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Chemical constituents, antimicrobial and antioxidant activities of *Leptoderris brachyptera* (Benth.) Dunn and *Leptoderris micrantha* Dunn essential oils

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ABSTRACT

This study examined the composition of the essential oils long with corresponding antioxidant and antimicrobial properties of the leaves and stems of *Leptoderris brachyptera and Leptoderris micrantha*. Essential oils were obtained by hydrodistillation method using Clevenger-type apparatus, while identification and characterization were done by gas chromatography-mass spectroscopy (GC-MS) technique. The antimicrobial property was evaluated by the agar diffusion method and antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. Essential oils yields range from 0.4%-0.8%. GC-MS analyses revealed twenty-six and five compounds in leaves and stem of *Leptoderris brachyptera* representing 96.4% and 92.6% of which phytol (11.2%) and 4-(1*H*-pyrazol-1-yl)benzeneamine (60.8%) as the most abundant components, respectively. Nineteen and thirteen volatile constituents were identified from leaves and stems of *Leptoderris micrantha* representing 94.3% and 94.1% with phytol (30.7%) and palmitic acid (36.4%), respectively. However, the essential oils exhibited moderate antimicrobial and antioxidant activities.

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1. Introduction

Since time immemorial, medicinal plants have been used traditionally in the treatment of numerous human diseases and are the primary source of medicine in rural areas of developing countries (Oloyede and Olatinwo 2014; Olaoluwa et al., 2018). Natural products derived from medicinal plants are also sources of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. World Health Organization reported that nearly 80% of the world population rely on traditional medicines for primary health care, most of which involve the use of plant extracts for therapeutic purposes. Essential oils are volatile naturally occurring, complex compounds found in various medicinal plants characterized by a pungent odor, rarely colored, and soluble in organic solvents with a density generally lower than that of water (Mohammadhosseini et al., 2017; Olaoluwa et al., 2018). Previous scientific reports have shown that essential oils exhibit pharmacological properties involving antiplasmodial, antioxidant, antimicrobial, antiviral, antimutagenic, anticancer, insecticidal, anti-inflammatory, and immunomodulatory activities (Dongmo et al., 2019; Mohadjerani and Asadollahi 2019; Mohammadhosseini et al., 2019). Also, the chemical constituents present in the essential oils could be linked to their therapeutic properties (Mohammadhosseini et al., 2017).

Leguminosae is one of the most common family found in tropical rainforests and dry forests in the Americas and Africa and includes several important agricultural and food plants, such as *Glycine max* (soybean), *Phaseolus vulgaris* (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), and *Glycyrrhiza*

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glabra (liquoric). Some species are also weedy pests in different parts of the world, including *Cytisus scoparius* (broom), *Robina pseudoacacia* (black locust), *Ulex europaeus* (gorse), *Pueraria montana* (kudzu), and some *Lupinus albus* (lupine) (Carlos and José 2015; Judd et. al., 2002). Morais et al. (2017) reported that methanol extracts from Leguminosae plants (*Mimosa pigra*) were potential sources of antifungal compounds, mainly the extract and fractions (*n*-hexane and ethyl acetate) from *Mimosa pigra*. The dichloromethane fraction from *Mimosa pigra* did not show *in vitro* toxicity according to the applied assays. Yhiya et al., (2015) stated that the Leguminosae family was reported to contain flavonoids, alkaloids, triterpenoids, steroids, fatty acid derivatives, cinnamyl phenols, and other miscellaneous compounds which exhibited a potential source of antimalarial, antifungal, antiparasitic, anti-inflammatory, anti-oxidant, and antibacterial activities.

Leptoderris micrantha is a climbing shrub with brown stems and purple flowers belonging to the family Leguminosae. It is found in Guinea, Ghana, and southwest Nigeria (Famobuwa et al., 2016). *Leptoderris micrantha*, the leaf is oblanceolate in shape, with entire margin, acute apex, glabrous surface, petiolate; flowers sessile and flat, fruit broadly elliptic (Sonibare et al., 2014). The stem bark of *Leptoderris micrantha* is used in the treatment of microbial infection such as cough, dropsy, swellings, gout, bronchial affections, and oedema, and it has a side effect of emetic and purgative (Burkill, 2000). The decoction from the leaves has been reported to invigorate men sexually (Famobuwa et al., 2016).

Leptoderris brachyptera is a woody species of plant or a climbing shrub 15-30 m tall. It belongs to the family Leguminosae (Addo-fordjour et al., 2008; Gabon, 2016). It is majorly found in gallery forests; areas near mangrove swamps, dense forests, secondary thickets, and the margins of the primitive forest. *Leptoderris brachyptera* leaf has been used in the treatment of pulmonary troubles, dropsy, swellings, oedema, gout and emetics, and laxatives (Rukangira, 2001; Addo-fordjour et al., 2008). It is also used in making fish poison, resin, and exudation gums (Burkill, 2000). *Leptoderris brachyptera* and *Leptoderris micrantha* plants are distributed across West Africa. Indigenous people of Guinea-Bissau use the sap of the plant in the treatment of neurologic disorders. It was reported that *Leptoderris brachyptera* and *Leptoderris micrantha* plants showed significant properties like antioxidant, antibacterial, anti-inflammatory, antispasmodic, analgesic, and antidiuretic activities (Famobuwa et al., 2016).

There is no literature study on the chemical constituents of the essential oil of the two species from Nigeria. Hence, the study aims to identify the chemical constituents of essential oils using the GC-MS technique and carrying out *in-vitro,* antioxidant, and antimicrobial activities to justify the ethnomedicinal claims of both plants.

2. Experimental

2.1. Collection and preparation of the plant materials

The whole plant material of *Leptoderris micrantha* was collected from Ilu-ogbon town, Ibadan, Oyo State (latitude: 7º 22' 39.22" N, longitude: 3º 54' 21.28" E), and *Leptoderris brachyptera* was collected from Odofin village, Ikire town, Osun State (latitude: 7º 22' 20.68" N, longitude: 4º 11' 14.60" E), Nigeria in March 2019. They were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) with the herbarium file FHI No: 112599 and 112540 respectively. The fresh plant materials of *Leptoderris micrantha* and *Leptoderris brachyptera* were separated into leaves and stems. Then plant parts were air-dried for a week to remove moisture and pulverized. Evaporation of the volatile constituents in the dried plant materials and contamination were avoided by storing the material in airtight bags before hydro-distillation.

