Phytochemicals Analysis and GC-MS Determination of Ethanolic Extracts of *Azadirachta indica* and *Mangifera indica* Stem Bark and their Biological Potentials

A.A. Ayoola[1*]; D.A. Ekunseitan[1]; S.B. Muhammad[2]; M.A. Oguntoye[3]; and Y.A. Adejola[4]

[1]Department of Animal Production and Health, Federal University of Agriculture, PMB 2240, Abeokuta, Nigeria.
[2]Department of Animal Nutrition, Federal University of Agriculture, PMB 2240, Abeokuta, Nigeria.
[3]Department of Animal Science, Taraba State University, Jalingo, Nigeria.
[4]Department of Animal Production and Technology, Federal College of Agriculture, Ibadan, Nigeria.

E-mail: ayoolaaa@funaab.edu.ng*
Telephone: 08062299054

ABSTRACT

This study serves to explore the chemical constituents of *Azadirachta indica* and *Mangifera indica* barks as a potential alternative to conventional antibiotics and promising precursors for pharmaceutical industries, especially for application in livestock production. Ethanolic extracts from *Azadirachta indica* (AI) and *Mangifera indica* (MI) stem bark were analyzed for their phytochemical constituents as well as identification of the compounds and their proportions via gas chromatography (GC)/mass selective (MS) analysis.

The concentration of secondary metabolites such as Tannin (1510.00 mg/kg), Oxalate (139.20 mg/kg), Phytate (15.55 mg/kg), Trypsin inhibitor (730.00 mg/kg), Flavonoids (78.50 mg/kg) and Saponins (17.71 mg/kg) contents of Mango stem bark were higher than in *Azadirachta indica*. However, *Azadirachta indica* contained higher Terpenoids (43.85 mg/kg), Total Phenol (34.00 mg/kg), Total Carotenoids (89.59 g/kg), Total Carotene Carotenes (69.88 g/kg), Xanthophyll (19.71 g/kg), Alkaloids (19.50 %) and Antioxidant (68.65 %) than *MI* stem bark.

Azadirachta indica had higher concentrations of crude protein (3.54), Crude fat (0.81), Crude fiber (1.07), Total Ash (0.22), and Carbohydrate (8.65) while the moisture content was higher in *Mangifera indica* (90.20). *Azadirachta indica* ethanolic stem bark extract has residents in it four major compounds which include Hexadecanoic acid, methyl ester (10.94%), Methyl stearate (6.51%), 9-Octadecenoic acid, methyl ester (E) (25.02%), and cis-11-Eicosenoic acid, methyl ester (2.22%) while seven prominent compounds in *Mangifera indica* are hexadecanoic acid, methyl ester (16.571%), Methyl 10-trans,12-cis-octadecadienoate (4.216%), 11-Octadecenoic acid, methyl ester (62.598%), Methyl stearate (3.825%), (E)-9-Octadecenoic acid ethyl ester (4.597%), cis-11-Eicosenoic acid, methyl ester (4.897%) and Bis (2-ethylhexyl) phthalate (3.295%). It can therefore be concluded that stem bark extracts, besides serving as good source of pharmacologically active phytochemicals may also be useful as supplements in human and animal nutrition particularly since components are biodegradable compared to synthetic antibiotics.

(Keywords: phytochemicals, active ingredient, ethanolic extract, biological activities)

INTRODUCTION

Plants apart from being a major food source for both humans and animals, also serve several other uses in medicine, horticulture, construction, textile industries, and others. Medicinal plants have been used in both traditional and modern medicine for centuries (Awotedu et al., 2018). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial agents has led to the screening of several medicinal plants and increased research geared towards understanding the components of these botanicals and also the determination of the efficacy as well as safety of their use.

Phytochemicals which may also be referred to as phytonutrients are present in diverse kinds of plants which are consumed as essential components of both human and animal diet (Kamba and Hassan, 2010; Edeoga, and Eriata, 2001). According to Awotedu, et al. (2018)

medicinal value of plants lies in their bioactive constituents which usually allow them to fight against several diseases and can be found in any part of the plant like bark, leaves, flowers, roots, fruits, seeds. (Awotedu et al., 2018). Neem (*Azadirachta indica*) and Mango (*Mangifera indica*) are plants of immense medicinal value and have been used extensively (Madunagu, et al., 1990; Aarati, et al., 2011; Tsabang, et al., 2012; Ayoola, et al., 2018).

Mangifera indica with the common name 'mango' has traditional medicinal applications. Mango extract has been reported to have anti-malaria effect by Tsabang, et al. (2012) and was found to display *in vitro* activity against *Plasmodium falciparum* (Rasoanaivo, et al., 2004). Olasehinde (2018) reported that phytochemical screening of the extracts of *M. indica* bark showed presence of active pharmacological components such as tannins, saponins, cardiac glycoside, flavonoid and alkaloids. This was corroborated by the findings of Madunagu, et al. (1990). These components are known to be biologically active because they protect the plant against infections and predations by animals. Phytochemicals generally exert their antimicrobial activities through different mechanisms from that of synthetic drugs (Scalbert, 1991).

