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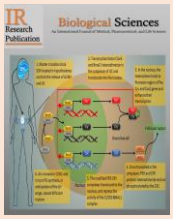

Phytochemical Analysis and Antioxidant Potential of Ethanol Extracts of *Anogeissus leiocarpus* (DC.) Guill & Perr and *Acacia nilotica* (L.) Delile

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ABSTRACT

Acacia nilotica (Gum Arabic) and *Anogeissus leiocarpus* (African birch) are multipurpose plants that have been used in the African traditional system of medicine. Various studies have proven their antibacterial, anti-diabetic, anti-diarrheal, and wound-healing effects. The aim of this research was to investigate the phytochemical constituents and antioxidant potential of the ethanol extracts of *Anogeissus leiocarpus* stem bark and *Acacia nilotica* pod. Phytochemical screening was carried out using the standard protocol and compounds were identified by GC-MS and FT-IR analysis methods on a spectrophotometer system. Antioxidant assay was carried out using the free radical scavenging of the 1-1-diphynyl-2-picrylhydrazyl radical (DPPH). The phytochemical screening revealed the presence of saponins, tannins, phenols, alkaloids, flavanoids and terpenoids in both the investigated plants extract. Results from GC-MS analysis identified 69 and 76 compounds in *A. Nilotica* and *A. leiocarpus* respectively. The FT-IR analysis of *A. Nilotica* confirmed the presence of O-H, N-H, C=O, C-O, C-H, C-C, and C≡C functional groups. The FT-IR analysis of *A. leiocarpus* also confirmed the presence of O-H, C≡C, C-H, N-H, C=C, N-O, C-H, C-N, O=H, C-O and C=O functional groups. The IC50 value of A.leiocarpus was found to be 0.27±0.719µg/ml and that of A.nilotica was 0.11±0.002µg/ml which showed no significant difference ($P<0.01$) with the control (Ascorbic acid) 1.53±1.37 µg/ml. Hence the investigated plants can be suggested as promising agents for antioxidant drug development.

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Introduction

Phytochemicals also known as secondary metabolites are organic compounds produced by plants that act as therapeutic agent for the treatment of various human diseases such as diabetes, heart disorder, wound healing, liver diseases and cancer (1). The medicinal effects of

plant materials to humans typically result from the action of these phytochemicals (2). Phytochemicals from medicinal plants have been widely studied and used as a source of antioxidants to scavenge excessive free radicals in various disease models (3). Plants rich in phenolic compounds like flavonoids have been demonstrated to have anti-inflammatory, anti-allergic, anti-viral, anti-aging, and anti-carcinogenic activities which can be attributed to their antioxidant properties (4). Hence the present study focused on Phytochemical screening and antioxidant analysis of ethanol Pod extract of *Acacia nilotica* and ethanol stem extract of *Anogeissus leiocarpus*.

Anogeissus leiocarpus (family combretaceae) is a deciduous tree that is sparsely distributed (5) and can grow up to 15–30 m in height and measure up to 1 m in diameter. The bark is greyish, and scaly, leaves are alternate, ovate–lanceolate in shape, 2-8 cm long and 1.3-5 cm across (6). Flowers are pentamerous, pale yellow, and fragrant. They are distributed across Africa and found in a large range of ecosystems from dry savannah to wet forest (7). In Nigeria, the plant is used as timber, fuel, and chew sticks. In the traditional system of medicine, stem bark has been used in the treatment of cough, diarrhea, fever, and wound healing (8). It is also a rich source of tanning and flavonoids (8). The ethanol extract of the stem bark exhibited antibacterial activity against some bacterial isolates from diabetic foot ulcers (9). The ethanol extract also showed antibacterial activity against infected wounds (10). Studies in Alloxan-induced diabetic rats reported that *A. leiocarpus* ethanolic extract exhibited antidiabetic and hypolipidemic effects (11). The methanol extracts of the leaves and stem bark also demonstrated antioxidant activity (12).

