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SHORT COMMUNICATION

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF VOLATILE OILS OF *PERISCOPSIS LAXIFLORA* LEAF AND STEM

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ABSTRACT. *Pericopsis laxiflora* (Fabaceae) is a savannah shrub used traditionally to treat headache, stomach ulcers, heart pain and abdominal pain. Air-dried leaves and stem of the plant were extracted by hydrodistillation and the oils obtained were characterized using gas chromatography-mass spectrometry. Antimicrobial assay was carried out by agar well diffusion method against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiellae pneumonia*, (gram negative bacteria), *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, (gram positive bacteria); *Candida albicans*, *Aspergillus niger*, *Penicillum notatum* and *Rhizopus stolonifer* (fungi) while antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. Oxygenated monoterpenes (32.92%), monoterpenes (22.58%) and sesquiterpenes (21.63%) hydrocarbons dominated the leaves' oil and monoterpenes (33.63%), sesquiterpenes (18.42%) and diterpenes (21.48%) hydrocarbons dominated the stem' oil. The leaf oil showed better scavenging activity at 1.0 mL (60.56%) than the stem oil (46.58%) comparing activity with ascorbic acid and butylated hydroxyl anisole (BHA) in the antioxidant assay. *P. laxiflora* stem oil showed highest activity against *K. pneumonia* and *E. coli* at 100 µg/mL and 50 µg/mL with comparable activity to gentamicin and tioconazole which are antibacterial and antifungal standards respectively. This study therefore showed that the colourless volatile oils exhibited comparable bioactivity with standards thus, justifying the plant's ethno medicinal application.

KEY WORDS: *Pericopsis laxiflora*, *Fabaceae*, Monoterpenes hydrocarbons, Antimicrobial, antioxidant

INTRODUCTION

Medicinal and aromatic plants are extensively used as natural organic compounds and as medicines [1]. The importance of volatile and essential oils is increasing day by day because they have been used for the treatment of various sorts of infectious diseases and are used in the beverage and food industries, cosmetics and fragrance industries for making valuable perfumes [2]. Volatile and essential oils have variable composition and are best known for their action as antispasmodic, antiviral negotiators, antimicrobial and carminative [3, 4].

The Fabaceae commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. It includes trees, shrubs, and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume) and stipulate leaves. Many legumes have characteristic flowers and fruits. The family is widely distributed and is the third-largest land plant family in a number of species, behind only the Orchidaceae and Asteraceae, with about 765 genera and nearly 20,000 known species [5]. The nutritional value of Fabaceae has been attributed to their ability to fix atmospheric nitrogen for protein synthesis. This advantage has led to protein concentrations in leaves and seeds which vary between 20% and 40% dry weight, depending on the species [6]. They are found in tropical rainforests and dry forests of the Americas and Africa [7]. Recent molecular and morphological evidence supports the fact that the Fabaceae is a single monophyletic family [8]. *Pericopsis* is a genus of legume in the family Fabaceae and comprises the following species: *Pericopsis angolensis* (Baker) Meeuwen, *Pericopsis elata* (Harms) Meeuwen—African teak, *Pericopsis laxiflora* (Baker) Meeuwen.

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Pericopsis mooniana (Thwaites)—Nandu wood [8]. *Pericopsis laxiflora* is a savannah, perennial, woody deciduous shrub or tree belonging to the family Fabaceae. This plant is native to Tropical America; however, it is now widely spread in tropical rain forests. It is mostly planted in the communities and villages due to the fondly shade capabilities. It is commonly called satin wood. It has several other names all over the world. In Nigeria, it is referred as - "Ayan" by the Yoruba people and "Abua-ocha" by the Igbo people. In Ghana, it is known as "esreso kokrodua" by the Asantes, "kiplig" by Ewes and "duakobi" by the Fantes. *P. laxiflora* usually grows to a height of 9-13 m tall but is occasionally only a shrub up to 2 m [9, 10]. In Nigeria, Côte d'Ivoire and Ghana, the roots and bark are used as abortifacient, aphrodisiac, tonic and are used to treat many other health conditions including headache, snake bites, jaundice, malaria, rheumatism, diarrhoea, stomach ulcers, gastritis, enteritis and heart pain [11, 12]. The dried and powdered root is applied externally to relieve pain and to treat oedema and tumours. The leaf sap is drunk as an anthelmintic [13, 14]. Parts of the plant such as leaves and stem barks are also regarded as medicine for the treatment of syphilis, diarrhoea, dysentery and other bacterial and parasitic agents [15]. *P. laxiflora* leaves and stem bark ethanolic crude extract has been reported to contain phytochemicals such as alkaloids, flavonoids, terpenoids, saponins and phenols [14, 16, 17]. Although many drugs have been derived from herbal medicines, several medicinal plants are yet to be exploited or fully investigated. *P. laxiflora* is one of such plants. The aim of this study is to determine the chemical constituents of *P. laxiflora* leaf and stem volatile oil since essential oils are used in aromatherapy as an alternative source of healing because of the presence of aromatic compounds [18] and to evaluate its antimicrobial and antioxidant activities.