Several researchers revealed that neem (*Azadirachta indica*) is perhaps the most useful traditional medicinal plant especially in India. Almost all parts of the plant are endowed with medicinal properties and is thus commercially exploitable. It has been used as traditional medicine or household remedies against various human aliments from time immemorial. Its crude extracts from different parts of neem have been used in traditional medicine, however, these days, it is regarded as valuable source of unique natural products or precursor for the development of various medicines against various diseases. (Aarati, et al., 2011). The bark contains sugiol, nimbiol, nimbosterol, deacetylnimbin, kulinone, methyl kulonate, sterols, polysaccharides, polydactyl derivatives and tricyclic diterpenoide.

The knowledge of the active ingredients and their abundance in these plants will give a better understanding of the potentials of this plant in human and animal medicine. Hence this study sought to investigate the phytochemicals and active ingredients resident in the two herbs to infer their potential use in livestock health.

METHODOLOGY

Sources and Collection of Plant Materials

The plants materials: *Mangifera indica* (MI) and *Azadirachta indica* (AI) bark were sourced from the school premises of Federal University of Agriculture Abeokuta (FUNAAB). The samples were air dried under the shade at room temperature for two weeks. The dried samples were chopped into smaller pieces in preparation for extraction.

Extraction of Plant Materials

The extraction was done mechanically by crushing and soaking the crushed stem bark in 70% ethanol for 3 days in a sealed container at a room temperature. 1kg of the stem bark was used against 2kg of 70% ethanol. The mixture was turned twice daily to ensure proper mixing, on the fourth day the extract was separated from the shaft with a strainer (cheesecloth). The solvent was removed at a temperature of 40°C in a water bath and the extracts were stored in a freezer at -20°C.

Phytochemical Screening of *Mangifera indica* (MI) and *Azadirachta indica* (AI) Extracts

Quantitative phytochemical test of the ethanolic extracts of both barks were carried out using standard procedures as described by Harborne (1973) and enunciated by Sofowora (1993) to determine saponin, terpenoid, alkaloid, phenol, carotene, carotenoid, phytate, xanthphyll, flavonoids, oxalate content, and antioxidant (DPPH scavenger).

Determination of Active Ingredient Present in *Mangifera indica* (MI) and *Azadirachta indica* (AI) Extract.

The extracts of the samples were subjected to gas chromatography (GC)/mass selective (MS) analysis; this group of powerful instruments interface helped to characterize the various compositions. The gas chromatograph Model: 7890A (GC) analysis was performed on an Agilent Technologies interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion-source temperature at 250^{o}C. Pure helium gas

(99.9% purity) was used as carrier gas, while HP-5ms (5% Phenyl 95% dimethylpolysiloxane) (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was at 80°C held for 5 minutes and increased to 250 degrees while holding for 16 minutes at the rate of 4 degrees/minute. 1µ/l was auto injected for a final run time of 50 minutes. Each element/compound present in the extract was identified since each compound had different retention time; these elements tried to spurt from the sample once its retention time had been attained in the gas chromatographic system.

Statistical Analysis

Data obtained from the phytochemical analysis were subjected to Independent T-test using SAS (2005).

RESULTS

Phytochemical Screening of *Azadirachta indica* (AI) and *Mangifera indica* (MI) Ethanolic Stem Bark Extract

Table 1 shows the result obtained from phytochemical screening of ethanolic bark extract of *Azadirachta indica* (AI) and *Mangifera indica* (MI). All the values were significantly ($p<0.05$) different between the two extracts. *Mangifera indica* (MI) bark extract had significantly ($p<0.05$) higher tannin (1510.00mg/kg), oxalate (139.20mg/kg), phytate (15.55mg/kg), trypsin inhibitor (730mg/kg), flavonoids (78.50g/kg) and saponins (17.71%) than extract of *Azadirachta indica* (AI).

However, terpenoids (43.85mg/kg), total phenol (34.00mg/kg), total carotenoids (89.59g/kg), total carotene carotenes (69.88g/kg), xanthophyl (19.71g/kg), alkaloids (19.50%) and antioxidant (DPPH scanvenger) (68.65%) were significantly ($p<0.05$) higher in extract of *Azadirachta indica* (AI).

Table 1: Phytochemical Screening of *Azadirachta indica* (*AI*) and *Mangifera indica* (*MI*) Ethanolic Stem Bark Extract.