Acacia nilotica (family fabaceae) is an evergreen medium-sized tree, 15-18m tall. The bark is fissured, dark brown to blackish (13). Leaves are alternate with yellow sweetly scented flowers. Pods are long, narrow, flattened, mostly narrowed between seeds, stalked at the base, short, pointed grey or black, mostly aromatic. Seeds are beanlike, rounded flattened, and blackish-brown. In Kano state, northern Nigeria, the pod is used to dye leather. In southeast Africa, the tender twig of the plant is used as a toothbrush (14). The stem bark of the plant is a good source of terpenoids, alkaloids, saponins glycosides (15). In folk medicine, the pods are used in the treatment of colds, coughs, diarrhea, diabetes, Post-partum wound healing, fever, and as aphrodisiac [15, 16]. It is reported to possess a significant antibacterial, anti-fungal, anti-plasmodial, and anti-mutagenic, anti-diabetic, anti-oxidant effect (17).

Materials and methods

Collection and preparation of the selected plant parts extracts

The Pod of *Acacia nilotica* and stem of *Anogeissus leiocarpus* were collected from their natural source and were identified by a botanist in the Department of Plant Biology, BUK. The fresh plant parts were air-dried at room temperature until a constant weight was obtained. The air-dried plant parts were powdered using a blender. Fifty grams of the powdered of each selected part were macerated in 500ml of ethanol for 72h. The extracts were filtered and evaporated at 40 ºC under reduced pressure to obtain crude extract. The yielded crude extract was stored at 4 ºC until use.

Qualitative phytochemical screening

Detection of Alkaloids

Hager's test: two drops of Hager's reagent were added to 1ml of each extract. A reddish-brown precipitate observed indicates the presence of alkaloids in each extract (18)

Detection of Tannins

Two drops of 5% FeCl3 were added to 1 ml of the extracts. A greenish precipitate indicated the presence of tannins in the three extracts (18)

Detection of Saponins

Frothing test: 2ml of the extracts in a test tube was vigorously shaken for two mins. Frothing observed in the extracts tested indicated the presence of saponins.

Emulsion test: 5 drops of olive oil were added to 3ml of the extracts in a test tube and the mixture was vigorously shaken. A stable emulsion formed indicating the presence of saponins (18)

Detection of Terpenoids

Salkowaski reaction: To 0.5 g of each of the plant part extracts 2 ml of chloroform were added. Then into it 3 ml of concentrated sulfuric acid was carefully added, to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.

Detection of Flavonoids

To 0.5 ml of filtrate of each of the plant parts extracts, 5 ml of dilute ammonia was added, followed by addition of 1 ml of concentrated sulfuric acid it. The

presence of flavonoids was detected by the yellow coloration of the solution that disappears on standing (19)

Detection of Phenols

Ferric chloride test: Small quantities of alcoholic and aqueous extracts were dissolved in 2 ml of distilled water separately, and into it, a few drops of 10% aqueous ferric chloride solution were added. A blue or green color was produced which indicated the presence of phenols (19)

Gas chromatography-mass spectrometry (GC-MS) analysis of A.nilotica (pod) and A.leiocarpus (stem) ethanol extracts

This was performed according to the method described by Akanji (20). It was performed using Hewlett-Packard (HP) 6890 series (Agilent) Gas Chromatography System, interfaced to HP 5973 series (Agilent) mass spectrometer, equipped with an autosampler and a single capillary injector. TR-FAME (Thermo 260 M142P) (70% cyanopropylpolysilphenylenesiloxane) capillary GC column (30 m x 0.25 mm, i.d., x 0.25 µm film thickness) was also used. This was done by dissolving the sample in the organic solvent till it dissolved completely. GC condition was maintained at 100 – 280 °C at 5°C/min. 2µl of the sample was injected into the column. The helium gas was allowed to move at 1 ml/min through the column and the compound split in the ratio of 1:10. After the program, the mass spectrometer scanned the compounds separated, and each peak area was measured to find the compounds present at the area. The results were represented as a chromatogram graph in the GC-MS.

