J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg. , 47(2): 311-323, 2023
Doi: 10.33483/jfpau.1205941

ORIGINAL ARTICLE / ÖZGÜN MAKALE

PHYTOCHEMICAL PROFILING AND HEAVY METALS COMPOSITION OF AQUEOUS AND ETHANOL EXTRACTS OF *ANOGEISSUS LEIOCARPUS*

ANOGEISSUS LEIOCARPUS'UN SULU VE ETANOLİK EKSTRELERİNİN FİTOKİMYASAL PROFİLİ VE AĞIR METAL BİLEŞİMİ

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ABSTRACT

Objective: *The present study aimed to investigate the phytochemical and heavy metals components of A. leiocarpus considering its applications in ethnomedicine.*
Material and Method: *The phytochemical components were determined qualitatively and quantitatively gravimetrically, while component identification was done using Gas spectrometer-mass spectrometer (GC-MS) technique. Heavy metals were quantified by atomic absorption spectrophotometer.*
Result and Discussion: *Saponins and flavonoids were detected in the aqueous extracts in concentrations of 10.22% ±0.48, and 38.67% ±0.17 respectively, and concentrations of 17.37% ±0.65 and 19.63% ±0.60 respectively in the ethanol extract. GC-MS analysis identified 16 and 26 compounds in the aqueous and ethanol extracts respectively. In the aqueous extract, 5-Hydroxymethylfurfural, 1,2,4-Benzenetriol, and cis-Vaccenic acid had the highest peak areas of 46.24, 17.12, and 15.13% respectively, while in the ethanol extract 5-Hydroxymethylfurfural (14.40%), 1,2,3-Benzenetriol (12.29%) and -methoxybenzene-1,4-diol (7.54%) were the highest. Chromium (0.548 ppm ±0.030) was detected only in the aqueous concentration, while Cadmium had a concentration of 0.002 ±0.001 and 0.006 ppm ±0.002 in the aqueous and ethanol extract respectively. Lead was present with aqueous and ethanol extracts concentrations of 0.096 ±0.020*

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Submitted / Gönderilme : 16.11.2022
Accepted / Kabul : 29.12.2022
Published / Yayınlanma : 20.05.2023

and 0.096 ppm ±0.040 respectively. Conclusively, the present study agrees with the claims for the traditional application of the plant in folkloric medicine to manage different ailments.
Keywords: *Anogeissus leiocarpus, GC-MS, heavy metals, phytochemicals, profiling*

ÖZ

Amaç: *Bu çalışma, A. leiocarpus'un fitokimyasal ve ağır metal bileşenlerinin etnomedikaldeki uygulamalarını göz önünde bulundurmayı amaçlamıştır.*
Gereç ve Yöntem: *Fitokimyasal bileşenler kalitatif ve kantitatif olarak gravimetrik olarak belirlenirken, bileşen tanımlaması Gaz spektrometresi-kütle spektrometresi (GC-MS) tekniği kullanılarak yapılmıştır. Ağır metallerin miktarı atomik absorpsiyon spektrofotometresi ile belirlenmiştir.*
Sonuç ve Tartışma: *Saponinler ve flavonoidler sulu ekstrelerde sırasıyla %10.22 ±0.48 ve %38.67 ±0.17 konsantrasyonlarında ve etanol ekstraktında sırasıyla %17.37 ±0.65 ve %19.63 ±0.60 konsantrasyonlarında tespit edildi. Ancak alkaloidler, steroidler, glikozitler ve terpenoidler tespit edilmedi. GC-MS analizi, sulu ve etanol ekstrelerinde sırasıyla 16 ve 26 bileşik tanımladı. Sulu ekstrede 5-Hidroksimetilfurfural, 1,2,4-Benzenetriol ve cis-Vaccenic asit sırasıyla 46.24, 17.12 ve 15.13 ile en yüksek pik alanlarına sahipken, etanol ekstresinde 5-Hidroksimetilfurfural (%14.40), 1,2,3-Benzenetriol (%12,29) ve -metoksibenzen-1,4-diol (%7,54) en yüksek değerlerdi. Krom (0.548 ppm ±0,030) sadece sulu konsantrasyonda tespit edilirken Kadmiyum, sulu ve etanol özütünde sırasıyla 0,002 ±0,001 ve 0.006 ppm ±0.002 konsantrasyona sahipti. Kurşun, sırasıyla 0.096 ±0.020 ve 0.096 ppm ±0.040'lık sulu ve etanol özütlerinde mevcuttu. Sonuç olarak, bu çalışma, bitkinin farklı rahatsızlıkları tedavi etmek için folklorik tıpta geleneksel uygulamasına ilişkin iddialarla hemfikirdir.*
Anahtar Kelimeler: *Ağır metaller, Anogeissus leiocarpus, GC-MS, fitokimyasal, profil oluşturma*