2.2. Isolation of essential oils

Pulverized plant material of *Leptoderris micrantha* and *Leptoderris brachyptera* leaves (500 g each) and stem (115.40 g each) were hydro-distilled for 3 h in an all-glass Clevenger type apparatus (with a thermo-regulated heating mantle) following the British Pharmacopeia specifications (1980) (Oloyede et al, 2010; Oloyede and Ojeyinka, 2019). The volatile oils distilled over were collected in the receiver arm of the apparatus containing water and analytical grade *n*-hexane (which dissolves the oil). The oils and the hexane were collected into sample bottles (Olaoluwa et al., 2018). The volatile oils were preserved in sealed glass vials and stored under refrigeration (at 4 °C) before analysis and bioassay. The oil yield was calculated relative to the dry matter (Carlos and José, 2015).

2.3. Gas chromatography/mass spectrometry (GC-MS) analysis and identification

Essential oils were subjected to GC-MS analysis on an Agilent 7809A Gas Chromatography hyphenated with an Agilent Mass Detector having split/splitless injector interfaced with mass selective detector operating at 70 eV. The ion source temperature was set to 200°C over a mass spectral range of m/z 50-700 at a scan rate of 1428 amu/sec. The column of the GC used was HP-5MS with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm. The oven temperature was programmed as follows: initial temperature of 80 °C for 2 min, increased at 10 °C/min to a temperature of 240 °C for 6 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min. Injection volume; linear velocity and pressure were adjusted at 1.0 µL, 362 cm/s, and 56.2 KPa respectively. The oven temperature was set at 60 °C, hold for 1 min to 180 °C for 3 min at 10 °C/min, the final temperature was 280 °C for 2 min at 10 °C/min both the injector and detector temperatures were fixed at 250 °C. Identification of the essential oil components was based on a comparison of their mass spectral fragmentation patterns (NIST database 14.L/ chemstation data system) with the data previously reported in the literature (Adams, 2007; Oloyede et al., 2010).

2.4. Antimicrobial assay

The essential oils were screened against ten (10) strains of microbes consisting of six bacteria; four Gram-negative (*Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, and Pseudomonas aeruginosa*) and two Gram-positive (*Staphylococcus aureus, Bacillus substilis*) with four fungi (*Candida albicans, Penicillum notatum, Aspergillus niger, and Rhizopus* spp.) were used in this study. All microbes were clinical isolates obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy of University of Ibadan, Nigeria. Agar diffusion and surface plate methods were employed for antibacterial and antifungal activities, respectively (Onocha et al., 2016). The oil sample was prepared such that 1 mL of the oil was regarded as 1000 µg/mL concentration; 0.5 mL of this essential oil was taken into 0.5 mL of DMSO to give 500 µg/mL concentration and 250 µg/mL, 125 µg/mL and 62.5 µg/mL concentrations were obtained using serial dilution. DMSO was used as a negative control, Gentamycin (10 µg/mL) as a positive control for bacteria, and Tioconazole (0.7 mg/mL) as a positive control for fungi. All tests were carried out in duplicates. Observed zones of inhibition of growth were measured and recorded in mm.

2.5. Antioxidant assay

Antioxidant potential of essential oils of *Leptoderris micrantha* and *Leptoderris brachyptera* were studied using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability method. Various concentrations (1000, 500, 250, 125, and 62.5) µg/mL of the essential oils were mixed with 100 µM methanolic DPPH solution (2.0 mL) prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol to give a purple solution. The mixture was shaken vigorously and left to incubate for 20 mins in the dark at room temperature. The absorbance at 517 nm was recorded as $A_{(sample)}$ using a UV-Vis spectrophotometer. In its radical form, DPPH absorbs but upon reduction by antioxidant species, its absorption reduces. A blank experiment was carried out applying the same procedure but without essential oils (DPPH + methanol) and the absorbance was recorded as $Abs_{(control)}$ (Oloyede and Ojeyinka, 2019). Each experiment was carried out in triplicates and the free radical scavenging activities of the essential oils were calculated as percentage inhibition according to the formula:

$$\%\text{Inhibition} = \frac{\text{Abs(control)} - \text{Abs(sample or standard)}}{\text{Abs(control)}} \times 100 \quad \text{(Eqn. 1)}$$

Where $Abs_{(control)}$ denotes the absorbance of the control (without sample), while $Abs_{(sample)}$ accounts for the absorbance of the sample.

The same experiment was carried out on vitamin C and butylated hydroxylanisole (BHA) used as standards for the antioxidant assay.

3. Results and discussion

3.1. Physicochemical properties and percentage yield of the essential oils.

The oil samples were obtained by hydrodistillation of the leaves and stems of *Leptoderris brachyptera* and *Leptoderris micrantha* in relatively moderately low yields (0.42-0.79% w/w) as shown in Table 1. The variation in the yield of the essential oils could be attributed to the number of volatile oils present in specialized structures such as glandular hairs on the epidermis, oil tubes in the pericarp, and the plant tissues. Light yellow with leafy scent essential oil from *Leptoderris brachyptera* leaf (LBL) recorded the highest yield followed by *Leptoderris micrantha* leaf (LML) essential oil as shown in Table 1. It was observed that both stem essential oils gave rise to low yield.

3.2. Chemical composition of essential oil

The quantitative and qualitative data on the chemical constituents of essential oil of *Leptoderris brachyptera and Leptoderris micrantha* (see Fig.s 1-4) were obtained from the GC-MS spectra data and compared with the spectra reference compounds from NIST 14 library. The essential oils constituents are summarized in Table 2. The GC analysis showed the presence of twenty-six and five compounds in leaves and stem of *Leptoderris brachyptera*, respectively which accounted for 96.4% and 92.6% of the whole essential oil content. Nineteen and thirteen volatile constituents were identified representing 94.3% and 94.1% of the total volatile constituents in leaves and stems of *Leptoderris micrantha* essential oils respectively. The mixture of terpenes and non-terpene derivatives mainly, aliphatic alcohols, ketones, aromatics, and aliphatic hydrocarbons were obtained.

