http://dx.doi.org/10.5455/sf.69187

Science Forum (Journal of Pure and Applied Sciences)

journal homepage: www.atbuscienceforum.com

Isolation of biologically active plant secondary metabolites in *Albizia chevalieri* using column chromatographic techniques

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ABSTRACT

Plants have served human beings as a natural source for treatments and therapies from ancient times to date; among them, medicinal herbs have gained attention because of its wide use and less side effects. Chromatographic techniques have a significant role in natural products chemistry and also contribute dramatically to the discovery of novel and innovative compounds of pharmaceutical and biomedical importance. In the present study, the phytochemical analysis of *Albizia chevalieri* was carried out for the three aerial parts of the plant extracted with three different solvents (methanol, ethyl acetate, and n-hexane). This study also focused on a step-by-step visual demonstration of fractionation and isolation of biologically active plant secondary metabolites in *A. chevalieri* using column chromatographic techniques. Isolation of bioactive compounds using column chromatography involves preparation of the sample; packing of column; pouring of sample into the column; elution of fractions, and analysis of each fractions using thin layer chromatography. Based on the column chromatographic technique, it was observed that in each fraction one band were detected. 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 garlic acid equivalent (GAE)/g], followed by methanol leaf extract (38.89 µg GAE/g) and methanol stem bark 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) followed through, 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 a high amount of phytochemical compounds suggests that the *A. chevalieri* plant has a 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. However, depending on the nature of research, compounds can be further purified using high-performance liquid chromatography, and nuclear magnetic resonance spectral analyses.

ARTICLE INFO

Article history:
Received 31 March 2021
Received in revised form
30 April 2021
Accepted 01 May 2021
Published 12 October 2021
Available online 12 October 2021

KEYWORDS

Phytochemical
Isolation
Column
Chromatography
Packing
Elution

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1. Introduction

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova et al., 2005). Extraction and characterization of several active phyto-compounds from these green factories have given birth to some high-activity profile drugs (Mandal et al., 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 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. 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 lesser 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, 2020). The constituents of plants are 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 (Chanda and Dave, 2009; Mojab et al., 2003).

Plants are healthy and natural resources of life (Russell and Duthie, 2011). In particular, medicinal plants are of great importance with endless therapeutic properties which are useful for curing various diseases with an advantage of being natural (El-Shemy et al., 2007). At present, there are uncountable products in the market with adverse side effects on one's heath. Therefore, the use of secondary metabolites from plant origin could be an advantage and best solution to narrow down the use of unhealthy products (Russell and Duthie, 2011).

In the past, the plant or microbial extracts in crude or partially purified forms were the only sources of medication available for the treatment of human and animal diseases. This gave an idea that the effect of a drug on human body is due to an interaction of drug with biological molecules. This opened new doors in pharmacology, as pure, isolated chemicals, instead of extracts, as the standard for the treatment of diseases. At present, there are innumerable bioactive compounds isolated form crude extracts and their chemical structures are elucidated (Bajpai et al., 2001). Moreover, plants have always been a source of a wide array of secondary metabolites with potential pharmacological properties (Russell and Duthie, 2011). Polyphenolic (flavonoids) compounds occurring ubiquitously in foods of plant origin have many beneficial health effects due to their potential antioxidant, anti-inflammatory and cancer-preventive activities (Li et al., 2014). Therefore, the objective of this study is to provide a step-by-step visual demonstration of fractionation and isolation of biologically active plant secondary metabolites using column chromatographic techniques.

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. It has an open and rounded or umbrella-shaped canopy, bark is pale-grayish, 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 tanning in Nigeria and Senegal. It is used in Borno, northeastern Nigeria, as a purgative, tenicide, and also as a remedy for cough. A decoction of the leaves is used in northern Nigeria as a 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 the 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 qualitative phytochemical constituents and also focused on a step-by-step visual demonstration of fractionation and isolation of biologically active plant secondary metabolites using column chromatographic techniques in *A. chevalieri.*

2. Materials and Methods

1. Cylindrical chromatographic column
2. Separating funnel

3. Silica gel 60 (Mesh 230–400)
4. *n*-Hexane (need for packing of column)
5. Cotton or silica sand
6. *n*-Hexane (need for packing of column)
7. Iodine chamber
8. UV light detector (254 nm)
9. Thin layer chromatography (TLC) plate-developing chamber
10. Lead pencil
11. Capillary tubes
12. Spatula
13. Organic solvents
14. Preparative TLC plates (20 × 20 cm)
15. Kim wipe tissue
16. Solvent mixer

2.1. Plant collection, authentication, and extraction

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. 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 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°C–37°C) away from direct sun light for 3 weeks and were later pulverized into coarse powder using mortar and pestle, and into fine powder using an electric grinder, the plant material was then stored in an air-tight container until use. Extraction was carried out via maceration using ethyl acetate, n-hexane, and methanol; the macerates after the extraction process were filtered twice through cotton wool and through What man No.1 filter paper; the residue after the filtration process were discarded and the filtrate was then concentrated using a rotary evaporator; the extracts were stored at 4°C, as described by Adoum (2001).