Parameter	Herbs Bark Extracts		
	Azadirachta indica	*Mangifera indica*	SEM
Tannin (mg/kg)	1495.50^{b}	1510.00^{a}	0.29
Oxalate (mg/kg)	128.68^{b}	139.20^{a}	0.14
Phytate (mg/kg)	13.90^{b}	15.55^{a}	0.28
Terpenoids (mg/kg)	43.85^{a}	13.54^{b}	1.01
Trypsin inhibitor (mg/kg)	494.71^{b}	730.00^{a}	0.21
Total phenol (mg/kg)	34.00^{a}	30.60^{b}	0.29
Total Carotenoids (g/kg)	89.59^{a}	54.17^{b}	0.14
Total Carotene Carotenes (g/kg)	69.88^{a}	42.26^{b}	2.10
Xanthophyll (g/kg)	19.71^{a}	11.92^{b}	0.28
Flavonoids (g/kg)	13.68^{b}	78.50^{a}	1.29
Alkaloids (%)	19.50^{a}	5.61^{b}	1.43
Saponins (%)	8.02^{b}	17.71^{a}	1.03
Antioxidant (DPPH Scanvenger) (%)	68.65^{a}	58.48^{b}	4.21

^{ab} Means with different superscript differs significantly ($p<0.05$)

Active Ingredient of *Mangifera indica* Stem Bark Extract

The active compounds with their retention time (RT), molecular formula, molecular weight (MW), peak area (%) and concentration (%) is presented in Table 2. A total of sixteen compounds were detected comprising of seven prominent and nine minor constituents.

The seven prominent compounds and their percentage abundance are hexadecanoic acid, methyl ester (16.571%), Methyl 10-trans,12-cis-octadecadienoate (4.216%), 11-Octadecenoic acid, methyl ester (62.598%), Methyl stearate (3.825%), (E)-9-Octadecenoic acid ethyl ester (4.597%), cis-11-Eicosenoic acid, methyl ester (4.897%) and Bis (2-ethylhexyl) phthalate (3.295%). GC–MS chromatogram of the ethanol extract of *Mangifera indica* (MI) bark extract (Figure 1) clearly showed seven peaks indicating the presence of prominent phytochemical compounds. The spectrum sketch out of GC-MS confirmed the presence of 7 components with the retention time 16.829, 18.921, 19.035, 19.280, 19.756, 21.756, and 24.319 minutes, respectively.

Active Ingredient Present in *Azadirachta indica* Stem Bark Extract

The active component with their retention time (RT), compound name, molecular weight, molecular formula, peak area (%) and concentration (%) in the ethanolic extract of *Azadirachta indica* stem bark were presented in Table 3. Eleven components were detected consisting of four prevailing compounds and seven minor constituents in ethanolic extract of *Azadirachta indica* stem bark. The four major compounds and their percentage abundance are Hexadecanoic acid, methyl ester (10.94%), Methyl stearate (6.51%), 9-Octadecenoic acid, methyl ester (E) (25.02%) and cis-11-Eicosenoic acid, methyl ester (2.22%). GC–MS chromatogram of the ethanolic extract of *A. indica* stem bark extract in Figure 1 clearly shows five peaks indicating the presence of prominent phytochemical compounds.

Table 2: Components Detected in *Mangifera indica* Ethanolic Stem Bark Extract.

S/N	RT (Minutes)	Compound Name	Molecular formula	Molecular Weight	Peak area (%)
1.	16.83	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	16.57
2.	18.92	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	1.41
3.	18.92	9,12-Octadecadienoic acid, methyl ester (E,E)	$C_{19}H_{34}O_2$	294	1.41
4.	18.92	9,12-Octadecadienoic acid (Z,Z)- methyl ester	$C_{18}H_{32}O_2$	280	1.41
5.	19.03	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	20.86
6.	19.03	Cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	20.86
7.	19.03	9-Octadecenoic acid, methyl ester (E)	$C_{19}H_{36}O_2$	296	20.86
8.	19.23	Methyl stearate	$C_{19}H_{38}O_2$	298	2.56
9.	19.23	Heptadecanoic acid, 16-methyl, methyl ester	$C_{19}H_{38}O_2$	298	1.26
10.	19.76	(E)-9-Octadecenoic acid ethyl ester	$C_{19}H_{38}O_2$	310	1.56
11.	19.76	Ethyl Oleate	$C_{20}H_{38}O_2$	310	3.02
12.	21.26	Cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	1.63
13.	21.26	Cis-Methyl 11-eicosenoate	$C_{21}H_{40}O_2$	324	1.63
14.	21.26	Cis-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	1.63
15.	24.32	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	2.23
16.	24.32	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390	1.07

Table 3: Biological Activities of Phyto-Components Identified in the Ethanolic Extracts of *Mangifera indica* Stem Bark.