To the best of our knowledge, the constituents of the leaves essential oils of *Leptoderris brachyptera* and *Leptoderris micrantha* have not been investigated previously. The essential oil of the leaves was characterized by a high percentage of oxygenated monoterpene and diterpene compounds. *Leptoderris brachyptera* leaves oil is dominated by phytol (11.2%) and α-ionone (10.5%). *Leptoderris micrantha* leaf oil has as major compounds phytol (30.7%) and hexahydrofarnesylacetone (17.6%). The most abundant constituents in the stem oil of *Leptoderris brachyptera* was 4-(1*H*-Pyrazol-1-yl) benzeneamine (60.8%), followed by three other major compounds are β-cedrene (12.1%), palmitic acid (11.1%), and Isothiazole-3-methyl-5-phenyl (7.3%). The other remaining component includes α-cedrenol (1.3%). The major components in the stem essential oil of *Leptoderris micrantha* were palmitic acid (36.4%), tetradecanoic acid (17.1%), oleic acid (9.0%), dodecanoic acid (7.6%), pentadecanal (6.1%), nerolidol (4.6%) and 1-pentadecyne (4.0%). Other chemical components in the leaf oil of *Leptoderris micrantha* were (*Z*)-9-tetradecenal, (2.4%), 6-tetradecyne (2.2%), phytol (1.9%), octadecenal (1.1%), eugenol (1.0%) and hexahydrofarnesylacetone (0.7%). Ten constituents were common to both the leaves essential oil of *Leptoderris brachyptera* and *Leptoderris micrantha* and they include phytol (11.2%, 30.7%), α-ionone (10.5%, 4.9%), isophytol (9.7%, 1.3%), geranyl acetone (8.6%, 2.8%), β-ionone (8.5, 2.4%), pentadecanal (6.8%, 6.8%), hexahydrofarnesylacetone (4.8%, 17.6%),

Table 1
Yields of essential oils hydrodistilled from Leaf and stem of *Leptoderris brachyptera* and *Leptoderris micrantha*.

Sample	Yields (%)	Color	Odor
LBL	0.8	Light yellow	Leafy
LBS	0.6	Colourless	Woody
LML	0.7	Light yellow	Leafy
LMS	0.4	Colourless	Woody

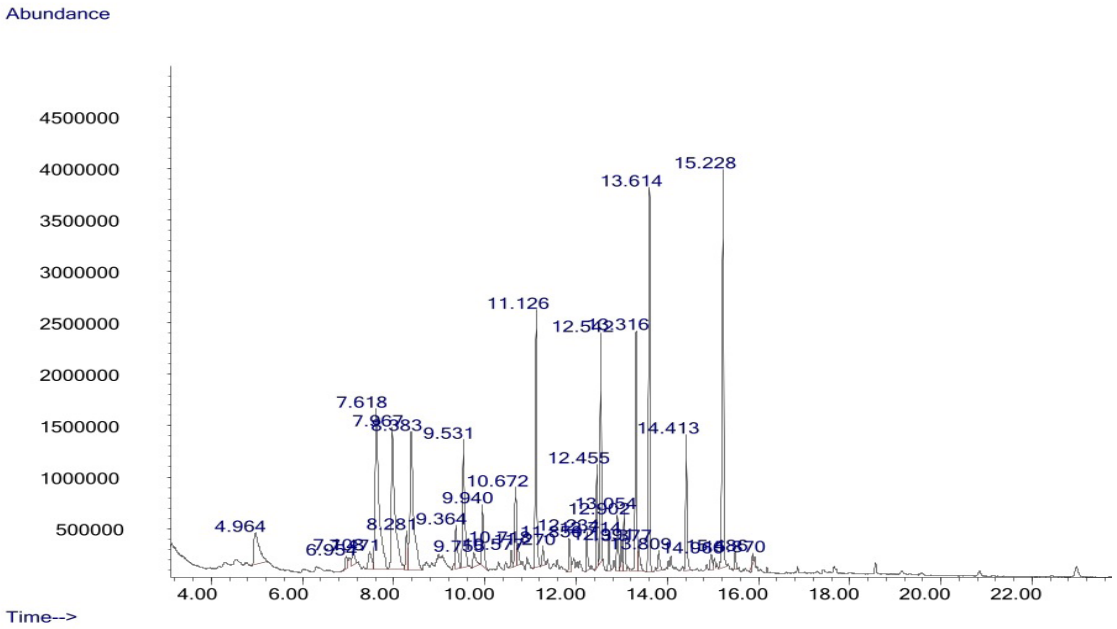

Fig. 1. The Chromatogram of essential oil of *Leptoderris brachyptera* leaf.

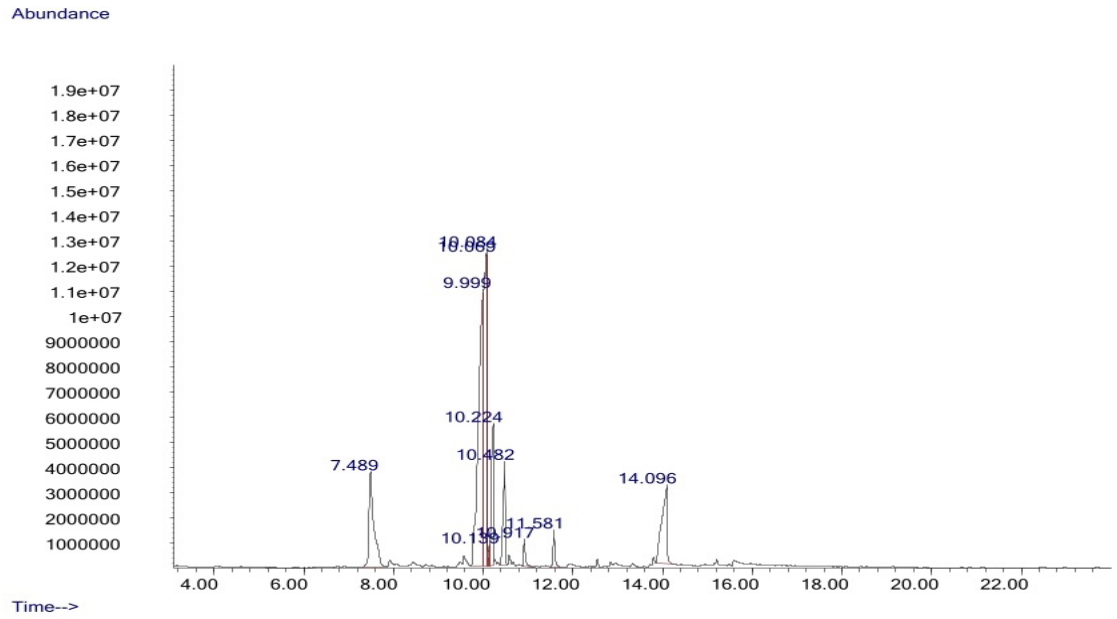

Fig. 2. The Chromatogram of essential oil of *Leptoderris brachyptera* stem.