2.2. 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 (1989). These include alkaloids, saponins, tannins, flavonoids, anthraquinones, and steroids.

2.2.1. Test for saponins

Saponins were detected using the froth test method. Exactly 1 g of the sample was weighed in a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 minutes. The mixture 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 stoppered and shaken vigorously for about 30 seconds. It was later boiled for 10 minute and the froth was observed for the confirmation of saponins and allowed to stand for half an hour; honey comb froth indicates the presence of saponins (Sofowora, 1993).

2.2.2. Test for tannins

About 0.5 g of the extract was mixed thoroughly with 10 ml distilled water and then filtered; 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.

2.2.3. Test for glycosides

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

2.2.4. Test for alkaloids

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

2.2.5. Test for flavonoids

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

2.2.6. Test for anthraquinones

Exactly 0.5 g each of the plant extract was shaken with 10 ml of benzene and filtered, then 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 indicated the presence of anthraquinones (Sofowora, 1993).

2.3. Quantitative phytochemical analysis

These tests were carried out on the secondary metabolites, flavonoids and alkaloids, to determine the total phenolic compounds.

2.3.1. Estimation of total alkaloids

2.3.1.1. 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 thrice; 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 and evaporated to dryness; the fraction containing ajmalicine was weighed and serpentine was expressed as mg/100 g (Harborne, 1973). The percentage was calculated as follows:

$$Alkalodis\ (\%) = \frac{weight\ of\ Alkalodis}{weight\ of\ Sample} \times 100$$

2.3.2. Determination of flavonoids

Flavonoids determination was carried out according to the method reported by Ejikeme et al. (2014) and Boham and Kocipai (2004).

2.3.2.1. 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 discarding the supernatant, the residue was re-extracted (thrice) 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 desicator and weighed until constant weight was obtained (Okwu, 2004). The percentage of flavonoid was calculated as follows:

$$\%\ flavonoids\ (\%) = \frac{weight\ of\ flavonoid}{weight\ of\ Sample} \times 100$$

2.3.3. Estimation of total phenols

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

2.3.3.1. 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 was 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's reagent (0.5 ml) was added and equal volumes 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 was also treated as above. The concentration of phenols was expressed as µg/g.

2.4. Test sample

Air-dried sample powder of the plant material was extracted using different solvents and vacuum-dried before loading into column. The amount of the sample was measured in order to define the yield of the desired extract.

2.4.1. Gradient solvent system

Gradient solvent system (non-polar to high polar solvent system) provides best elution and best separation of various organic compounds from plant-organic extract. However, the volume of solvent can be dependent on the amount of the sample to be purified.

2.4.2. Organic solvents

They were ready to use and purchased commercially from Sigma (St. Louis, MO)

2.5. Column chromatography

A cylinder-shaped glass column containing stationary phase (silica gel) is encountered slowly from the top with a liquid solvent (mobile phase) that flows down the column with the help of gravity or external pressure applied. This technique was used for the purification of compounds from a mixture. Once the column was ready, the sample was loaded inside the top of the column. The mobile solvent was then allowed to flow down through the column. The compounds in mixture have different interaction abilities with the stationary phase (silica gel), and mobile phase, thereby flowing along the mobile phase at different time intervals or degrees. In this way, the separation of compounds from the mixture was achieved. The individual compounds are collected as fractions and analyzed further for structure elucidation.