S/N	Name of compound	Molecular formula	Compound nature	Biological Activities
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid	Anti-oxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti androgenic, flavor, hemolytic-5-α reductase inhibitor.
2.	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	Conjugated Linoleic Acid Methyl Ester	Indicate its activities
3.	9,12-Octadecadienoic acid, methyl ester(E,E)	$C_{19}H_{34}O_2$	linoleic (omega-6 fatty acid)	Antiinflammatory, Nematicide, Insectifuge, Antiacne, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiarthritic, Anti-eczemic
4.	9,12-Octadecadienoic acid (Z,Z)-methyl ester	$C_{18}H_{32}O_2$	Linoleic acid	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic and Anticoronary
5.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		Absorption and distribution in human plasma and lipoprotein lipids
6.	Cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O2$	Fatty acid	Antinflammatory, Hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
7.	9-Octadecenoic acid, methyl ester (E)	$C_{19}H_{36}O_2$	Fatty acid	Raises VLDL And Lowers HDL Cholesterol
8.	Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid	Anti-diarrheal, cytotoxic and Anti-proliferative activity
9.	Heptadecanoic acid, 16-methyl, methyl ester	$C_{19}H_{38}O_2$		Antioxidant, anti-microbial and anti-inflammatory
10.	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	Elaidic acid	Antioxidant, anti-inflammatory activities.
11.	Ethyl Oleate	$C_{20}H_{38}O_2$	Ester	Indicate its activities
12.	Cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$		Antioxidant, Pesticide, Flavor, 5- Alpha Reductase inhibitor, Antifibrinolytic , Hemolytic, Lubricant, Nematicide, Antialopecic
13.	Cis-Methyl 11-eicosenoate	$C_{21}H_{40}O_2$		Indicate its activities
14.	Cis-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	an omega-7 fatty acid	
15.	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$		Antimicrobial, antibacterial, plasticizer
16.	Phthalic acid, di(2-propylpentyl) ester	$C_{21}H_{38}O_4$		Oral toxicity during pregnancy and sucking

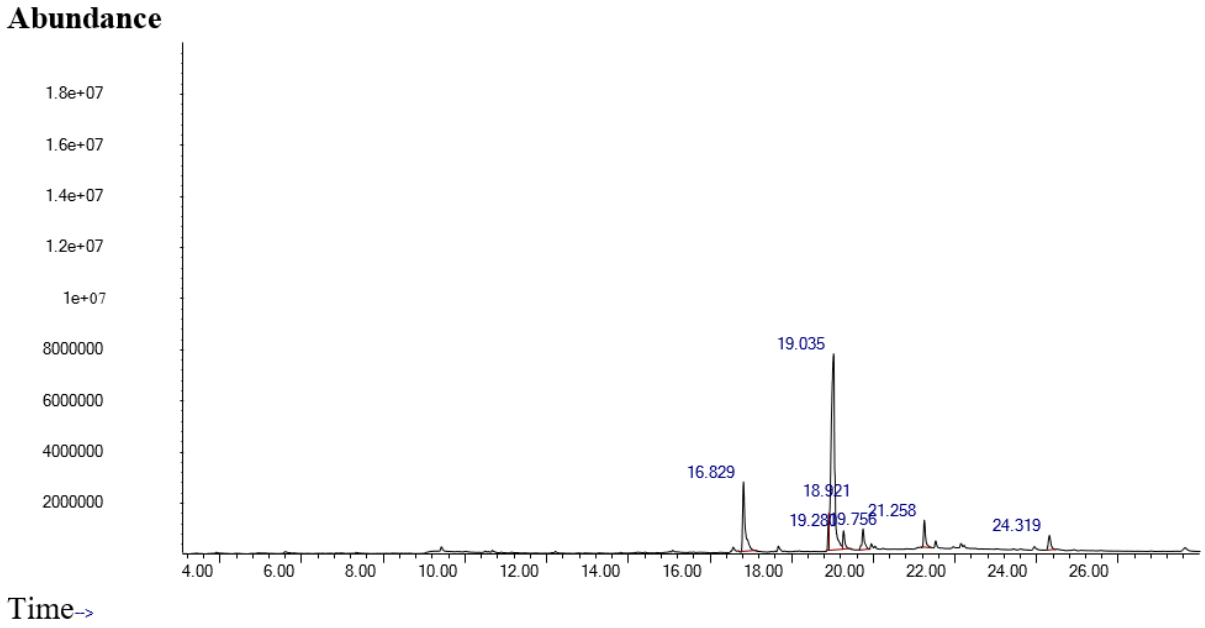

Figure 1: GC-MS Chromatogram of the Ethanol Stem Bark Extract of MI.

Table 4: Components Detected in *Azadirachta indica* Ethanolic Stem Bark Extract.

S/N	RT (Minutes)	Compound Name	Molecular formula	Molecular Weight	Peak area (%)
1.	16.876	Hexadecanoic acid, methyl ester.	$C_{17}H_{34}O_2$	270	10.943
2.	16.876	Pentadecanoic acid, 14-ethyl ester	$C_{17}H_{34}O_2$	270	1.695
3.	18.991	9-Octadecenoic acid, methyl ester (E)	$C_{19}H_{36}O_2$	296	25.022
4.	18.991	Cis-13-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	25.022
5.	18.991	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.49	25.022
6.	19.303	Methyl stearate	$C_{19}H_{38}O_2$	298	6.509
7.	19.782	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	0.84
8.	19.782	Ethyl Oleate	$C_{20}H_{38}O_2$	310	0.823
9.	19.782	9,17-Octadecadienal, (Z)	$C_{18}H_{32}O$	264	0.788
10.	21.308	cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	2.226
11.	21.308	Methyl 9-eicosenoate	$C_{21}H_{40}O_2$	324	1.113