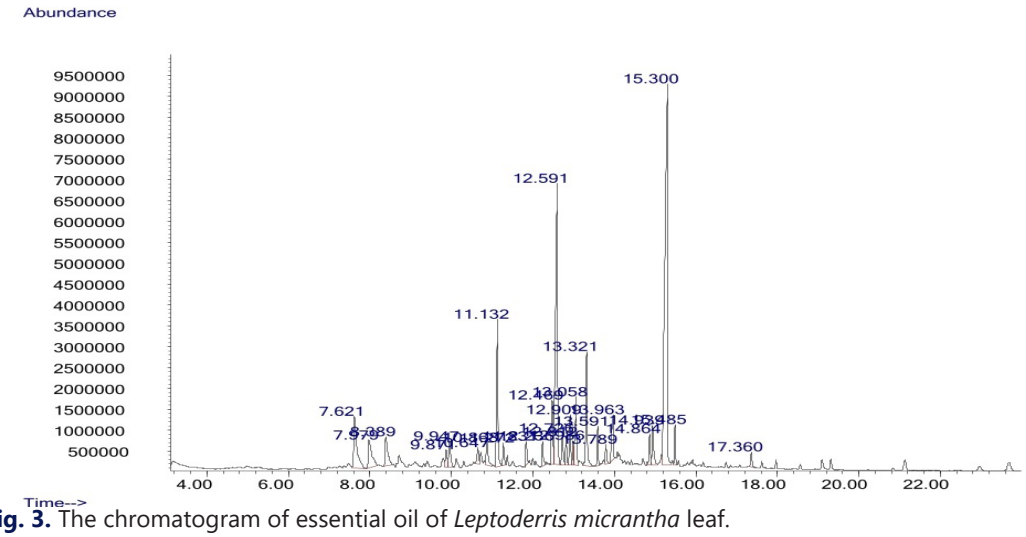

Fig. 3. The chromatogram of essential oil of *Leptoderris micrantha* leaf.

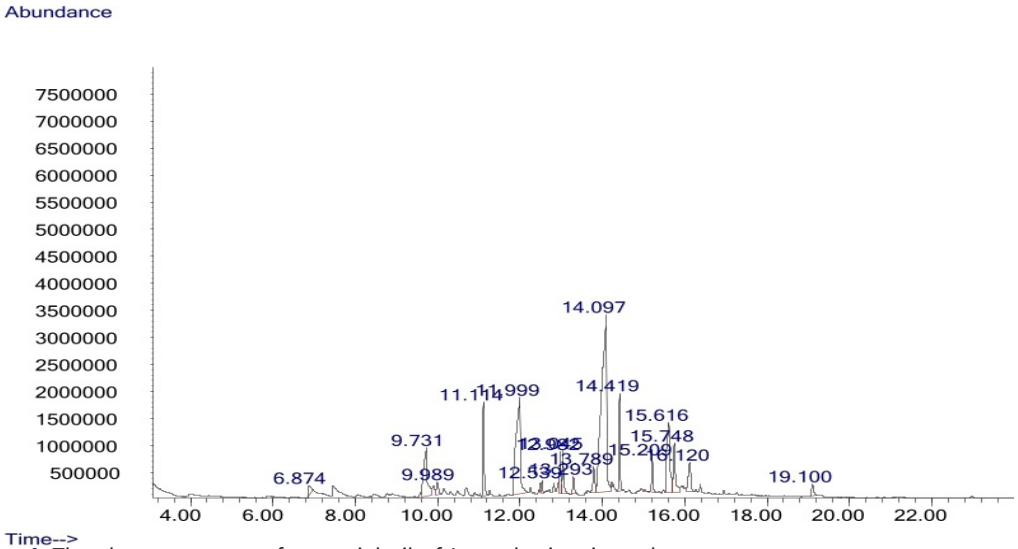

Fig. 4. The chromatogram of essential oil of *Leptoderris micrantha* stem.

neophytadiene (3.6%, 5.0%) 1,13-tetradecadiene (2.1%, 2.8%), calamenene (0.6%, 2.0%) respectively. One of the components, palmitic acid was common to both stem essential oil of *Leptoderris brachyptera* and *Leptoderris micrantha* having 36.4% and 11.1%, respectively. The stem oil is deficient in hydrocarbons and oxygenated monoterpene and diterpene compounds.

The Leguminosae family has palmitic acid as one of the major components (Carlos and Jose, 2015). Yhiya et al. (2015) also reported that many Leguminosae species host bacteria in their stem. These bacteria, known as rhizobia, can take nitrogen gas (N_2) out of the air and convert it to a form of nitrogen that is usable to the host plant (NO_3^- or NH_3). This process is called nitrogen fixation. The legume, acting as a host, and rhizobia, acting as a provider of usable nitrate, form a symbiotic relationship that may justify the high percentage of nitrogen compounds (non-terpenes) in the stem. Three different compounds were found to be present in three different samples with different proportions, hexahydrofarnesylacetone was absent in *Leptoderris brachyptera* stem oil, palmitic acid was found to be absent in *Leptoderris* brachyptera leaf oil while phytol was absent in *Leptoderris* brachyptera stem oil.

A comparison of our results with the previous reports on the chemical composition of the Leguminosae family shows differences in the chemical constituents. For instance, Limonene, o-ethyl toluene, methyl xylene, cymene, β-calarene, and 9-octadecenamide, which were present in the oils of *Hildegardia barteri* and *Ononis natrix* L are also of pharmacological importance (Balogun et al., 2017; Khallouki et al., 2002). Limonene is rapidly absorbed in the gastrointestinal tract in humans and distributed to various tissues of the body, where it elicited anti-inflammatory, anticancer, antioxidant, and anti-atherosclerotic activity (Magiatis et al., 1999; Maetins et al., 2000), as well as cymene, β-calarene, and 9-octadecenamide, have been implicated for substantial antioxidant and antimicrobial activities (Mevy et al., 2007).

Table 2
Chemical composition of the leaf and stem essential oils of *Leptoderris brachyptera* and *Leptoderris micrantha*.