Fourier transform infrared spectrophotometer (FTIR) analysis of *A. Nilotica* (pod) and *A. leiocarpus* (stem) ethanol extracts

Dried powders of ethanol extracts of the selected plant parts were used for FTIR analysis. 10mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare a translucent sample disc. The powdered sample of each plant specimen was loaded in an FTIR Spectroscope (Agilent Technology), with a scan range from 650 to 4000 cm-1 with a resolution of 8cm-1(21).

In vitro antioxidant evaluation

Free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH)-scavenging test

The total antioxidant capacity of the extracts was determined using the DPPH radical as a reagent, according to the procedure described by Breghente et al. (22) with some modifications. This method is based on the ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) to decolorize in the presence of antioxidants. 100 µL of the test compound at concentrations ranging from 15.63 to 1000 µg/mL were mixed with 0.1 mL of the DPPH solution (0.004% in ethanol). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm and converted into a percentage of antioxidant activity (AA) using the following formula:

AA% = 100–{[(Abssample–Absblank)×100] / Abscontrol}.

A methanol (2.0mL) and plant extract (1.0mL) solution was used as the blank (Absblank). A DPPH (2.0mL) and methanol (1.0mL) solution was used as the control (Abscontrol). Ascorbic acid was used as standard. The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC50). The IC50 value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration (22).

Results and Discussions

Phytochemical analysis

Table 1 depicts the phytochemical compounds in the ethanol extract of pod of *A. Nilotica* and the stem of A.leiocarpus. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponin, tannin, terpenoid and phenol in all the selected plants part extracts. Studies by many researchers also confirmed the presence of these phytochemicals on the studied plants extract. A report by Sabah et al. (23) confirmed the presence of alkaloid, flavonoid, tannin and saponin in aqueous and ethanol extract of *A. Nilotica*. Victor et al. (24) reports the presence of alkaloids, saponins, tannins, flavonoid, terpenoid and phenol in *A. leiocarpus*. These phytochemicals have been proven to possess many biological activities. An individual phytochemical compound may have more than one biological function (25). Saponin, flavonoid and phenol were reported to be responsible for anti-inflammatory, antiplasmodic, analgesic and diuretic activities (26). Flavanoids were also reported to possess anti-oxidative, anti-inflammatory, anti-mutagenic, antimicrobial, anti-carcinogenic, vascular activities, free radical scavenging abilities, and other medicinal properties

Table 1. Phytochemical compounds of the ethanol extract of *A. Nilotica* Pod and *A. leiocarpus* stem bark.

Plant extract	Phhytochemical					
	Saponin	Tannin	Phenol	Flavanoid	Alkaloid	Terpenoid
A. nilotica	+	+	+	+	+	+
A. leiocarpus	+	+	+	+	+	+

+ = present

Table 2. GC-MS analysis of *A. Nilotica* ethanol Pod extract.

S/N	Retention time	Peak area %	Compound name	Compound Type
1	12.250	0.14	Hexadecanoic acid, methyl este	Fatty acid
2	6.906	0.18	1,2,4-Benzenetriol	Phenol
3	10.250	0.49	Decanoic acid, 3-methyl-	Fatty acid
4	15.054	0.59	11-Octadecenoic acid, methyl ester	Fatty acid ester
5	31.763	0.62	Dodecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Fatty acid
6	24.439	0.67	Hexadecenoic acid, Z-11-	Fatty acid
7	24.084	3.66	9-Octadecenoic acid (Z)-, 2,3-dihy droxypropyl ester	Fatty acid ester
8	12.893	0.96	n-hexadecanoic acid	Fatty acid
9	14.266	1.01	13-Hexyloxacyclotridec-10-en-2-one	
10	32.310	10.08	Dodecanoic acid, 1,2,3-propanetriy l ester	Fatty acid
11	34.217	8.75	Cyclododecanol, 1-aminomethyl-	phenol
12	34.311	2.82	Cyclodisilazane-2,2,4,4-tetramine, N,N,N',N'-tetramethyl-1,3-bis[tris(methylamino)silyl]-	Amide

Table 3. GC-MS analysis of *A. leiocarpus* Stem ethanol extract.