2.6. Isolation and purification of bioactive compounds from Albizia chevaleri methanol leaf and root extracts

A suitable size long cylindrical glass column (based on the amount of the sample) was set to stand firm on a column chromatography stand. A completely dried leaf extract was mixed with silica gel to make a fine powdered form for easy distribution of sample in already packed silica gel column. Sample powdered mass was placed on top of the pre-packed silica column and the sample was covered with a layer of cotton. Then solvents of different polarities were passed through the column at uniform rate under gravity to fractionate the sample extract. Each fraction was collected separately in a test tube and numbered consecutively for further analysis on TLC. TLC provides partial separation of both organic and inorganic materials using thin-layered chromatographic plates, especially useful for checking the purity of fractions. Each fraction is applied on activated TLC plates with the help of capillary tube at half-inch apart from the lower edge of TLC plate, and the plate is kept in a developing chamber containing methanol for 5 minutes until the developing solvent reaches the top of the upper edge of TLC plate. The plate was taken out from the developing chamber, dried, and the solvent front was marked by lead pencil. Compound bands/spots were visualized on TLC chromate plate and can be detected by visual detection, under UV light (254 nm), in the iodine chamber for the presence of specific compounds. The visualized spots of the components in the chromate plate were marked and the R*f* value of each spot was calculated by the following formula: R*f* = distance travelled by the sample (cm) / distance travelled by the solvent (cm). The TLC plate showing the number of bands (compounds) for each fraction can be further purified using high-performance liquid chromatography (HPLC). Based on the nature of the compounds, further spectral analyses such as infrared (IR), mass spectrometry, and nuclear magnetic resonance (NMR) will be carried out to elucidate the chemical structure of target compounds.

3. 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 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 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.

Based on the column chromatography technique carried out, the TLC plate showing the number of bands (compounds) for each fraction and further study can be purified using -HPLC and based on the nature of the compounds, further spectral analyses such as IR, mass spectrometry, and NMR will be carried out to elucidate the chemical structure of target compounds.

4. Discussion

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 phytochemical determination in this study is shown to contain a significant amount of phenolic compounds. This research has shown that the leaf, stem bark, and the root of *A. chevalieri* contain significant amounts of flavonoids. It can be seen in Table 2. The fresh leaves, stem bark, and root of *Albizia chevalieri* were collected in the month of September, 2018 at Kurba-North, in Yamaltu-Deba

Table 1. The ratio of gradient solvent used in column chromatography. st samples.

Solvent system	Ratio	Volume (ml)	Fraction
Hexane	100%	50	1
Hexane: ethyl acetate	10:1	50	2
Hexane: ethyl acetate	5:1	48	3
Hexane: ethyl acetate	1:1	50	4
Hexane: ethyl acetate	1:5	48	5
Hexane: ethyl acetate	1:10	50	6
Ethyl acetate	100%	50	7
Ethyl acetate: methanol	10:1	50	8
Ethyl acetate: methanol	5:1	48	9
Ethyl acetate: methanol	1:1	50	10
Ethyl acetate: methanol	1:5	48	11
Ethyl acetate: methanol	1:10	50	12
Methanol	100	50	13

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 2 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 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 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 the stem bark of n-hexane extract. It was observed after quantifying alkaloids, as seen in Table 3 and Figure 1, that methanol root extract had 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, which were 25.72%, 21.34%, 16.30%, and 10.78%, respectively.

Pharmacologically important phytochemicals in different plant parts of *A. chevalieri* as shown in Figures 1 to 4 were quantified, and total phenolic content was found to be in good amounts. The root methanol extract showed maximum phenolic content as 44.48 µg garlic acid equivalent (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 amounts of phenolic compounds were found in the methanolic leaf extract of *A. chevalieri* (Hajara et al., 2017).

Not all secondary metabolites or natural products such as penicillin, morphine, and paclitaxel (Taxol) can be fully synthesized due to their very complex

Table 2. Qualitative phytochemical screening in three parts of *Albiziachevalieri* 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	+	+	+	–	–	–	+	–	–

S/bark Stem bark.
+ = Positive; – = Negative.

Table 3. Phytochemical composition in three parts of *Albiziachevalieri* 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