S/N	RT	Compounds	M. Formula	*Leptoderris brachyptera* TIC(%)		*Leptoderris micrantha* TIC(%)	
				Leaf	Stem	Leaf	Stem
1	4.96	Carvacrol methylether	$C_{11}H_{16}O$	2.9	-	-	-
2	6.87	Eugenol	$C_{10}H_{12}O$	-	-	-	1
3	7.11	1,6-Octadiene,2,5,-dimethyl-(*E*)	$C_{10}H_{18}$	0.6	-	-	-
4	7.47	Caryophyllene	$C_{15}H_{24}$	1	-	-	-
5	7.49	β-Cedrene	$C_{15}H_{24}$	-	12.1	-	-
6	7.62	α-Ionone	$C_{13}H_{20}O$	10.5	-	4.9	-
7	7.96	Geranylacetone	$C_{13}H_{22}O$	8.6	-	2.8	-
8	8.28	Germacrene D	$C_{15}H_{24}$	1.2	-	-	-
9	8.38	β-Ionone	$C_{13}H_{20}O$	8.5	-	2.4	-
10	9.53	(3*E*,7*E*)-4,8,12,-trimetyltrideca-1,3,7,11,-Tetraene	$C_{16}H_{26}$	4.7	-	-	-
11	9.73	Dodecanoic acid	$C_{12}H_{24}O$	-	-	-	7.6
12	9.75	Hexadecane	$C_{16}H_{34}$	0.6	-	-	-
13	9.93	1,13-Tetradecadiene	$C_{14}H_{26}$	2.1	-	2.8	-
14	9.99	4-(1H-Pyrazol-1-yl) benzeneamine	$C_9H_9N_3$	-	60.8	-	-
15	10.22	Isothiazole,3-methyl-5-phenyl	$C_{10}H_9NS$	-	7.3	-	-
16	10.57	6-Tetradecyne	$C_{14}H_{26}$	0.4	-	-	4.6
17	10.67	8-Heptadecene	$C_{17}H_{34}$	0.6	-	-	-
18	10.72	Cyclotetradecane	$C_{14}H_{28}$	1.5	-	-	-
19	10.86	Acorenone	$C_{15}H_{24}O$	-	-	1.5	-
20	10.91	Cedrenol	$C_{15}H_{24}O$	-	1.3	-	-
21	11.13	Pentadecanal	$C_{15}H_{30}O$	6.8	-	6.8	6.1
22	11.26	7-Tetradecene	$C_{14}H_{28}$	0.6	-	-	-
23	11.27	p-Diisopropylbenzene	$C_{12}H_{18}$	-	-	0.9	-
24	11.99	Tetradecanoic acid	$C_{14}H_{28}O_2$	-	-	-	17.1
25	12.23	Tetradecanal	$C_{14}H_{28}O$	-	-	1	-
26	12.23	Hexadecanal	$C_{16}H_{32}O$	0.9	-	-	-
27	12.45	Neophytadiene	$C_{20}H_{38}$	3.6	-	5	-
28	12.54	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	4.8	-	17.6	0.7
29	12.72	9-Octadecyne	$C_{18}H_{34}$	-	-	1.3	-
30	12.99	*cis*-9-hexadecanal	$C_{16}H_{30}O$	0.7	-	-	-
31	13.04	9-tetradecenal, (Z)-	$C_{14}H_{26}O$	-	-	-	2.4
32	13.05	1,5-Cyclodecadiene,(E,Z)-	$C_{10}H_{16}$	1.7	-	-	-
33	13.05	7-Tetradecenal,(Z)-	$C_{14}H_{26}O$	-	-	3.5	-
34	13.31	Geranylgeraniol	$C_{20}H_{34}O$	6.7	-	-	-
35	13.32	Farnesyl acetone	$C_{18}H_{30}O$	-	-	5.7	-
36	13.37	Methylpamitate	$C_{17}H_{34}O$	0.9	-	-	-
37	13.61	Isophytol	$C_{20}H_{40}O$	9.7	-	1.3	-
38	13.78	Octadecanal	$C_{18}H_{36}O$	-	-	-	1.1
39	14.09	Palmitic acid	$C_{16}H_{32}O_2$	-	11.1	2.3	36.4
40	14.41	Nerolidol	$C_{15}H_{26}O$	5	-	-	2.2
41	14.86	Phytanol	$C_{20}H_{42}O$	-	-	1.2	-
42	14.95	Calamenene	$C_{15}H_{22}$	0.6	-	2	-
43	15.21	Phytol	$C_{20}H_{40}O$	11.2	-	30.7	1.9
44	15.61	Oleic acid	$C_{18}H_{34}O_2$	-	-	-	9
45	15.74	1-Pentadecyne	$C_{15}H_{28}$	-	-	-	4

Table 2
Continued

S/N	RT	Compounds	M. Formula	*Leptoderris brachyptera* TIC(%)		*Leptoderris micrantha* TIC(%)	
				Leaf	Stem	Leaf	Stem
46	17.35	4,8,12,16-tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	-	-	0.6	-
Oxygenated Monoterpenes				**30.5**	-	10.2	-
Sesquiterpene Hydrocarbons				11	12.1	7	-
Oxygenated Sesquiterpenes				5	1.3	7.2	4.7
Oxygenated Diterpenes				27.6	-	**33.2**	1.9
Fatty acid and Fatty Acid Esters				14.2	11.1	33	**80.3**
Non-Terpenes				8.1	**68.1**	3.7	7.2
Total				**96.4**	**92.6**	**94.3**	**94.1**

These differences in the volatile composition of other plants could be attributed to genetic (genus, species, and ecotype), chemotype, part, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods (Khallouki et al., 2002; Yhiya et al., 2015).
Oxygenated mono-, sesqui-, and diterpenoids identified in this study are of pharmaceutical importance. For instance, hexahydrofarnesylacetone identified in the leaf of *Leptoderris brachyptera,* leaf along with the stem of *Leptoderris micrantha*, was reported as the major compound present in the oil extracted from *Hildegardia barteri* that demonstrated a broad-spectrum inhibition against various strains of bacteria and fungi, allopathic and pest control (Balogun et al., 2017). Ionone and pentadecanal inhibited the growth of algae (Kumar et al., 2010), while acorenone was the major constituent in *Acorus calamus* L. rhizome essential oil and has been shown to possess gastrointestinal disorders properties (Neńić et al., 2018). Also, geranyl acetone has medicinal implications such as antimicrobial activity (Ghannadi et al., 2003).