INTRODUCTION

Medicinal plants sources of important bioactive compounds with pharmacological roles attributed to their phytochemical compositions. Plants are exploited as therapeutics for the management of different ailments in traditional medicine due to their phytochemical components. Formulations in form of decoctions or powder are taken orally or through topical applications in phytotherapy. The presence of phytochemicals offer a diversification of pharmacological activities which allows for the development of novel drugs for application in modern medicine [1]. Worldwide, medicinal plants are processed into different finished plant-based products of various efficacy for use at different doses as commercial products for export [2]. More than 70.000 species of plants were reported to be used in the management of various ailments worldwide [3]. Notably, in rural areas, herbalists take advantage of the diversity of medicinal plants and utilized them for various ailments [4]. Modern pharmaceutical industries rely on the supply of bioactive compounds for production, thus, providing information on these compounds through research on medicinal plants is important [5]. Different plants of varying efficacy were reported to possess different pharmacological activities in comparison to modern medicine applied in the treatment of several ailments [6].

Anogeissus leiocarpus which is called African birch in English and *Marke* in the native language (Hausa) of Northern Nigeria. Aqueous extract of *A. leiocarpus* has been reported to exert pharmacological effects against African trypanosomiasis which was credited to the phytochemicals present in the plant [7]. In another study, anti-microbial, anti-inflammatory, anti-diabetic, and wound healing were among the pharmacological activities of aqueous extract of *A. leiocarpus* reported [8]. In a similar study, the antifungal activity of ethanol extract of *A. leiocarpus* was reported which was accredited to the application of the plant in the folkloric treatment of candidiasis [9]. In another study, the butanol, hexane, and aqueous extracts of *A. leiocarpus* exerted pharmacological activities against *Klebsiella* spp., *Escherichia coli*, and *Pantoea agglomerans*, which was attributed to the phytochemical constituents of the plant, and was suggested to be a source of therapeutics against drug-resistant bacteria [10]. In a previously reported study, the methanol and aqueous extracts of *A. leiocarpus* was reported to possess antioxidant potential and suggested to be a source for the development of novel therapeutic agents [11]. A combined administration of *A. leiocarpus* and *Khaya senegalensis* indicated a better

treatment option against trypanosomosis supporting the utilization of the plants by pastoralists in the treatment of trypanosomosis [12]. Hydro alcoholic extract of *A. leiocarpus* was reported as an antioxidant and anti-diabetic agent and could be developed into an alternative for the management of diabetes [13].

Therefore, in the present study we aimed to investigate the phytochemical profile and heavy metals composition of aqueous and ethanol extracts of Anogeissus leiocarpus due to the extensive application of the plant in the management of different ailments.

MATERIAL AND METHOD

Plant Material

A sample of *A. leiocarpus* plant was collected from Girei Local Government, Adamawa state, Nigeria, and was identified by a Forest Technologist from the Forestry Technology Department of Adamawa State Polytechnic, Yola. A voucher specimen was kept in the departmental herbarium with voucher number ASP/FT/101. The drying of the stem bark was done under shade and ground to powder using a blender.

Reagents and Chemicals

All the chemicals and reagents used in this research were of AnarlaR (Xilong Scientific Co., Ltd. Guangdong, China).

Extraction

Extraction was done by maceration of 400 g of *A. leiocarpus* bark powder in 1.5 l of ethanol and distilled water for 2 days at room temperature, followed by filtration and concentration to dryness under reduced pressure at 40°C [14].

Qualitative Phytochemical Analysis

The detection of phytochemicals present aqueous (ASBE) and ethanol (ESBE) stem bark extracts of *A. leiocarpus* was carried out using a method reported previously to detect alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids [14].

Alkaloids

To 2 ml of the extract, 2 ml of 10% HCl was added, followed by the addition of 2 ml of Meyer's reagent. Formation of an orange precipitate indicate a positive result.

Saponins

To 2 ml of the extract, 2 ml distilled water was added. The mixture was agitated in a test tube for 5 min. Appearance of a layer of foam indicated a positive result.

Steroids

To 2 ml of the extract, 10 ml of chloroform was added and then, 10 ml of concentrated sulphuric acid was added by the side of the test tube. Formation of a reddish upper layer and yellow sulphuric acid layer with green fluorescence indicate a positive result.

Glycosides

To 2 ml of acetic acid, 2 ml of the extract was added. The mixture was cooled in cold water bath, and then 2 ml of concentrated H_2SO_4 was added. Colour development from blue to bluish green indicate the presence of glycosides.

Terpenoids

To 2 ml of the extract, 2 ml of chloroform and 1ml of concentrated sulphuric acid were carefully added to form a layer. A clear upper and lower layer with a reddish-brown interphase indicate a positive result.

Flavonoids

To 2 ml of the extract, 10% sodium hydroxide was added. A yellow color was formed which turned colorless upon addition of 2 ml of dilute hydrochloric acid indicating a positive result.