EXPERIMENTAL

Plant material. *Pericopsis laxiflora* fresh leaf and stem were collected in Ikire, Osun state, in September 2021. The plant was identified and authenticated by Mr Odewo at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves and stems were separated; air dried under ambient temperature, ground into fine powder and kept in air tight bags until further analysis.

Chemicals and reagents. Methanol, hexane, butylated hydroxylanisole (BHA), vitamin C, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were used.

Test organisms. Bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiellae pneumonia*, (gram negative bacteria), *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, (gram positive bacteria) and fungi: *Candida albicans*, *Aspergillus niger*, *Penicillum notatum* and *Rhizopus stolonifer* were collected from the Department of Pharmaceutical Microbiology, University of Ibadan, Oyo State, Nigeria. The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4 ºC. Aliquots (100 mL) of nutrient broth were inoculated with the culture of test micro-organisms using a loop and then incubated at 37 ºC for 24 hours.

Reference standards. The antibacterial and antifungal standards, gentamicin® (10 µg/mL) and tioconazole® (70%) obtained from the University Medical Hospital, Jaja Clinic Pharmacy, were used. Ascorbic acid (Vitamin C) and Butylated hydroxyl anisole were used as antioxidant standards.

Equipment. Gas chromatography-mass spectrometer (GC-MS) (Agilent Technologies GC-MS (HP 7890), Clavenger Apparatus (hydro distiller), UV-Visible spectrophotometer (721S visible spectrophotometer) were used.

Extraction of volatile oil. The air dried, ground leaf and stem of *Pericopsis laxiflora* were subjected to hydrodistillation for three hours using the Clavenger apparatus as described by the 2022 British Pharmacopoeia specifications. Air dried leaf and stem (200 g each) of *P. laxiflora*

was separately transferred into a 10 L round bottom flask fitted on Clavenger apparatus with the heater set at a thermo-stated temperature of 100 °C, the volatile oil was collected after 3 hours and trapped with 100% analytical grade 10 mL n-hexane. The oil samples were kept in the refrigerator at a temperature of 4 °C until further analyses.

Analysis of the volatile oil by gas chromatography-mass spectrometry (GC-MS). The oils were analysed using a GC model of Agilent technologies 7890 coupled with mass spec. 5975 Agilent technologies. The mobile phase is helium gas and the stationary phase is the column HP5MS of length 30 m, internal diameter of 0.320 mm and thickness of 0.25 µL. For the oven temperature, the initial temperature is 80 °C held for 2 min at 12 °C per minute to the final temperature of 240 °C held for 6 min. The scan range is 50 to 550 while the interface temperature between GC and MS is 250 °C. The volume of sample injected is 1 µL.

Identification of components. The constituents of the oils were identified on the basis of their retention indices determined with a reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns (NIST database/chemstation data system) with data previously reported in literature [19-21].

Antioxidant activity. The oils were analysed using DPPH (2,2-diphenyl-1-picryl hydrazyl) radical free scavenging method. 2 mL DPPH solution prepared in methanol was added to different concentrations (1.0-0.25 mL) of the oil samples. The scavenging activity of the oils was obtained by recording the decrease in absorbance at 517 nm after 10 min of incubation using the UV-Visible spectrophotometer. The same procedure was carried out on ascorbic acid (vitamin C) and butylated hydroxyanisole (BHA) which are known antioxidant agents. The analyses were run in triplicates and the mean was used to calculate the % inhibition [22].