3.3. Antimicrobial activity of the essential oils

Essential oils obtained from leaves and stem of *Leptoderris brachyptera* and *Leptoderris micrantha* were screened for their antibacterial activity against four Gram negative (*E. coli*, *S. typhi, K. pneumoniae, and P. aeruginosa*) and two Gram positive (*S. aureus, B. substilis*) bacteria and antifungal activity against four fungi (*C. albicans, P. notatum, A. niger,* and *R.* spp.). Testing was carried out at 1000-62.5 µg/mL. The antimicrobial potency was assessed by measuring the inhibition zones around the well of the essential oils. The results are shown in Table 3. According to the result shown in Table 3, *Leptoderris brachyptera* leaf essential oil showed no inhibition zone at 1000 µg/mL against four Gram-negative bacteria (*E. coli*, *S. typhi, K. pneumoniae, and P. aeruginosa*) and two Gram-positive bacteria (*S. aureus,* and *B. substilis*) and four fungi (*C. albicans, P. notatum, A. niger,* and *R.* spp.).
Low inhibition zones were observed at 125-62.5 µg/mL against the fungi *A. niger* and *R.* spp. *Leptoderris brachyptera* leaf essential oil shows moderate inhibition zones at 500-125 µg/mL against *S. aureus, B. substilis*, *K. pneumoniae, and C. albicans*. At 62.5 µg/mL, growth inhibition against *S. aureus,* and *B. substilis* was 10 mm. The absence in the activity at a higher concentration (1000 µg/mL) might be that only a small amount is needed to attack a specific site in the organism and high concentration will cause accumulation and blockage of the sensitive site thereby cause no activity (Youmans et al., 1967). Table 3 shows the inhibition zones observed in *Leptoderris brachyptera* stem essential oils at 1000-62.5 µg/mL. At 1000 µg/mL there was no inhibition against all the microorganisms used except gram-negative *S. typhi* which inhibition zone was 16 mm against the standard drug with 38 mm. *Leptoderris brachyptera* stem essential oils exhibit growth inhibition against all the microorganisms used at 500-125 µg/mL. Low inhibition zones were observed at 125-62.5 µg/mL against *E. coli*, *P. notatum* and *R.* spp.
Leptoderris micrantha leaf essential oil antimicrobial screening result was shown in Table 3. At 1000 µg/mL, a 17 mm inhibition zone was exhibited against *E. coli* while no growth inhibition was observed against the remaining microorganisms. No inhibition was observed against *S. typhi* and *R.* spp. at 1000 µg/mL and 125 µg/mL, no inhibition was observed against *S. typhi, P. aeruginosa, P. notatum, A. niger,* and *R.* spp. Table 3 shows the inhibition zones observed in *Leptoderris micrantha* stem essential oil. At 1000 µg/mL growth inhibition against *S. typhi,* and *P. aeruginosa* was 18 mm while 38 mm was observed for the standard drug. No growth (inhibition zones) was observed against *R.* spp. at 1000 µg/mL. The findings in these results revealed that the stems' essential oils showed potential antimicrobial activity against all the microorganisms used while the leaves demonstrated moderate activity. The leaves did not show any activity against *S. typhi*. On the other hand, the moderate inhibition zone observed in this studied can be attributed to polar compounds that have been identified in the essential oils. A similar trend was reported by Olaoluwa et al. (2013) that polar compounds possess better interaction with microorganisms' cell walls leading to their inhibition.

Table 3
Antimicrobial properties of essential oil from leaf and stem of *Leptoderris brachyptera* and *Leptoderris micrantha* (Inhibition zone diameter in mm).

Concentration (mg/mL)	Inhibitory Zone (mm) of Tested Microorganisms										Samples
	Bacterial strains						Fungi Strains				
	EC	PS	ST	SA	BS	KP	CA	AN	PN	RS	
1000	-	-	-	-	-	-	-	-	-	-	LBL
500	-	-	-	14±0.001	14±0.002	15±0.001	15±0.003	12±0.002	12±0.001	-	
250	-	-	-	12±0.002	12±0.001	12±0.005	13±0.001	10±0.002	10±0.002	-	
125	-	-	-	12±0.001	11±0.003	10±0.001	12±0.002	-	-	-	
62.5	-	-	-	10±0.001	10±0.005	-	-	-	-	-	
1000	-	-	16±0.001	-	-	-	-	-	-	-	LBS
500	13±0.002	14±0.001	15±0.003	14±0.003	16±0.001	15±0.004	15±0.001	13±0.03	12±0.003	12±0.001	
250	10±0.001	12±0.006	13±0.003	12±0.001	14±0.001	13±0.001	13±0.002	11±0.001	10±0.002	10±0.001	
125	-	11±0.001	11±0.001	11±0.003	12±0.000	12±0.001	11±0.003	10±0.001	-	-	
62.5	-	10±0.005	10±0.002	10±0.003	12±0.000	10±0.001	-	-	-	-	
1000	17±0.000	-	-	-	-	-	-	-	-	-	LML
500	15±0.001	14±0.003	-	16±0.000	15±0.001	14±0.002	14±0.001	12±0.003	12±0.004	-	
250	13±0.001	12±0.003	-	14±0.000	12±0.003	12±0.002	12±0.001	10±0.001	10±0.000	-	
125	11±0.001	-	-	-	-	-	-	-	-	-	
62.5	-	-	-	-	-	-	-	-	-	-	
1000	-	18±0.000	18±0.002	-	-	-	-	-	-	-	LMS
500	13±0.001	16±0.000	15±0.003	14±0.001	15±0.000	16±0.001	15±0.003	13±0.004	12±0.001	-	
250	11±0.000	14±0.001	13±0.003	13±0.000	13±0.001	13±0.003	13±0.002	11±0.001	10±0.000	-	
125	10±0.002	12±0.001	11±0.000	11±0.003	11±0.001	12±0.002	11±0.000	10±0.001	-	-	
62.5	-	10±0.001	10±0.003	-	10±0.000	10±0.002	10±0.001	-	-	-	
	-	-	-	-	-	-	-	-	-	-	-ve control
	38±0.001	38±0.000	38±0.001	36±0.000	38±0.002	40±0.002	28±0.001	28±0.000	28±0.002	28±0.003	+ve control

Keys: **EC**= Escherichia coli **PS**= Pseudomonas aeruginosa **ST**= Salmonella typhi **SA**= Staphylococcus aureus **BS**= Bacillus substilis
KP= Klebsiella pneumoniae **CA**= Candida albicans **AN**= Aspergillus niger **PN**= Penicillum notatum **RS**= Rhizopus spp.
-ve = negative control (DMSO) **+ve** = positive control (Gentamicin (10 µg/mL) for bacteria and Tioconazole (0.7 mg/mL) for fungus) *The values given are means of duplicate analysis (mean± Standard deviation)

Table 4
Absorbance measurement and IC_{50} in the DPPH scavenging activity of leaf and stem essential oils of *Leptoderris brachyptera* and *Leptoderris micrantha*.

Sample	Mean Absorbance at each concentration mg/mL					IC_{50}
	1	0.5	0.25	0.125	0.063	
LBL	0.583±0.001	0.661±0.001	0.683±0.000	0.709±0.000	0.712±0.001	0.95
LBS	0.585±0.001	0.724±0.000	0.729±0.001	0.720±0.001	0.778±0.001	1.02
LML	0.498±0.002	0.626±0.002	0.669±0.000	0.686±0.001	0.695±0.001	0.75
LMS	0.545±0.001	0.611±0.001	0.651±0.001	0.664±0.000	0.664±0.000	0.82
VIT C	0.076±0.001	0.078±0.001	0.080±0.001	0.104±0.001	0.105±0.000	0.05
BHA	0.081±0.001	0.088±0.000	0.094±0.000	0.095±0.000	0.102±0.001	0.05

*The values given are means of triplicate analysis (mean-absorbance. ± Standard deviation), IC_{50} = Inhibition concentration at 50%.