Quantitative Phytochemical Analysis

The quantification of phytochemicals in ASBE and ESBE of *A. leiocarpus* was carried out by the following methods:

Saponins Content

Saponins quantification was done by the method previously described [15]. Briefly, 0.5 g extract was introduced into a conical flask and 10 ml of 20% aqueous ethanol was added. The sample was heated over a water bath for 1 h with continuous stirring at about 550°C. The concentrate was transferred into a 250 ml separator funnel and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded. About 10 ml of n-butanol was then added followed by addition of 2 ml of 5% aqueous NaCl. The remaining solution was heated over a water bath. After evaporation, the sample was dried in the oven to a constant weight and calculated as follows:

$$\% \text{ Total Saponins} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

Flavonoid Content

Flavonoid quantification was carried out according to a method described previously [16]. About 0.5g of the extract was mixed with 10 ml of 80% aqueous methanol. The whole solution was filtered through Whatman filter paper. The filtrate was transferred to a pre-weighed crucible and evaporated into dryness over a water bath and weighed, and calculated as follows:

$$\% \text{ Total Flavonoids} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

Gas chromatography-mass spectrometry analysis was carried out with a combination of a Gas chromatography-mass spectrophotometer (Agilent 19091-433HP, USA). The system was fitted fused with a silica column. A column flow velocity of 1.6 ml/min was set for the carrier gas (Helium). Ion-source temperature was set to 250°C while the pressure was 8.6 psi. A split mode injection (1 µl) at 250°C was used. The initial temperature of the column was set at 100°C, and gradually increased to 180°C at 20°C/min, then 10°C/min to 280°C. The total elution time was 16 min. The National Institute of Standards and Technology (NIST) database was used for the identification and comparison of the unknown spectrum of the detected compounds with that of known standards.

Determination of Heavy Metals Composition

Atomic absorption spectrophometric method principled on the absorption of of light by different elements at different wavelenths was used to determine the concentration of heavy metals [17]. A gram of the samples was ashed at 500ºC for 1 h, which was dissolved in 25 ml of 10% HCl and made up to 100 ml. Chromium (Cr), cadmium (Cd), and lead (Pb) contents were quantified by the method previously described [17] using Atomic Absorption Spectrophotometer (AAS) (Buck Scientific AAS210).

Statistical Analysis

Data obtained in the present study were expressed as mean ± standard error of triplicate determinations' mean (± SEM) evaluated with Statistical Package for the Social Sciences (SPSS) version 22 Software.

RESULT AND DISCUSSION

The phytochemicals detected in ASBE and ESBE of *A. leiocarpus* are shown in Table 1. Saponins and flavonoids were detected in both ASBE and ESBE of *A. leiocarpus*, while alkaloids, steroids, glycosides, and terpenoids were absent in both extracts.

Table 1. Qualitative phytochemical composition of aqueous (ASBE) and ethanol (ESBE) stem bark extracts of *A. leiocarpus*

Phytochemical	**Inference**	
	Aqueous	**Ethanol**
Alkaloids	-	-
Saponins	+	+
Steroids	-	-
Glycosides	-	-
Terpenoids	-	-
Flavonoids	+	+

+ = present, - = Absent.

The phytochemicals quantified in ASBE and ESBE of *A. leiocarpus* are shown in Table 2. Although both saponins and flavonoids were detected in ASBE and ESBE of *A. leiocarpus*, saponins were in higher amounts in ESBE (38.67% ±0.17) than in the ASBE (10.22% ±0.48) of *A. leiocarpus*. Flavonoids were also detected in higher concentrations in the ESBE (19.63% ±0.60) than ASBE (17.37% ±0.65) of *A. leiocarpus*.

Table 2. Quantitative phytochemical composition of aqueous (ASBE) and ethanol (ESBE) stem bark extracts of *A. leiocarpus*

Phytochemical	**Concentration of extracts (%)**	
	Aqueous	**Ethanol**
Saponins	10.22 ±0.48	38.67 ±0.17
Flavonoids	17.37 ±0.65	19.63 ±0.60

Concentration values are in triplicates determinations (± SEM)

Although only saponins and flavonoids were present in both ASBE and ESBE of *A. leiocarpus*, their concentrations were different. Both saponins (38.67% ±0.17) and flavonoids (19.63% ±0.60) were present in higher concentrations in the ESBE than in ASBE (10.22% ±0.48 and 17.37% ±0.65 respectively). The difference in polarity of the solvents used might be attributed to the difference in the concentration of the phytochemicals quantified in the present study [18]. Saponins were reported to poses several pharmacological activities [19]. Saponins were reported to exert antitumor activities through multiple signaling pathways by blocking cellular proliferation, promoting apoptosis, and controlling the tumor microenvironment [20]. The saponin trillin was reported to exert antioxidant activity through the elevation of superoxide dismutase, catalase, and glutathione peroxidase which are antioxidant enzymes, thus protecting the heart against oxidative stress [21]. In another study, saponins were reported to raise the activity of several antioxidant enzymes, responsible for cardiac protection against reactive oxygen species [21]. Dioscin which is a saponin has been reported to be an active agent against a tumor, microbes, inflammation, and oxidative stress [22].

