) kinases and the CTD of RNA pol II suggest a role for ESS1 in transcription initiation and elongation in saccharomyces cerevisae. Genetics 2004; 167(1):93–105.

Wurdig G, Woller R. Chemie des weines. Eugen Ulmer, Stuttgart, Germany, 1989. Available via http://www.fao.org/ag/AGP/AGPC/doc/GBASE/Data/pf00366 (Accessed 21 May 2013)