Table 5: Biological Activities of Phyto-Components Identified in the Ethanol Extracts of the Plant of *Azadirachta indica.*

S/N	Name of compound	Molecular formula	Compound nature	Biological Action
1.	Hexadecanoic acid, methyl ester.	$C_{17}H_{34}O_2$	Palmitic acid methyl ester.	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor Hemolytic,5-Alpha reductase inhibitor.
2.	Pentadecanoic acid, 14-ethyl ester	$C_{17}H_{34}O_2$	Palmitic acid methyl ester.	Anti-oxidant, antifungal and antimicrobial activities
3.	9-Octadecenoic acid, methyl ester (E)	$C_{19}H_{36}O_2$	Fatty acid	Raises VLDL and Lowers HDL Cholesterol
4.	cis-13-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	Fatty acid	Indicate its activities
5.	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	Fatty acid	Absorption and distribution in human plasma and lipoprotein lipids
6.	Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid methyl Esters	Anti-diarrheal, cytotoxic and antiproliferative activity.
7.	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	Elaidic acid	Antioxidant, anti- inflammatory activities.
8.	Ethyl Oleate	$C_{20}H_{38}O_2$	Esters	Indicate its activities
9.	9,17-Octadecadienal, (Z)	$C_{18}H_{32}O$	long chain unsaturated aldehyde compound	Antimicrobial, Anti-inflammatory
10.	cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	Monounsaturated fatty acids	Antioxidant, Pesticide, Flavour, 5-Alpha Reductase inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antialopecic
11.	Methyl 9-eicosenoate	$C_{21}H_{40}O_2$		Indicate its activities

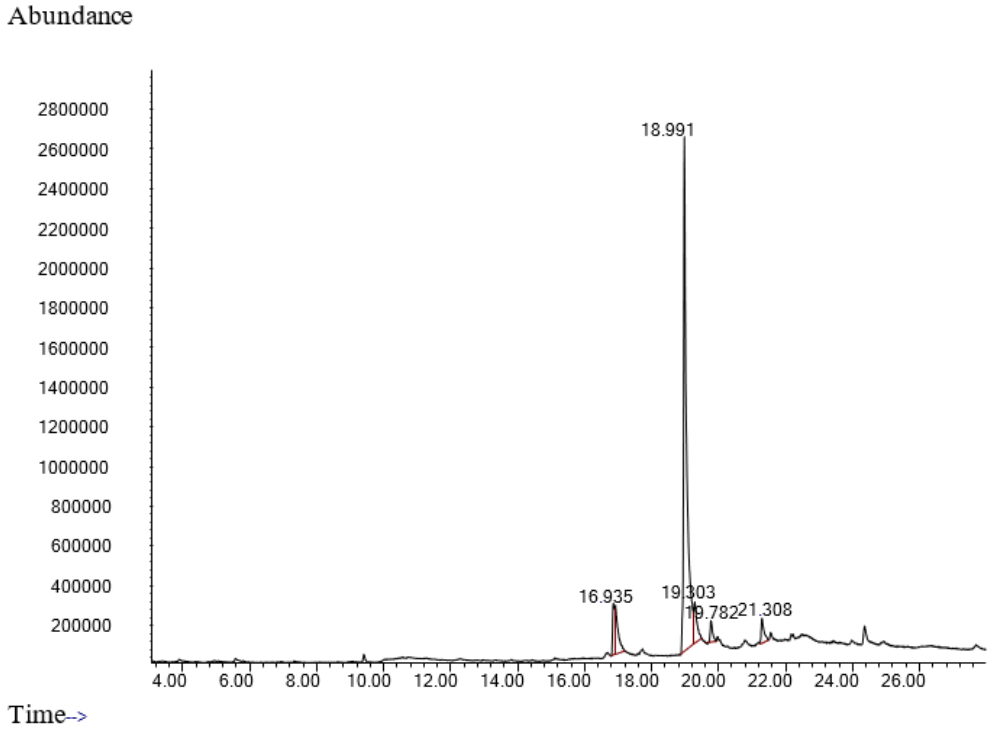

Figure 2: GC-MS Chromatogram of the Ethanol Stem Bark Extract of *Azadirachta indica.*

Table 6: Summary of Important Bioactive Constituents in Ethanolic Extract of *Mangifera indica* and *Azadirachta indica* Stem Bark

Mangifera indica	***Azadirachta indica***
Hexadecanoic acid, methyl ester	Hexadecanoic acid, methyl ester
Methyl 10-trans,12-cis-octadecadienoate	Methyl stearate
11-Octadecenoic acid, methyl ester	9-Octadecenoic acid, methyl ester (E)
Methyl stearate	cis-11-Eicosenoic acid, methyl ester
(E)-9-Octadecenoic acid ethyl ester	
cis-11-Eicosenoic acid, methyl ester	
Bis(2-ethylhexyl) phthalate	

Summary of Important Bioactive Constituent in Ethanolic Extract of Mangifera indica and Azadirachta indica Stem Bark Extract

Major bioactive constituents in both ethanolic extract of *Mangifera indica* and *Azadirachta indica* were presented in Table 6. Hexadecanoic acid, methyl ester, Methyl stearate and cis-11-Eicosenoic acid, methyl ester were found in both extracts. However, Mangifera indica contained other four bioactive constituents or compounds which are absent in Azadirachta indica. 9-Octadecenoic acid, methyl ester (E) was only found in Azadirachta indica.