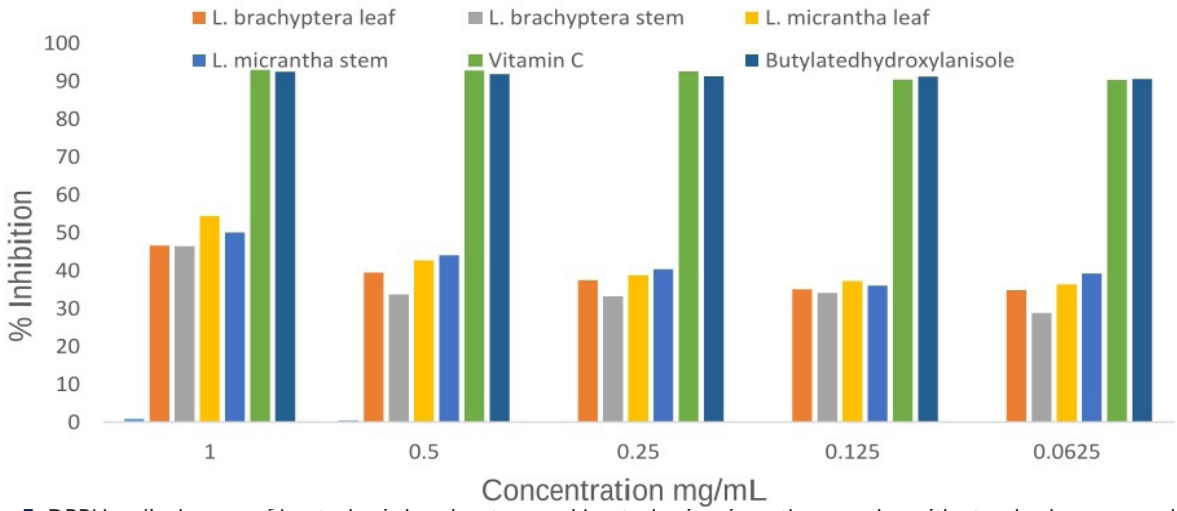

Fig. 5. DPPH radical assay of Leptoderris brachyptera and Leptoderris micrantha samples with standard compounds.

3.4. Antioxidant activity of the essential oils

Table 4 shows the absorbance measurement in the free radical scavenging assay of the essential oils of *Leptoderris brachyptera* and *Leptoderris micrantha* at 1.0-0.0625 mg/mL. The change in color of DPPH from purple to yellow suggests the ability of these oils to act as donors of hydrogen atoms or electrons in the transformation of DPPH into its reduced form DPPH-H. The antioxidant potential was concentration-dependent. Percentage inhibition of each oil sample was calculated from the absorbance (Fig. 5). The antioxidant assay result of *Leptoderris brachyptera* and *Leptoderris micrantha* samples shown in Table 4 and Fig 5 indicated that the activity of the tested oils and standard compounds increased as follows based on the IC_{50}, which ranges from 0.05-1.02: Vit C > BHA > LML > LMS > LBL > LBS. *Leptoderris micrantha* leaf and stem oils showed higher percentage inhibition 54.48% and 50.18% respectively at 1.0 mg/mL. Vitamin C showed significantly higher activity (93.05-90.40%) compared to others at the tested concentration of 1.0-0.0625 mg/mL.

The percentage inhibition of the essential oils *Leptoderris brachyptera* leaf obtained ranges from (46.71-34.92%) and stem ranges from (46.53-28.88%). The percentage inhibition of the essential oil of *Leptoderris micrantha* leaf observed ranges from (54.48-36.47%) and stem ranges from (50.18-39.31%).

The moderate activity of the oils could be a link to the moderate amount of oxygenated mono-, sesqui- and diterpenoids like phytol and isophytol. However, phytochemicals with the presence of OH moieties have been reported to exhibit antioxidant activity (Ogunlana et al., 2008). Phytol is an example of a compound with OH moiety, hence, it has been reported to exhibit antioxidant activity because of its ability to donate electron/hydrogen atom to DPPH radical easily, thereby showing remarkable antioxidant activity (Camila et al., 2013).

4. Concluding remarks

Comparative study of essential oils composition, antioxidant and antimicrobial activities of *Leptoderris*

brachyptera and *Leptoderris micrantha* from different parts (leaf and stem) is reported for the first time. Forty-six compounds were identified altogether in all the oil samples which are mainly monoterpenoids, sesquiterpenoid, fatty acid, fatty acid esters as well as non-terpene derivatives. *Leptoderris brachyptera* leaves oil is dominated by phytol (11.2%) and α-ionone (10.5%) while, *Leptoderris micrantha* leaf oil, phytol (30.7%), and hexahydrofarnesylacetone (17.6%) were the most abundant. The most abundant constituents in the stem oil of *Leptoderris brachyptera* was 4-(1*H*-pyrazol-1-yl) benzeneamine (60.8%), followed by three other major compounds are β-cedrene (12.1%), palmitic acid (11.1%), and isothiazole-3-methyl-5-phenyl (7.3%). 4-(1*H*-pyrazol-1-yl) benzeneamine (60.8%) from *Leptoderris brachyptera* stem, which has also been reported in many other plants of Leguminosae family in high concentration. The presence of different phytochemicals in these chemical profiles proved that both plants are of biological importance. In this study, the essential oils from the studied plants exhibited moderate antimicrobial and antioxidant activities that may buttress the ethnomedicinal applications.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

Adams R.P., 2007. Identification of Essential Oil Components by GC-MS. Allured Publication Corp., Carlo Stream, II. USA.

Addo-fordjour, P., Anning, A.K., Amaniampong, E., Serwaa-Agywei, P., 2008. Diversity and distribution of climbing plants in a semi-deciduous Rain Forest, FNUST Botanic Garden, Ghana. Inter. J. Botan. 2, 186-195.

Balogun, O.S., Ajayi, O.S., Adeleke, A.J., 2017. HexahydrofarnesylAcetone-rich extractives from *Hildegardia barteri*. J. Herbs, Spices Med. Plants. 1-8. doi: 10.1080/10496475.2017.1350614.

Burkill, H.M., 2000. The Useful Plants of West Tropical Africa, Vol 3; 2nd Ed. Kew, UK, Royal Botanical Gardens.

Camila, C.M.P.S., Mirian, S.S., Vanine, G.M., Luciana, M.C., Antonia, A.C.A., Guilherme, A.L.O., Jéssica, P.C., Damião, P.S., Rivelilson, M.F., Reinaldo, N.A., 2013. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. J. Neurosci. 2013, 1-9.