Flavonoids exerts antibacterial action against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* [23]. The anticancer activity of flavonoids against human hepatocarcinoma was

previously reported to be through induction of apoptosis via the mitochondria-dependent apoptotic pathway and death receptor-dependent apoptotic pathways, thereby suppressing tumor growth [24]. Flavonoids were reported to exert antibacterial activity against different species by destroying the cell membrane, blocking energy metabolism and nucleic acid synthesis, however, the antiviral activity of flavonoids against HIV was reported to be by blocking the phosphorylation of protein by cytokine II, preventing the integration of the virus [25]. In another study, flavonoids were reported to prevent cardiovascular diseases due to their antioxidant properties by modulating the functions of many inflammatory mediators and inhibiting immune cells [26]. The results reported in our study agree with the study reported previously where saponins and flavonoids were detected [27]. In a similar study, flavonoids were detected in the ESBE of *A. leiocarpus* while alkaloids were absent which agrees with the present study [28]. Previous studies on phytochemical components of stem bark extracts of *A. leiocarpus* reported results similar to our study [29, 30].

The bioactive compounds identified in the aqueous (ASBE) of *A. leiocarpus* using GC-MS with their retention time, peak area, molecular weight, and formula are presented in Table 3. In the ASBE, 16 compounds were detected with 5-Hydroxymethylfurfural having the highest (46.24%) peak area, followed by 1,2,4-Benzenetriol (17.12%) and cis-Vaccenic acid (15.13%). Maltol and Methyl 14-methylpentadecanoate had peak areas of 5.14% and 3.96% respectively. Zidovudine, Ascorbyl dipalmitate, and Octadecanal were also identified in ASBE of *A. leiocarpus*. The structures of the identified compounds from ASBE of *A. leiocarpus* showing the different groups present are also shown in Figure 1, while the chromatogram of the GC-MS analysis showing different peaks and retention times of the identified compounds is present in Figure 2.

Table 3. Bioactive compounds identified in ASBE of *Anogeissus leiocarpus* using GC-MS

S/N	Name of compound	Retention Time	Peak Area (%)	Molecular weight	Formula
1	5-Hydroxymethylfurfural	3.613	46.24	126.11184	$C_6H_6O_3$
2	Maltol	4.535	5.14	126.11184	$C_6H_6O_3$
3	1,2,4-Benzenetriol	5.194	17.12	126.11184	$C_6H_6O_3$
4	3-Methyl-1H-pyrazole-5-carboxylic acid	6.818	1.04	126.11484	$C_5H_6N_2O_2$
5	Methyl 14-methylpentadecanoate	6.978	3.96	270.45576	$C_{17}H_{34}O_2$
6	1,3,5-Benzenetriol	7.481	2.04	126.11184	$C_6H_6O_3$
7	1,2,3-Benzenetriol	7.785	0.33	126.11184	$C_6H_6O_3$
8	9-Octadecenoic acid (Z)-, methyl ester	8.431	1.87	296.49364	$C_{19}H_{36}O_2$
9	Zidovudine	8.609	1.30	267.24432	$C_{10}H_{13}N_5O_4$
10	4-Ethylcyclohexanone	8.923	1.07	126.19856	$C_8H_{14}O$
11	1-(4-Bromobutyl)piperidin-2-one	9.124	0.83	234.13614	$C_9H_{16}BrNO$
12	Ascorbyl dipalmitate	10.749	0.30	652.95312	$C_{38}H_{68}O_8$
13	Octadecanal	11.933	0.08	268.48324	$C_{18}H_{36}O$
14	cis-Vaccenic acid	14.788	15.13	282.46676	$C_{18}H_{34}O_2$
15	cis-11-Hexadecenal	15.692	2.03	238.4136	$C_{16}H_{30}O$
16	Oxirane, tetradecyl-	15.692	2.03	240.42948	$C_{16}H_{32}O$

The compounds identified in ESBE of *A. leiocarpus* using GC-MS and their various, retention times, peak area, molecular weight, and formulas are presented in Table 4. A total of 26 compounds were detected with 5-Hydroxymethylfurfural and 1,2,3-Benzenetriol having peaks of 14.40% and 12.29% respectively. 2-methoxybenzene-1,4-diol (7.54%), 3-Methyl-1H-pyrazole-5-carboxylic acid (5.56%), and Hexadecanal (5.01%) were among the compounds detected. Methyl palmitate, cis-vaccenic acid, oleic acid, squalene, erusic acid, and hexadecanal were also identified in the ESBE of *A. leiocarpus*. The structural formula of the identified compounds is shown in Figure 3, while Figure 4

shows the chromatogram of the identified compounds with their various peaks and retention time.