DISCUSSION

The phytochemical screening of the stem bark extracts of *Azadirachta indica* and *Mangifera indica* revealed the presence of tannin, oxalate, phytate, terpenoids, trypsin-inhibitor, total phenol, total carotenoids, total carotene carotenes, xanthophyll, flavonoids, alkaloids, saponin and antioxidant. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals (Surh, 2003).

Plants used in the treatment of diseases are said to contain bioactive molecules with biological activity which are responsible for the characteristic odor, pungencies and color of plant, while others give each plant its culinary, medicinal or poisonous virtue (Evans, 2002).

A study conducted by Meressa (2017) on phytochemicals of neem bark also confirmed the presence of tannin, saponin, flavonoid and alkaloid.

Saponins are known bioactive substances that can reduce the uptake of cholesterol and glucose at the gut through intra-lumenal physiochemical interaction (Price et al., 1987). Saponins as a class of natural products are also involved in complexation with cholesterol to form pores in cell membrane bilayers (Francis et al., 2002) as such may be used as anticholesterol agents or cholesterol lowering agent. The higher quantity of saponins contained in *Mangifera indica* is an indication that it has a better hypocholesterimic potential than *Azadirachta indica*. Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents their antimicrobial activity.

Flavonoids were also determined in the two extracts and they in general serve as flavoring ingredients in plants. Besides their role as flavoring agents, they are also expressed in plants in response to microbial infection suggesting their antimicrobial activity (Kujumgiere et al., 1999). Flavonoids have also been implicated as antioxidants both in physiological and disease states. For instance, tea flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides (Erdman, 2007). This bioactive compound is known to have potential anti-viral activity (Cheng et al., 2003) as well as potential prophylactic and therapeutic effect against cancer cells (Narayanan et al., 1999).

Phytic acid and Oxalate are present in low concentration in all the samples studied and this also makes them safe for consumption. Oxalate should be consumed in small quantity because oxalic acid binds with other mineral such as calcium to form oxalate salt which has been

postulated to be the cause of kidney stone according to (Bridget, 2010). However, in comparison to Harry-Asobara et al. (2014), his study revealed the presence of Alkaloid (1.22%), Flavonoid (0.36 0%), Saponin (0.32 %), Phenols (0.18 %), Phytate (0.15 %) and Tannin (0.26%) in dry ash extraction method of *Azadirachta indica* stem bark which contradicts the percentage concentration of the present study. Also, absence of carotenoids, xanthophyll, terpenoids, oxalate and phytate were reported by Adetuyi et al. (2013) on *Mangifera indica* stem bark contrary to the result of this study. In addition, authors also reported percentage composition of tannins, Saponins, flavonoids and nonflavonoids to be 0.35mg/g, 9.78mg/g, 12.9mg/g and 8.60mg/g, respectively, which were lower in concentration compared to values obtained in this study.

The bioactive components on the other hands of this study reveals the presence of sixteen bioactive components of (MI) while (AI) reveal the presence of eleven bioactive compounds as a whole from GC-MS technique, each component has specific functions and act as a drug for various disease. The presence of these phytochemical constituents justifies the use of this plant for various ailments by traditional practitioners.

Saturated fatty acids are synthesized by both plants and animals from acetyl coenzyme A as a form of long-term energy storage. Palmitic acid is a common 16-carbon saturated fat that represents 10-20% of the normal human dietary fat intake, and approximately 25% of the total plasma fatty acids in plasma lipoproteins (Santos, et al., 1995). Palmitic acid methyl ester is a fatty acid ester whose concentration in cells is modulated by methanol. In studies with isolated Kupffer cells, Palmitic acid inhibits phagocytosis and decreases cell viability. In cells treated with lipopolysaccharide, it also decreases secretion of interleukin-10, TNF-α, nitric oxide, and prostaglandin E2. This effect is thought to occur by the inhibition of NF-κB (Cai, et al., 2005).

Ethyl oleate is a fatty acid ester formed by the condensation of oleic acid and ethanol. It is a colorless to light yellow liquid. Ethyl oleate is produced by the body during ethanol intoxication (Dan and Laposata, 1997). Ethyl oleate is used as a solvent for pharmaceutical drug preparations involving lipophilic substances such as steroids (Ory, et al., 1983). It also finds use as a lubricant and a plasticizer. Ethyl oleate has been identified as a primer pheromone in honeybees (Leoncini et al., 2004).