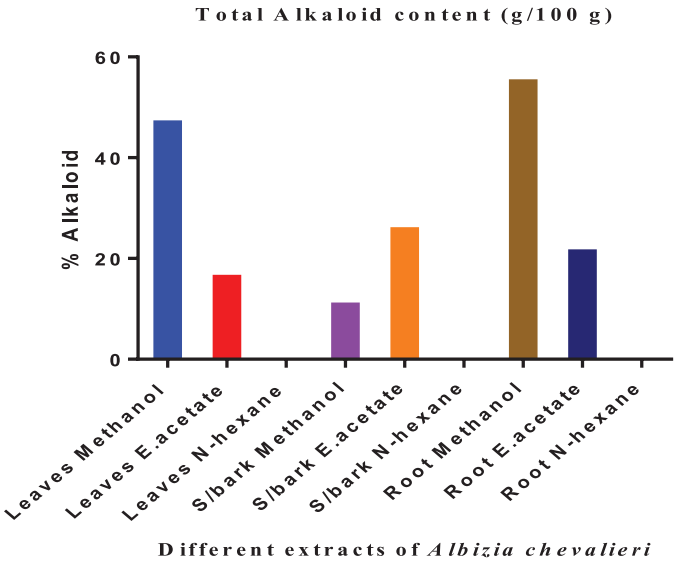

Figure 1. Total alkaloid content of different extracts of *A. chevalieri.*

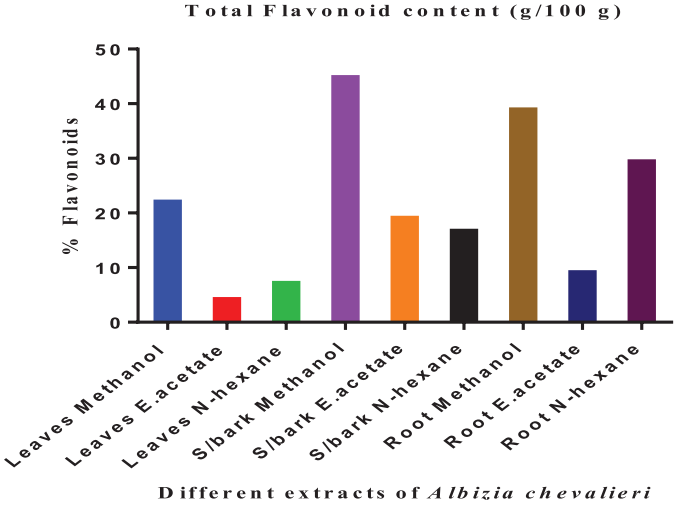

Figure 2. Total flavonoid content of different extracts of *A. chevalieri.*

structures that are too difficult and expensive on industrial scale. Hence, there is an urgent need to search for alternative remedies as naturally occurring biologically active secondary metabolites from plant origin. Certain phytochemicals act in many ways on various types of disease complex, and may potentially contribute to the field of pharmacology as natural supplements to control various infectious diseases in future as the fast and reliable alternatives. Many non-natural and synthetic drugs cause severe side effects that are not acceptable except as treatments of last resort for terminal diseases such as cancer. The metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs because they must accumulate within living cells (El-Shemy et al., 2007).