Chaverri, C., Cicció, J., 2015. Leaf and flower essential oil compositions of *Gliricidia sepium* (Fabaceae) from Costa Rica. Amer. J. Essen. Oils Nat. Prod. 2(3), 18-23.

Famobuwa, O.E., Oloyede, H.O., Bello, I.J., 2016. Synergistic antioxidant and anti-bacterial activities of *Croton zambesicus* MülLeptoderris-Arg. (Euphorbiaceae) *Leptoderris micrantha* Dunn (Fabaceae) and *Carpolobia lutea* G. Don (Polygalaceae). Int. J. Pharm. Sci. Res. 1(4), 05-08.

Gabon, F.I., 2016. Conservatoire et Jardin botaniques & South African National Biodiversity Institute, 49, 220-225.

Ghannadi, A., Tavakoli, N., Mehri-Ardestani, M., 2003. Volatile constituents of the leaves of *Ziziphus spinachristi* (L.) Willd. From Bushehr, Iran. J. Essen. Oil Res. 15, 191-192.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and Donoghue, M.J., 2002. Plant systematics: a phylogenetic approach. Sinauer Axxoc. 287-292.

Khallouki, F., Younos, C., Soulimani, R. and Bessière, J., 2002. Chemical composition of the essential oil of *Ononis natrix* L. Fabaceae. J. Essent. Oil Res. 14. 431-432.

Kumar, P.P., Kumaravel, S., Lalitha, C., 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr. J. Biochem. Res. 4(7), 191-195.

Magallón, S.A., Sanderson, M.J., 2001. Absolute diversification rates in angiosperm clades. Evolution 55(9), 1762-1780.

Magiatis, P., Melliou, E., Skaltsounis, A.L., 1999. Chemical composition and antibacterial activity of essential oils of *Pistacia lentiscus* var. *chia*. Planta Med. 65(8), 749-752.

Martins, A.P., Salgueiro, L.R., Goncalves, M.J., 2000. Antibacterial activity and chemical composition of the bark oil of *Croton stelluifer*, and endemic species from St. Tome and Principy. Planta Med. 66(7), 647-650.

Mevy, J.P., Bessiere, J.M., Dherbomez, D., Millogo, J., Viano, J., 2007. Chemical composition and some biological activities of the volatile oils of a chemotype of *Lippia chevalieri* Moldenke. Food Chem. 101(2), 682-685.

Mohadjerani, M., Asadollahi, S., 2019. *Veronica cristagalli* Steven and *Veronica persica* Poir. as anticancer and antioxidant plants *in-vitro*. Trends Phytochem. Res. 3(1), 61-66.

Mohammadhosseini, M., 2017. The ethnobotanical, phytochemical and pharmacological properties and medicinal applications of essential oils and extracts of different *Ziziphora* species. Ind. Crops Prod. 105, 164-192.

Morais, C.B., Scopel, M., Pedrazza, G.P.R., da Silva, F.K., Dalla Lana, D.F., Tonello, M.L., Miotto, S.T.S., Machado, M.M., De Oliveira, L.S., Fuentefria, A.M., Zuanazzi, J.A., 2017. Anti-dermatophyte activity of Leguminosae plants from Southern Brazil with emphasis on *Mimosa pigra* (Leguminosae). J. Med. Mycol. 27(4), 530-538.

Nahar, L., Sarker, S.D., 2019. UPLC in phytochemical analysis. Trends Phytochem. Res. 3(1), 1-2.

Nangue, Y.D., Llorent-Martínez, E.J., Fernández-de Córdova, M.L., Ngangoum, D.A.M., Nguelefack, T.B., Azebaze, A.G.B., Dongmo, A.B., 2019. Phytochemical study and anti-inflammatory activity of the roots of *Mangifera indica* L. in lipopolysaccharide (LPS)-stimulated peritoneal macrophages. Trends Phytochem. Res. 3(1), 53-60.

Neńić, M., Stojanović, N., RanĎelović, P., Radulović

N., 2018. Effects of acorenone-type *Acorus calamus* essential oil on rat gastric fundus contractions and intestinal transit. 49th International Symposium on Essential Oils (ISEO2018). Phys. Chem. Tech. 16(1), 139.
Ogunlana, O.E., Ogunlana, O.O., Farombi, E.O., 2008. *Morinda lucida*: Antioxidant and reducing activities of crude methanolic stem bark extract. Adv. Nat. Appl. Sci. 2, 49-54.
Olaoluwa, O.O., Aiyelaagbe, O.O., Irwin, D., Reid, M., 2013. Novel anthraquinone derivatives from the aerial parts of *Antigonon leptopus* Hook & Arn. Tetrahedron 69(33), 6906-6910.
Olaoluwa, O., Moronkola, D., Taiwo, O., Iganboh, P., 2018. Volatile oil composition, antioxidant and antimicrobial properties of *Boerhavia erecta* L. and *Euphorbia hirta* L. Trends in Phytochem. Res. 2(3), 171-178.
Oloyede, G.K., Oladosu, I.A., Shodia, A.F., 2010. Chemical composition and cytotoxicity of the essential oils of *Crinum ornatum* (Ait.) Bury. Afri. J. Pure and App. Chem. 4(3), 035-037.
Oloyede, G.K., Olatinwo, M.B., 2014. Phytochemical investigation, toxicity and antimicrobial screening of essential oil and extracts from leaves and stem bark of *Hura crepitans* (Euphorbiaceae). Academ. Arena 6(5), 7-15.
Oloyede, G.K., Ojeyinka, B.K., 2019. Effect of solvent on chemical composition and antioxidant activities of *Abelmoschus esculentus* (L.) Moench Pods oils extracts. J. App. Sci. 19, 133-139.
Onocha, P.A., Oloyede, G.K., Akintola, J.A., 2016. Chemical composition, free radical scavenging and antimicrobial activities of essential oil of *Mariscus alternifolius* Vahl. Open Conf. Proc. J. 7, 160-167.
Rukangira, E., 2001. Medicinal Plants and Traditional Medicine in Africa: Constraints and Challenges. Sustainable Development International, 4, 179-184.
Sonibare, M.A., Oke, T.A., Soladoye, M.O., 2014. A pharmacobotanical study of two medicinal species of Fabaceae. Asian Pacific J. Tropic. Biomedicin. 4(2), 131-136.
Yhiya, M.A., Amani, M.M, Mona, G.Z., Mohamed, S.A., 2015. The genus *Machaerium* (Fabaceae): Taxonomy, phytochemistry, traditional uses and biological activities. Nat. Prod. Res. 29(15), 1388-1405.
Youmans, G.P., Peterson, P.Y., Soumers, H.M., 1967. The Biological and Clinical Basis of Infectious Diseases. Longman London, pp. 721-725.