S/N	Retention time	Peak area %	Compound name	Compound Type
1	26.862	0.92	Oleic acid	Fatty acid
2	24.871	0.90	Octadec-9-enoic acid	Fatty acid
3	27.258	3.38	2-Ethylacridin	
4	25.837	1.04	9-Octadecene, 1,1-dimethoxy-, (Z)	
5	26.310	2.33	9-Octadecenoic acid (Z)- 2,3-dihy droxypropyl ester	Fatty acid ester
6	26.939	1.05	Benzo[h]quinoline, 2,4-dimethyl-	
8	31.956	1.24	1H-Indole, 5-methyl-2-phenyl-	phenol
9	24.801	1.32	6-Octadecenoic acid, (Z)-	Fatty acid
10	26.114	1.54	1-methyl-4-phenyl-5-thioxo-1,2,4-t riazolidin-3-one	
12	15.366	1.65	9-Octadecenoic acid (Z)-, methyl ester	Fatty acid ester
13	26.310	2.33	9-Octadecenoic acid, (E)-	Fatty acid
14	26.962	2.75	2,3-Dihydroxypropyl elaidate	
15	31.153	1.99	Carvacrol, TBDMS derivative	phenol
16	28.037	2.13	acetic acid, 2-[bis(methylthio)met hylene]-1-phenylhydrazide	Fatty acid
17	27.738	3.15	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	Volatile organic compound
18	6.523	2.40	Carbonic acid, ethyl 2-mercaptoethyl ester	Fatty acid
19	27.435	2.61	Silicic acid, diethyl bis(trimethy lsilyl) ester	
20	27.395	3.71	2-(Ethyl)oxybenzylidene acetophenone	
21	9.615	5.45	beta.-D-Ribopyranoside, methyl	

(27). Alkaloids were reported to have anti-diabetic and antioxidant activity (28), anti-hypertensive, antimalarial activity (29). Tannins are an important source of astringents to cure wounds and are used as anti-inflammatory, antioxidant, and antimicrobial compound (30). Terpenoids have also been proven to have antidiabetic, wound healing (28), and antimicrobial activity (31).

GC. MS analyses of *Acacia nilotica* (Pod) and *A. leiocarpus* (stem) ethanol extract

Results of the identification of compounds by GC-MS analysis from the ethanol extract of *A. Nilotica* pod and *A. leiocarpus* stem revealed the presence of 69 and 76 compounds respectively. The Major compounds present in the extract, their retention time (RT), peak area and compound type were provided in Table 2 and Table 3 respectively.

Interestingly, review literature of some of the identified compounds revealed that they were also found to possess some biological activities. Hexadecanoic acid, methyl ester (0.14%), Decanoic acid, 3-methyl- (0.49), n-hexadecanoic acid (0.96%) were reported to possess anti-inflamatory, antimicrobial, and antioxidant activity (32, 33, 34). Dodecanoic acid, 1,2,3-propanetriy l ester (10.08%) was reported to have Hypercholesterolemic, antiarthritic, nematocide, hepatoprtective activity (35). Carvacrol, TBDMS derivative (1.99%), .beta.-D-Ribopyranoside (5.45%), methyl, Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (3.15%),2-Ethylacridine (3.38%), Benzo[h]quinoline, 2,4-dimethyl-(1.05%) and Octadec-9-enoic acid (0.90%) have been reported to possess antibacterial, antioxidant and anti-tumor activity (36, 34, 37, 33, 38). 9-Octadecenoic acid (Z)-, methyl ester (1.65%), 6-Octadecenoic acid, (Z)-(1.32%), 9-Octadecenoic acid, (E)-(1.07%), and Oleic acid (0.89%) possess anti-inflammatory and cancer preventive property (39, 40, 41).

FTIR analysis of *Acacia nilotica* (Pod) and *Anogeissus leiocarpus* (stem) ethanol extracts

The result of FTIR analysis of *Acacia nilotica* is presented in Table 4. The ethanol extract of *Acacia nilotica* showed characteristics absorption band of 3190 cm-1for O−H stretching, 2113cm-1for C≡C stretching, 2005cm-1for multi bonding, 1894 cm-1for C−H bending, 1693 cm-1for C=O stretching, 1607cm-1for N−H bending, 1532 cm-1for C−C stretching, 1447-1316 cm-1for C−H bending, 1190-1030 cm-1for C−O stretching, 870-767 cm-1 for C=O bending.