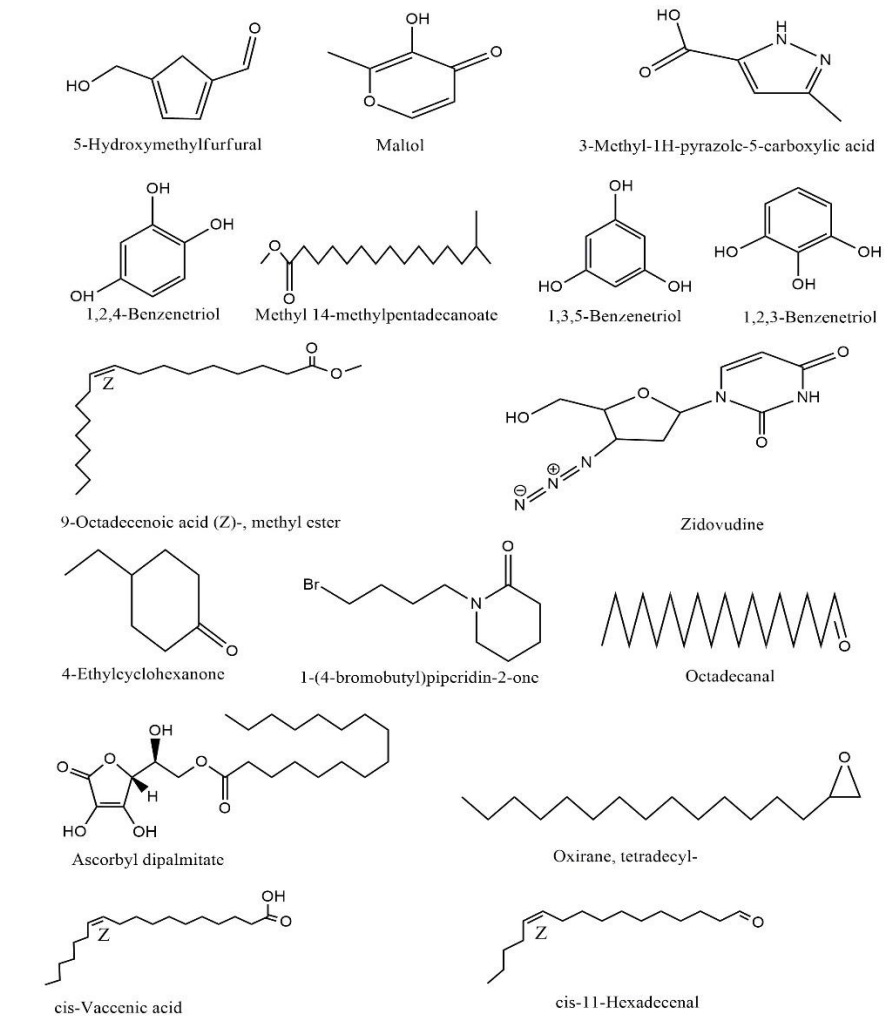

Figure 1. Structures of compounds identified in ASBE of *Anogeissus leiocarpus*

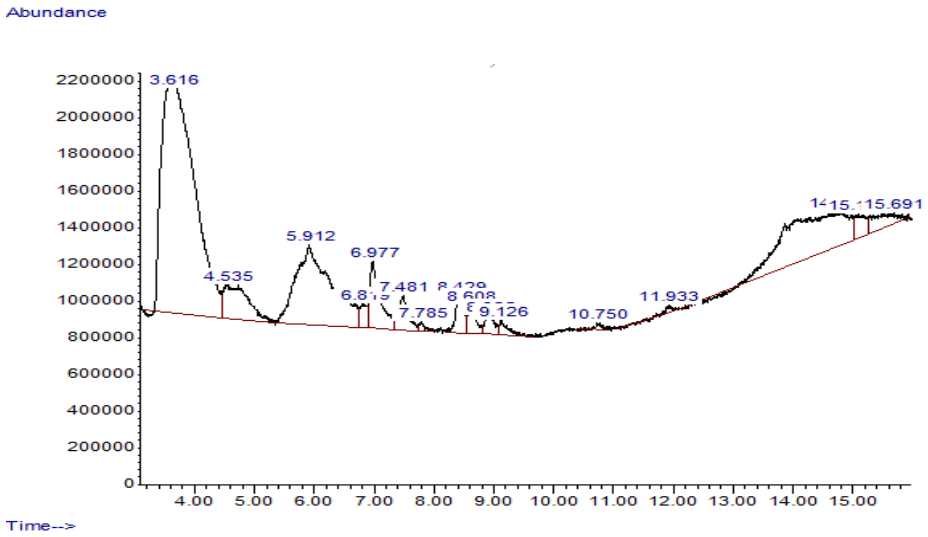

Figure 2. GS-MS Chromatogram for compounds identified in ASBE of *A. leiocarpus*

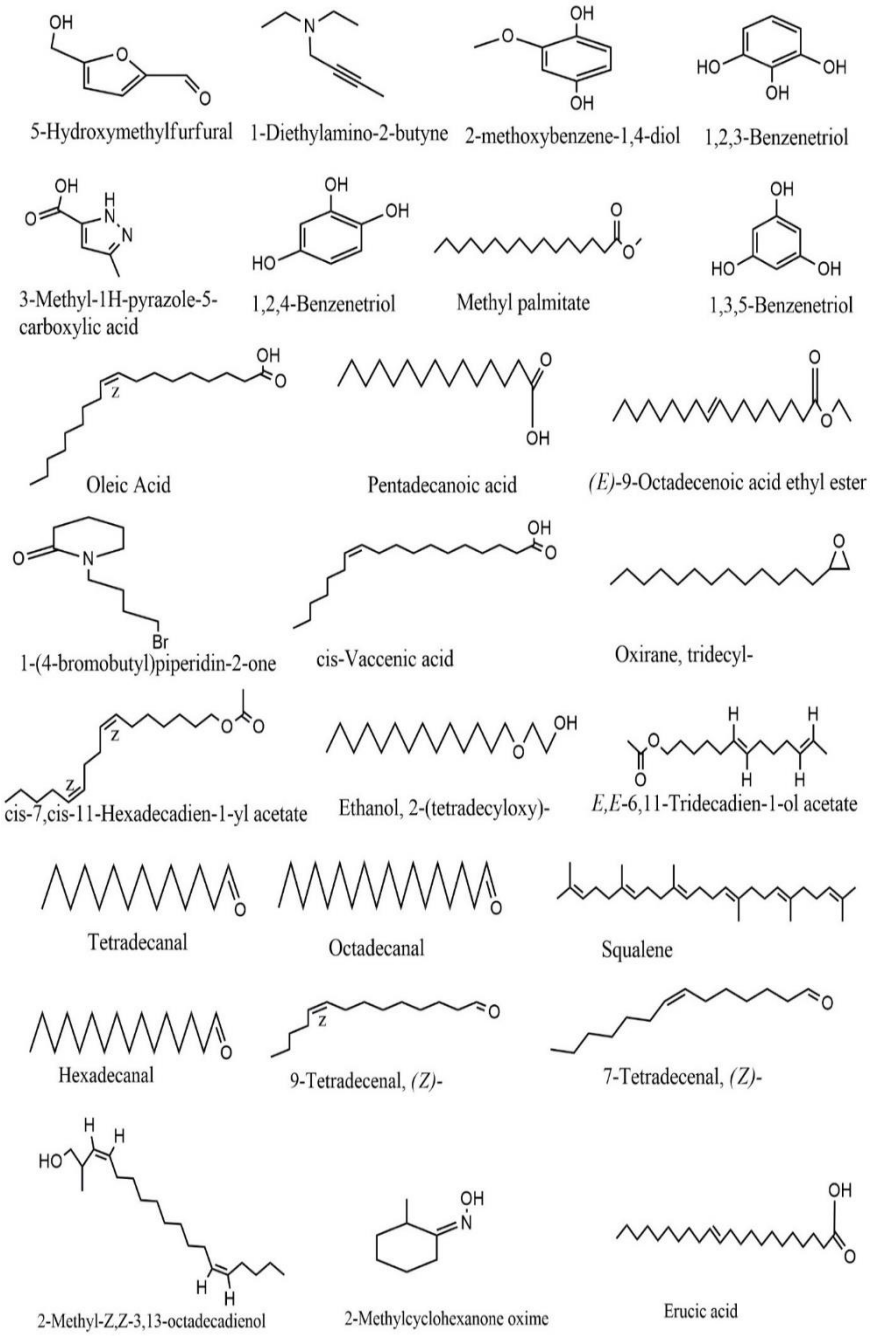

Figure 3. Structural formulas of compounds detected in ESBE of *Anogeissus leiocarpus*

GC-MS analysis of ASBE of *A. leiocarpus* identified 16 compounds (Table 3). The various compounds detected are associated with different pharmacological properties. 5-Hydroxymethylfurfural was reported to show anticancer activity against cancer cells by induction of cell apoptosis and cell circle arrest. This compound also demonstrated antioxidant potential by scavenging free radicals and increasing the activity of antioxidant enzymes [31]. The anti-inflammatory activity of 5-Hydroxymethylfurfural against lung injury was reported to be mediated through blocking of endoplasmic reticulum stress and activating of inflammasome [32]. Cis-vaccenic acid has been reported to be associated with antibacterial activity and anti-hyperglycemic effects in rats [33]. GC-MS analysis of ESBE of *D. leiocarpus* showed the presence of 26 compounds (Table 4). 1,2,3-Benzenetriol otherwise called pyrogallol demonstrated anti-malarial activity by its auto-oxidation in the presence of metallic

ions (Cu^{2+}, Fe^{3+}, and Mn^{2+}) to produce free radicals, thus inhibiting the growth of parasite, which is a characteristic of anti-malarial drugs [34]. Anti-bacterial study of pyrogallol reported inhibition of two strains of *Staphylococcus aureus* [35]. Methyl palmitate demonstrated pharmacological activity by decreasing inflammation through the reduction of the expression of cytokines promoting inflammation and increasing the expression of anti-inflammatory cytokines [36]. Methyl palmitate was reported to show antioxidant activity by reducing markers of oxidative stress and elevating the activities of the innate antioxidants [33]. Oleic acid exerts anti-inflammatory activity by acting on different pathways of immune cells that modulate inflammation [37]. Squalene detected in ethanol extract was reported to possess anti-diabetic activity against type 2 diabetes, and peroxidation [38].