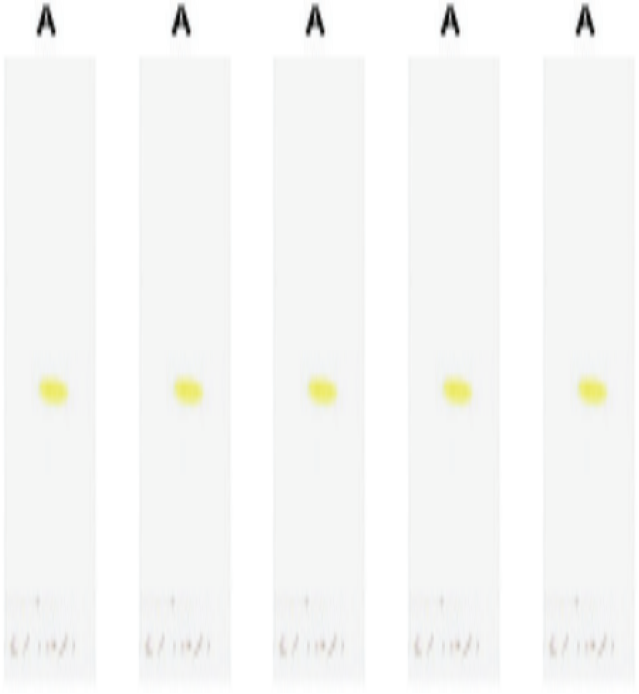

Figure 3. Total phenolic content of different extracts of *A. chevalieri.*

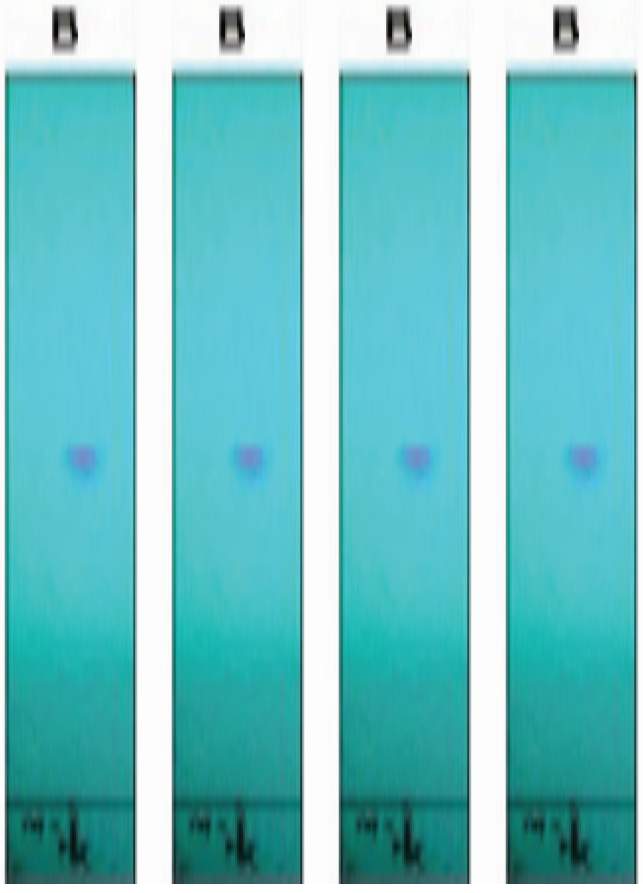

Figure 4. Demonstration of isolation pattern of pure compounds on TLC plate from leaf and root extracts of *A. chevalieri* using iodine (A), UV-detection (B).

Table 4. Spots of pure compounds in leaf and root extracts of *Albizia chevalieri.*

Solvent system	Ratio	Volume (ml)	Fraction	Bands/ spots
Hexane	100%	50	1	1
Hexane: ethyl acetate	10:1	50	2	1
Hexane: ethyl acetate	5:1	48	3	1
Hexane: ethyl acetate	1:1	50	4	1
Hexane: ethyl acetate	1:5	48	5	1
Hexane: ethyl acetate	1:10	50	6	1
Ethyl acetate	100%	50	7	1
Ethyl acetate: methanol	10:1	50	8	1
Ethyl acetate: methanol	5:1	48	9	1
Ethyl acetate: methanol	1:1	50	10	1
Ethyl acetate: methanol	1:5	48	11	1
Ethyl acetate: methanol	1:10	50	12	1
Methanol	100	50	13	1

Considering the deleterious effects of synthetic antibiotics, isolation, purification, and characterization of novel types of plant secondary metabolites could be safer alternatives to synthetic compounds (Bajpai and Kang, 2011) based on the current research spots of pure compounds in leaf and root extracts of A. Chevalieri were identified using thin layer chromatography and the results showed individual bands/spots that tell us the presence of various compounds in the extracts as shown in Table 4 and Figures 3 and 4. Various chromatographic techniques have been used for successful fractionation and purification of biologically active compounds from a variety of samples. Column chromatography is one of the most popular and widely used separation techniques to characterize both organic and inorganic materials suggesting its potential usefulness in chemical analysis of complex extract material. This research visualized successful application of column chromatographic techniques for the isolation of biologically active secondary metabolites from leaf and root of *A. chevalieri*. However, ongoing investigations of toxic or irritant properties are imperative, especially when considering any new biologically active compound for human use, be them medicinal or otherwise.

Acknowledgment

The authors are thankful to Gombe State University for the laboratory support and also to Musa Muktar and Sadisu Abubakar of the biochemistry laboratory for their technical support.

Conflict of interest

There is no conflict of interest among the authors in whatever form.

References

Adoum S, Coker H. Plants used in traditional medicine against malaria. Niger J Pharm 2001; 32:50–63.

Bajpai VK, Kang SC. Isolation and characterization of biologically active secondary metabolites from *Metasequoiaglyptostroboides*Miki ex Hu. J Food Saf 2011; 31:276–83.

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

Burkill H. The useful plants of west tropical Africa. Fam J R Bot Gard 1995; 3:207–8.

Chanda S, Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties. Afr J Microbiol Res 2009; 3:981–96.

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.

El-Shemy HA, Aboul-Enein AM, Aboul-Enein KM, Fujita K. Willow leaves' extracts contain antitumor agents effective against three cell types. PLoS One 2007; 2:e178.

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.

Hertog MG, Feskans EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993; 342(8878):1007–11.

Jeyachandran R, Mahesh A. Antimicrobial evaluation of *Kigelia africana* (llam). Res J Microbiol 2007; 2(8):6499.

Le Houerou H. Albizia chevalieri. Harms, 2009. Available via http://www.fao.org/ag/AGF/AGPC/doc/GBAS/E/Data/Pf000366.HTM (Accessed 21 March 2009).

Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB. Resources and biological activities of natural polyphenols. Nutrients 2014; 6:6020–47.

Mandal L, Martinez-Agosto JA, Evans CJ. A hedgehog and annepediadependent niche maintenance drasophila haematopoietic precursors. Nature 2007; 446(7133):320–4.

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.

Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 2020; 85(5):770–803.

Russell W, Duthie G. Plant secondary metabolites and gut health: the case for phenolic acids. Proc Nutr Soc 2011; 70:389–96.

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

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

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