The result of the FTIR analysis of *Anogeissus leiocarpus* is presented in Table 5. The ethanol extract of *Anogeissus leiocarpus* showed characteristics absorption bands of 3245-2929 cm-1 for O−H stretching, 2118 cm-1for C≡C, 1998-1722 cm-1for C−H bending, 1607 cm-1 for N−H bending and C=O stretching, 1514cm-1for N−O stretching, 1451cm-1for C−H bending, 1313 cm-1for C−N stretching and O−H bending, 1175 cm-1for C−O, 1033 cm-1for C−F stretching and 866-750 cm-1for C=O bending

FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation (42).Various functional groups observed in the ethanol extract of *A. leiocarpus* indicated the presence of carboxylic acids, alkane, alkyne, aromatic compound, ketone, amine, nitro compound, aromatic ester, phenol, alkyl amine, hydroxyl ester, alkyl halide and alkene. The functional groups of ethanol extract of *A. Nilotica* also indicated the presence carboxylic acid, alkyne, nitrile compound, aromatic compound, aldehyde, amine, conjugated alkene, ketone, alkane, phenol, hydroxyl ester, alcohol, alkyle halide and alkene. In addition, presence of O-H is an indication of different phytochemicals such as steroid, terpenoids, alkaloid, saponins, flavonoid, phenols and carbohydrates, the presence of C-N and C-H functional group indicated alkaloid and C-H stretching of aromatic hydrocarbons indicated terpenoid, steroid, saponins, and glycosides. Most of the functional groups observed in the ethanol extract of A.leiocarpus were also observed in the ethanol extract of *A. Nilotica* may be because these plants possessed similar bioactive compounds which were responsible for their similar bioactivity in this study.

In vitro antioxidant activity

The antioxidant activity of the studied plant extracts which includes the DPPH radical scavenging activity (% of inhibition) and the IC_{50} is presented in Table 6 and Table 7 respectively. The percentage inhibition of all the studied plant extracts was found to be increasing with an increase in the concentration of the extracts. Interestingly, the IC50 values of both *A. Nilotica* (0.11±0.02 µg/ml) and *A. leiocarpus* (0.27±0.17 µg/ml) were found to be lower than that of the control ascorbic acid (1.53±1.37 µg/ml) which statistically showed no significant difference ($P < 0.05$). It was reported that plant extract with IC50 values of below 5mg/ml will be regarded as a good antioxidant agent. The antioxidant potential of A.nilotica was in conformity with the study of Khan and Gohel (43) who reported the DPPH radical scavenging activity of the ethanol extract of A. niloca pod with an IC50 value of 2.16 µg/ml which is lower than the control ascorbic acid (3.89 µg/ml). A research by Hassan et al., (8) reported the

Table 4. Table 4 : FTIR absorption bands of *Acacia nilotica* Pod ethanol extract.
+ = present

Absorption peak (cm^{-1})	Functional group	Compound class
3190	O−H stretching	Carboxylic acid
2113	C≡C stretching	Alkyne
2005	Multibonding	Nitrile compound
1894	C−H bending	Aromatic compound
1693	C=O stretching	Conjugated aldehyde
1607	N−H bending, C=O stretching	Amine, Conjugated alkene and Ketone group
1532	C−C	Aromatic compound, Alkane
1447	C−H bending	Alkane
1316	C−N stretching, C−H bending	Aromatic amine, Phenol
1190	C−O stretching	Hydroxy ester
1078	C−O stretching	Alcohol, hydroxyl ester
1030	C−O stretching, C-F stretching	Hydroxy ester, Alkyl Halide
870	C=O bending	Alkene
762	C=O bending	Alkene

Table 5. FTIR absorption bands of *Anogeissus leiocarpus* stem ethanol extract.