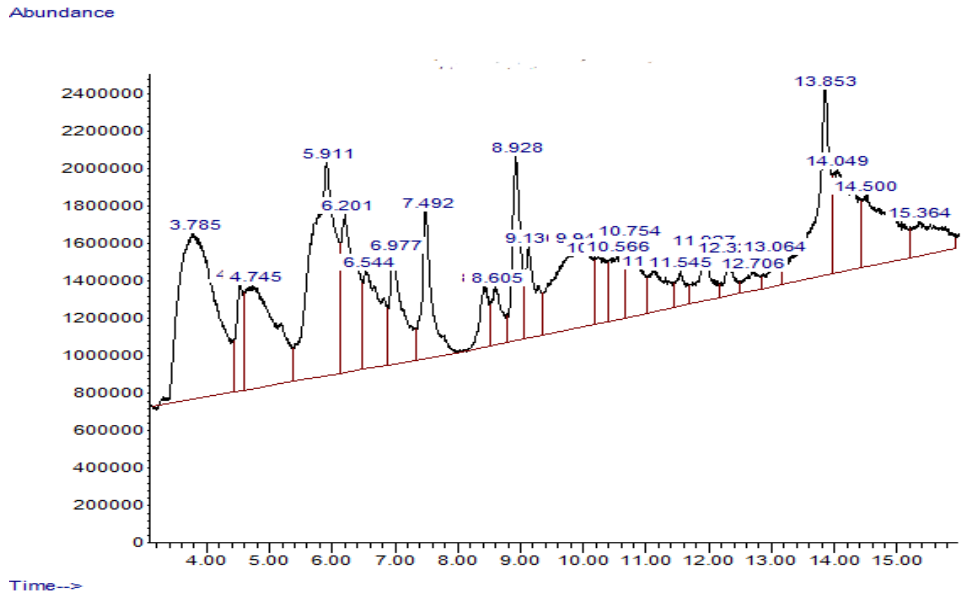

Figure 4. GS-MS Chromatogram for compounds identified in ESBE of *A. leiocarpus*

The heavy metals detected in ASBE and ESBE of *A. leiocarpus* and their respective concentrations are presented in Table 4. Chromium (Cr) was present in the highest concentration (0.548 ppm ±0.030) among all the heavy metals, though it was not detected in ESBE. Cd was detected in the least concentrations of 0.002 ppm ±0.001and 0.006 ppm ±0.002 for ABSE and ESBE respectively, while lead had concentrations of 0.096 ppm ±0.02 and 0.096 ppm ±0.04 in ASBE and ESBE respectively. Heavy metals exposure leads to acute and chronic toxicity targeting different organs of the body with effects such as cancer, gastrointestinal disturbance, and birth defects [39]. Exposure to heavy metals at lower continuous doses or high doses generate of reactive oxygen species (ROS) subsequently leading to oxidative stress and subsequently damaging the DNA, causing lipid peroxidation and modification of proteins [40]. Exposure to chromium leads to damage to the DNA by generation of ROS subsequently causing cancers of the kidney, bone, testicle, and thyroid [41]. Cd binds to the protein metallothionein, subsequently absorbed by the kidney leading to chronic toxicity of the kidney [42]. Cd exposure might also lead to carcinogenesis due to oxidative stress mediated by the generation of ROS, disturbance in gene expression and cell proliferation, and resistance to apoptosis [43]. The toxicity of lead (Pb) might be due to exposure to air and drinking water, which lead to disturbance of normal body processes [44].

The levels of heavy metals reported in our study were below the regulatory limits which are1.30, 0.02, and 2 ppm for Cr, Cd, and Pb respectively [45]. Although these heavy metals are present in the plant, traditional use of the plant occasionally might be said to be safe. In a previous study, the concentrations of lead and cadmium in the trunk bark of *A. leiocarpus* were reported to be 0.255 ppm and 0.22 ppm respectively [46]. The present study doesn't agree with this result as the concentration of lead and cadmium were lower both in the aqueous and ethanol extracts. In a similar study, the concentrations Cr, Cd, and Pb in leave of *A. leiocarpus* collected from a quarry site was reported to be

< 0.04, <0.01, and 0.060±0.01 mg/l [47], higher than values reported in the present study. In another study, the levels of Cr, Cd, and Pb in herbal drugs prepared *A. leiocarpus* were 0.061 ±0.001, 0.016 ±0.00, 0.386 ±0.001 mg/Kg [48]. The values for Cr, and Cd reported in their study were lower than the value (Table 5) reported in our study. However, the concentration of Pb was lower in our study. Weather conditions [49], location for sample collection [50], and environmental conditions [51] such as PH, temperature and dissolved oxygens were reported to influence the levels of heavy metals in soil samples. Heavy metals are absorbed by plants from the soil, the difference between concentration values reported in previous studies and our sudy might be due to the factors such as weather condition, location fro sample collection and environmental conditions.