Ethyl oleate is one of the fatty acid ethyl esters (FAEE) that is formed in the body after ingestion of ethanol. There is a growing body of research literature that implicates FAEEs such as ethyl oleate as the toxic mediators of ethanol in the body (pancreas, liver, heart, and brain) (Laposata, 1998). The oral ingestion of ethyl oleate degrade rapidly in the digestive tract and hence appears safe for oral ingestion (Saghir and Rapid, 1997).

Stearic acid is a saturated fatty acid commonly found in animal and vegetable fats that is frequently used in cosmetics, candles, soaps, plastics, oil pastels, and for softening rubber. Stearic acid ethyl ester (ethyl stearate) is the neutral, more lipid soluble form of the free acid. It perturbs the cell cycle and induces apoptosis in Hep-G2 cells and is a marker of excessive alcohol consumption that can be isolated from an individual's hair (Aydin et al., 2005).

The 9, 12-octadecadienoic acid, methyl ester (a linoleic acid), had been reported to possess antiinflammatory, nematicide, insectifuge, antiacne, hypocholesterolemic, anticancer, hepatoprotective, antihistaminic, antiarthritic and antieczemic activities (Sarker and Nahar, 2007). From the foregoing this work therefore indicates that the stem bark extracts, besides serving as good source of pharmacologically active phytochemicals may also be useful as supplements in human and animal nutrition. In addition, the components are biodegradable compared to synthetics conventionally used, environmentally friendly, cost-effective and would meet the current demand for organic product.

CONCLUSION

The revelations in terms of secondary metabolites and other bioactive compounds identified from the GC-MS analysis of the two bark extracts has indicated their potentials and relevance in phytopharmaceutical for both man and animals particularly livestock facing the challenge of reduced potency of antibiotics due to increased growing resistance by the causing organisms. Their uses are not limited to antimicrobial, anti-inflammatory, Hypo-cholesterolemic, Cancer preventive and Hepatoprotective, they can also be found useful

in production of cosmetics, pesticides, nematicide, lubricants, etc.

REFERENCES

1. Adetuyi, F.O., I.B.O. Dada, and T.A. Ibrahim. 2013. "Phytochemical Analysis and Antioxidant Activities of Hot and Cold Water Extracts of Mango (*Mangifera indica* L.) Leaf and Stem Bark". *Journal of Basic Medical Sciences*. 1:16 - 21

2. Aarati, N., N.N. Ranganath, G.B. Soumya, B. Kishore and K. Mithun. 2011. "Evaluation of Antibacterial and Anticandidal efficacy of aqueous and alcoholic extract of Neem (*Azadirachta indica*) an *in vitro* Study". *International Journal of Research in Ayurveda & Pharmacy*, 2(1): 230-235.

3. Awotedu, O.L., P.O. Ogunbamowo, I.B. Emmanuel and I.O. Lawal. 2018. "Phytominerals and Phtychemical studies of *Azadirachta indica*, *Leea guineensis* and *Parkia biglobosa* Leaves" *International Annals of Science*. 6(1): 28-34

4. Aydin, A., H.A. Celik, and R. Deveci. 2005. "Induction of Apotopsis by Fatty Acid Ethyl Esters in HEP-G2 Cells". *Food Chemical Toxicology*. 43: 139-145.

5. Ayoola, A.A., L.T. Egbeyale, D.A. Ekunseitan, A.V. Adegoke, and O.P Adeyeri. 2015. "The Effect of Neem (*Azadirachta indica*) Leaf Meal on the Growth Performance and Carcass Characteristics of Broiler Chickens". *Nigerian Journal of Animal Production*. 42(2):141-150.

6. Bridget, C. 2010. "Oxalates and Diets." http://www.livestrong (accessed on Nov. 2017)

7. Cai, P., B.S. Kaphalia, and G.A.S. Ansari. 2005. "Methyl Palmitate Inhibitor of Phagocytosis in Primary Rat Kupffer Cells". *Toxicology*. 210: 197-204.

8. Cheng, H.Y., C.C. Lin and T.C. Lin. 2003. "Anti-Herpes Simplex Virus Type 2 Activity of Casuarinin from the Bark of *Terminalia arjuna* Linn". *Antiviral Research*. 55: 447–455.

9. Dan, L. and M. Laposata. 1997. "Ethyl Palmitate and Ethyl Oleate are the Predominant Fatty Acid Ethyl Esters in the Blood After Ethanol Ingestion and their Synthesis is Differentially Influenced by the Extracellular Concentrations of their Corresponding Fatty Acids". *Clin. Exp. Research*. 21(2): 286–92.

10. Edeoga, H.O., D.E Okwu, and B.O. Mbaebie. 2005. "Phytochemical Constituents of some Nigeria Medicinal Plants". *African Journal of Biotechnology*. 4:685-688.

11. Erdman, J.W. 2007. "Flavonoid and Heart Health" *2005: Proceedings of the ILSI North America Flavonoid Workshop*, May 31 – June 1. Journal Nutrition, 137(3): 718s-737s.