Absorption peak (cm^{-1})	Functional group	Compound class
3245	O−H stretching	Carboxylic acid
2929	C−H Stretching, O−H stretching	Alkane, Carboxylic acid
2118	C≡C	Terminal alkynes
1998	C−H bending	Aromatic compound
1901	C−H bending	Aromatic compound
1722	C−H bending, C=O stretching	Aromatic compound, ketone
1607	N−H bending, C=O stretching	Amine, Ketone compound
1514	N−O stretching	Nitro compound
1451	C−H bending, C=C−C	Alkane, Aromatic compound
1313	C−N stretching, O−H bending	Aromatic ester, Phenol
1175	C−O	Hydroxy ester, Alkyle amine
1033	C−f stretching	Alkyle halide
866	C=C bending	Alkene
750	C=C bending	Alkene

Table 6. Showed DPPH radical scavenging activity of the ethanol extracts *A. Nilotica* Pod and A.leiocarpus Stem.

Plants extract	Concentrations (µg/ml) and % inhibition						
	1000.00	500.00	250.00	125.00	62.50	31.25	15.63
A. Nilotica	94.6±1.90	93.7±0.63	92.2±2.91	91.4±1.78	91.3±1.17	90.4±0.53	79.7±4.32
A.leiocarpus	94.0±1.02	93.7±1.63	93.6±2.30	93.5±2.46	93.1±1.49	92.8±0.12	79.4±8.76
Control	95.5±0.14	94.8±0.38	90.2±0.65	90.5±1.43	89.1±0.97	87.0±3.35	68.0±8.36

Data given are mean of three replicates±standard deviation.

Table 7. showed IC50 values of the ethanol extracts *A. Nilotica* pod and A.leiocarpus stem.

S/N	Plant extract	IC_{50}
1	*A.nilotica*	0.11 ± 0.02^{a}
2	*A.leiocarpus*	0.27 ± 0.17^{a}
3	Control (Ascorbic Acid)	1.53 ± 1.37^{a}

Data given are mean of three replicates±standard deviation. Superscript with the same letters indicates statistically no significant differences in the Turkey test, with ($P< 0.05$).

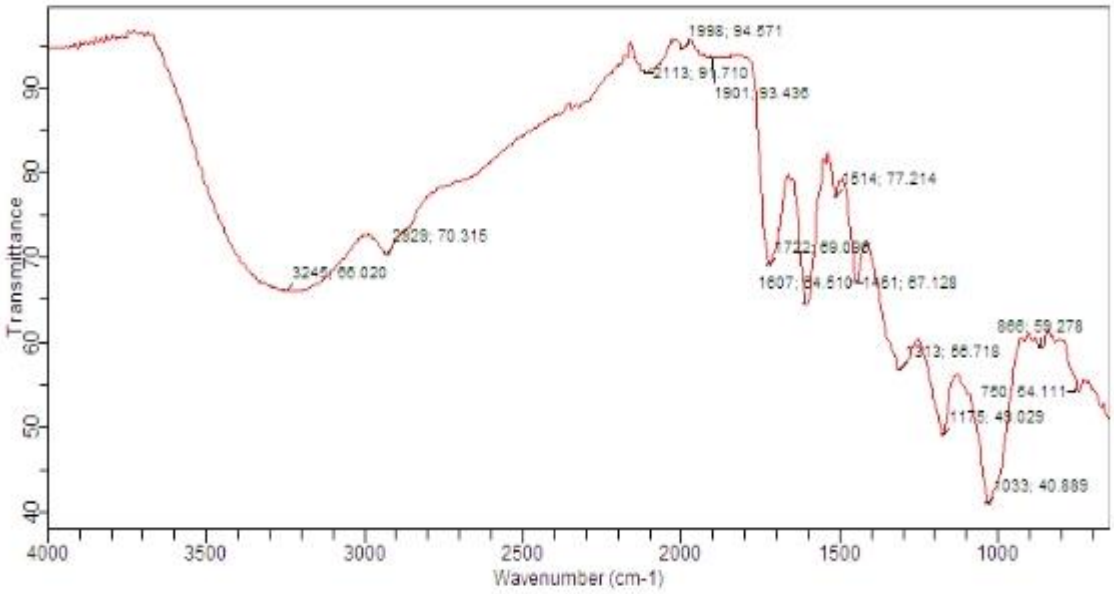

Figure 1. FT-IR chromatogram profile of ethanol Stem bark extract of A.leucarpus.