Table 4. Bioactive compounds identified in ESBE of *Anogeissus leiocarpus*

S/N	Name of compound	Retention Time	Peak Area (%)	Molecular weight	Formular
1	5-Hydroxymethylfurfural	3.785	14.40	126.11184	$C_6H_6O_3$
2	1-Diethylamino-2-butyne	4.540	1.85	125.2138	$C_8H_{15}N$
3	2-methoxybenzene-1,4-diol	4.746	7.54	140.13872	$C_7H_8O_3$
4	1,2,3-Benzenetriol	5.913	12.29	126.11184	$C_6H_6O_3$
5	3-Methyl-1H-pyrazole-5-carboxylic acid	6.200	5.56	126.11484	$C_5H_6N_2O_2$
6	1,2,4-Benzenetriol	6.543	4.01	126.11184	$C_6H_6O_3$
7	Methyl palmitate	6.978	3.44	270.45576	$C_{17}H_{34}O_2$
8	1,3,5-Benzenetriol	7.493	3.62	126.11184	$C_6H_6O_3$
9	Oleic Acid	8.425	1.37	282.46676	$C_{18}H_{34}O_2$
10	Pentadecanoic acid	8.603	1.39	242.402	$C_{15}H_{30}O_2$
11	(E)-9-Octadecenoic acid ethyl ester	8.929	3.62	310.52052	$C_{20}H_{38}O_2$
12	1-(4-bromobutyl)piperidin-2-one	9.129	2.22	234.13614	$C_9H_{16}BrNO$
13	cis-Vaccenic acid	9.942	7.11	282.46676	$C_{18}H_{34}O_2$
14	Oxirane, tridecyl-	10.234	1.61	226.4026	$C_{15}H_{30}O$
15	cis-7,cis-11-Hexadecadien-1-yl acetate	10.565	2.17	280.45088	$C_{18}H_{32}O_2$
16	Ethanol, 2-(tetradecyloxy)-	10.754	2.52	258.44476	$C_{16}H_{34}O_2$
17	E,E-6,11-Tridecadien-1-ol acetate	11.120	1.68	238.37	$C_{15}H_{26}O_2$
18	Tetradecanal	11.544	0.84	212.37572	$C_{14}H_{28}O$
19	2-Methyl-Z,Z-3,13-octadecadienol	11.927	1.60	280.5	$C_{19}H_{36}O$
20	2-Methylcyclohexanone oxime	12.322	0.85	127.18632	$C_7H_{13}NO$
21	7-Tetradecenal, (Z)-	12.705	0.65	210.35984	$C_{14}H_{26}O$
22	Octadecanal	13.066	0.81	268.48324	$C_{18}H_{36}O$
23	Squalene	13.856	6.64	410.727	$C_{30}H_{50}$
24	Erucic acid	14.050	4.83	338.57428	$C_{22}H_{42}O_2$
25	Hexadecanal	14.502	5.01	240.42948	$C_{16}H_{32}O$
26	9-Tetradecenal, (Z)-	15.366	2.35	210.35984	$C_{14}H_{26}O$

Table 5. Heavy metals composition of *Anogeissus leiocarpus*

Heavy metal	Concentration (ppm)	
	Aqueous extract	**Ethanol extract**
Chromium (Cr)	0.548 ±0.030	-
Cadmium (Cd)	0.002 ±0.001	0.006 ±0.002
Lead (Pb)	0.096 ±0.02	0.096 ±0.04

Concentration values are in triplicates determinations (± SEM), - = Absent.

This study revealed that different bioactive compounds are present in the aqueous and ethanol extracts of *A. leiocarpus*. These are associated with different pharmacological activities, with low levels of heavy metal concentrations. Thus, this study justified the claims for the folkloric application of this plant in traditional medicine to manage different ailments. Additionally, the bioactive compounds detected might be utilized in the development of novel therapeutics with different pharmacological activities.

ACKNOWLEDGEMENTS

The authors show their highest gratitude to Tertiary Education Trust Fund (TETfund) Nigeria for funding the research through the Institution based research (IBR) fund. The authors are also thankful to the Department of Science laboratory technology, Adamawa State Polytechnic, for Institutional support and assistance.

AUTHOR CONTRIBUTIONS

Concept: M.M.D., E.B.B., N.M.; Design: M.M.D., N.M.; Control: E.B.B.; Sources: M.M.D.; Materials: M.M.D., E.B.B.; Data Collection and/or Processing: M.M.D., E.B.B., N.M.; Analysis and/or Interpretation: M.M.D., E.B.B., N.M.; Literature Review: M.M.D.; Manuscript Writing: E.B.B., N.M.; Critical Review: M.M.D., E.B.B., N.M.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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