12. Evans, W.C. 2002. *Trease and Evans Pharmacognosy, 15th edition*. W.B. Sauders Company Limited: London, UK. 137-139.

13. Francis, C., G. George, K. Zohar, P.S. Harinder, L.M. Makhar, and B. Klaus. 2002. "The Biological Action of Saponins in Animal System: A Review". *British Journal Nutrition*. 88(6):587-605l.

14. Harry-Asobara, L. and E. O. Samson. 2014. "Comparative Study of the Phytochemical Properties of *Jatropha curcas* and *Azadirachta indica* Plant Extracts". *Journal of Poisonous and Medicinal Plants Research*. 2(2):20-24.

15. Harborne, J.B. 1973. *Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis*. 2nd Edition. London, UK. 49-188.

16. Kamba, A.S. and L.G. Hassan. 2010. "Phytochemical Screening and Antimicrobial Activities of *Euphorbia balsamifera* Leaves, Stems and Root Against Some Pathogenic Microorganisms". *African Journal of Pharmacy and Pharmacology*. 4(9): 645- 652.

17. Kujumgiev, A., T.S. Tseveikoval, D.E. Serkedjivay, V. Bankora, R. Christo, and S. Popov. 1999. "Antibacterial, Antifungal and Antiviral Activity of Propolis Geographic Origin". *Journal of Ethnopharmacol*. 44: 35-40.

18. Laposata, M. 1998. "Fatty Acid Ethyl Esters Nonoxidative Metabolites of Ethanol". *Addiction Biology*. 3: 5-14.

19. Leoncini, I., Y. Le Conte, G. Costagliola, E. Plettner, E. Toth, A. Wang, , M. Huang and Z. Becard. 2004. "Regulation of Behavioral Maturation by a Primer Pheromone Produced by Adult Worker Honey Bees". *Proc. National Academic Science*. 101(50):17559–17564.

20. Madunagu, B.E., R.U.B. Ebana, and E.D. Ekpe. 1990. "Antibacterial and Antifungal Activity of some Medicinal Plants of Akwa Ibom State". *West Af. J. Biol. Appl'd Chem*. 35: 25-30.

21. Narayanan, B.A., O. Geoffrey, M.C. Willingham, and D.W. Nixon. 1999. "Expression and its Possible Role in GI Arrest and Apoptosis in Allergic Acid Treated Cancer Cells". *Cancer Letters*,. 136(2):215 - 21.

22. Olasehinde, G.L., K.J. Sholotan, J.O. Openibo, O.S. Taiwo, O.A. Bello and A.A. Ajayi. 2018. "Phytochemical and Antimicrobial Properties of

Mangifera indica Leaf Extract". *Covenant Journal of Physical and Life Sciences*. 6(1): 55-63.

23. Ory, S.J., C.B. Hammond, S.G. Yancy, R.W. Hendren, and C.G. Pitt. 1983. "The Effect of a Biodegradable Contraceptive Capsule (Capronor) Containing Levonorgestrel on Gonadotropin, Estrogen, and Progesterone Levels". *American Journal Obstet. Gynecology*. 145(5): 600–5.

24. Price, K.R., L.I. Johnson, and H. Feriwick, 1987. "Chemical and Biological Significance of Saponin in Food Science". *Nutrition*. 26:127-135.

25. Rasoanaivo, P.D., H. Ramanitrahasimbola, D. Rafatro, B. Rakotondramanana, A. Robijaona, et al. 2004. "Screening Extracts of Madagascan Plants in Search of Antiplasmodial Compounds". *Phytothera. Res.*18(9):742–747.

26. Saghir, M. and A. Rapid. 1997. "*In vivo* Hydrolysis of Fatty Acid Ethyl Esters, Toxic Nonoxidative Ethanol Metabolites". *American Journal of Physiology*. 273: G184–190.

27. Santos, M., J. Lopez, M. Jurado, and J. Lopis, 1995. "Influence of Dietary Supplementation with Fish Oil on Plasma Fatty Acid Composition in Coronary Heart Disease Patients". *Animal Nutrition Metabolism*. 39:52-62.

28. Sarker, S.D. and L. Nahar. 2007. *Chemistry for Pharmacy Students General, Organic, and Natural Product Chemistry*. John Wiley and Sons: London, UK. 283-359.

29. Scalbert A. 1991. "Antimicrobial Properties of Tannins". *Phytochemicals*. 30:3875-3883.

30. Sofowara, A. 1993. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd.: Ibadan, Nigeria. 289.

31. Tsabang, N., P.V.T. Fokou, L.R.Y. Tchokouaha, B. Noguem, I. Bakarnga-Via, M.S.D. Nguepi, et al. 2012. "Ethnopharmacological Survey of Annonaceae Medicinal Plants used to Treat Malaria in Four Areas of Cameroon". *Journal of Ethnopharmacology*. 139(1):171–180.

32. Meressa, M.G. 2017. "Phytochemical and Antibacterial Evaluation of Ethanol Stem bark extract of *Azadirachta indica* Grown in Dibila Kebere, Tigray Ethopia". *International Journal of Engineering Development and Research*. 5(4): 1129- 1132.

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