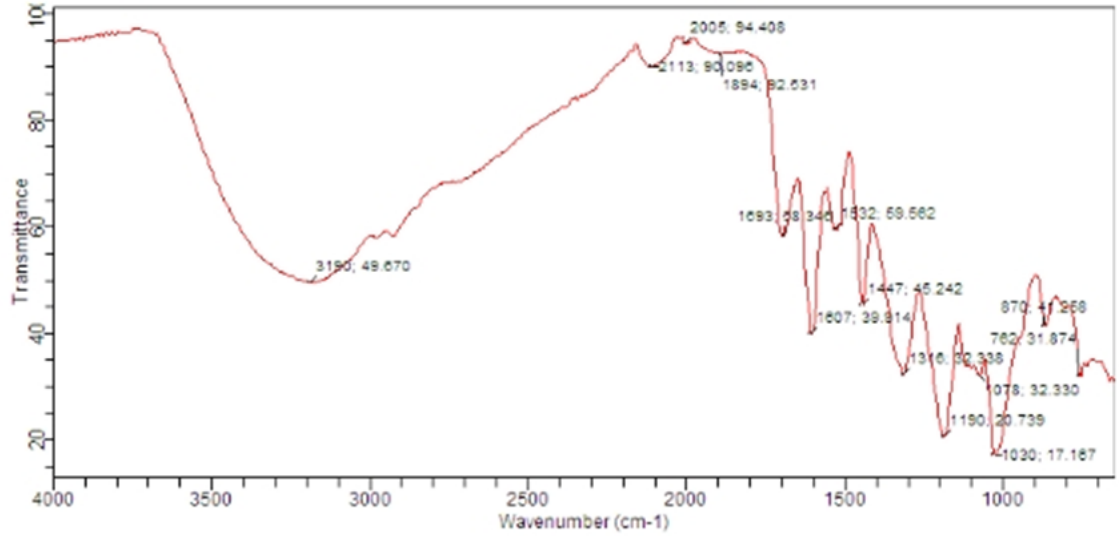

Figure 2. FT-IR chromatogram profile of ethanol Pod extract of *A. Nilotica*.

antioxidant activity of A.leiocarpus leaves and stem extract in DPPH scavenging assay with an IC50 value of 29.87µg/ml and 43.34 µg/ml respectively, which is contrary to our findings. However, the varying concentrations used in their study were 200-12.5µg/ml which was lower than the concentrations used in our research.

Conclusion

The present study reveals the presence of alkaloids, phenols, flavonoids, terpenoids, tannins, and saponins in the ethanol extracts of both A.nilotica pod and A.leiocarpus stem. The GC-MS and FTIR analysis revealed the presence of Fatty acids, phenols, and esters in both the ethanol extracts of *A. Nilotica* and *A. leiocarpus*. The study also showed the antioxidant activity of the studied extract by free radical scavenging effect. The activity may be attributed to the presence of these identified phytochemicals in the extracts. Hence the studied plants can be suggested as promising agents for antioxidant drug development.

Contribution of authors

Sani, S.B. designed and carried out the research, analysed the data, wrote the manuscript, Surayya Lawan Idris and Binta Muhammad Saidu contributed to the implementation of the research, Namadina, M.M. assisted in interpretation of FT-IR result, Sani, S.B. and Taufiq kyari Mazadu prepare the final version of the manuscript with contribution from Mardiyya Auwal Yakasai, Aisha Tijjani, Mujittafa Lawan, Ladan Wada Hayatu, Hamisu Abba, Aisha

Muhammad Galalain, Hadiza Rabiu Isihaq and Hassan Ibrahim Muhammad.

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Conflict of Interest

The authors declare no conflict of interest.

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