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

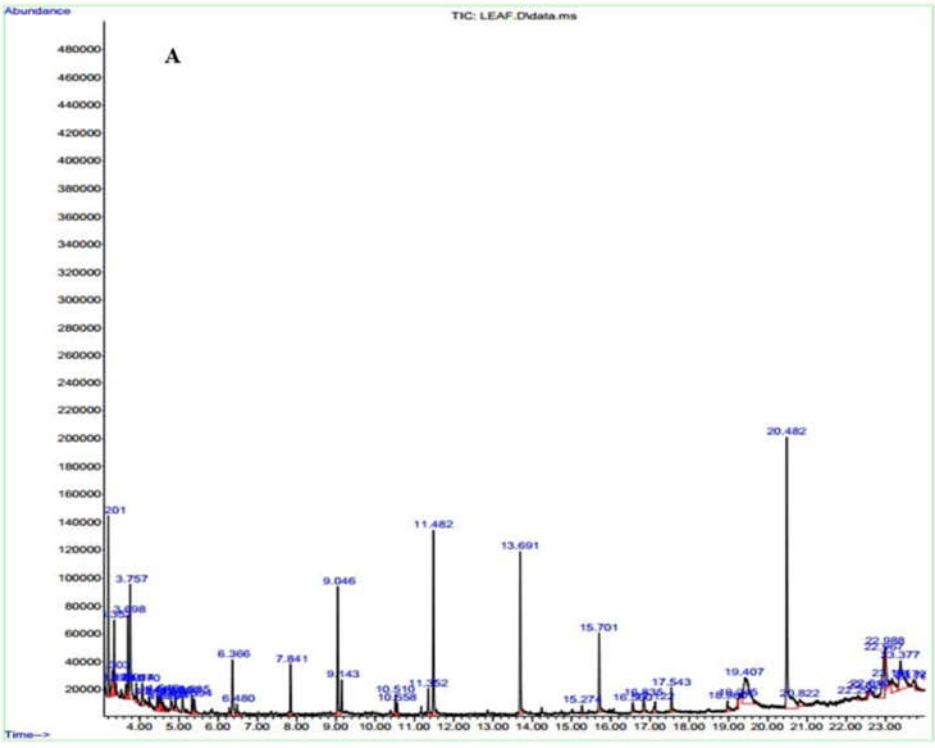
Antimicrobial activity by agar well diffusion method. Antimicrobial activities of volatile oil of *Pericopsis laxiflora* stem and leaf were carried out using the agar well diffusion method. 0.2 mL of an overnight broth culture of the test micro-organism was added to 20 mL of cooled molten agar. It was mixed and poured into a sterile petri-dish and allowed to set. The stock was maintained on nutrient agar slant and sub-cultured in nutrient broth for incubation at 37 °C prior to each antimicrobial testing. This stock solution was diluted serially to obtain concentrations 100 – 6.25 µg/mL and dispensed into each respective well. Gentamicin (10 µg/mL) and tioconazole (1%) were used as positive controls for the antibacterial and antifungal assay, respectively. Inoculation of the test organisms on nutrient agar-prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using a Grade No. 1 Whatman filter paper. 100 discs were obtained by punching and putting in vials-bottles and sterilizing in an oven at 150 °C for 15 min. Thereafter, the cups (9 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions of oils (50 µL) at concentration of 40 g/mL were then introduced into each of the designated cups on each plate ensuring that no spillage occurred. The same amount of the standard antimicrobial agent and solvents were introduced into the remaining cups on each plate to act as positive and negative controls respectively. The plates were left at room temperature for 1 hour, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37 °C for 24 h in an incubator. Clear zones of inhibition were observed. Activity or inactivity of the oil was tested in triplicate and the diameters of zones of inhibition were measured in millimeter [22].

RESULTS AND DISCUSSION

The volatile oils were obtained from *Pericopsis laxiflora* leaves and stem at 0.9% and 0.75% yields, respectively. The oils were colourless with a herbal-like smell. Chemical constituents identified in the chromatograms (Figure 1) of volatile oil of leaves and stem of *P. laxiflora* were 24 and 19, respectively (Table 1). Oxygenated monoterpenes (28.94%), monoterpenes hydrocarbons (22.58%), sesquiterpenes hydrocarbons (21.63%), diterpenes hydrocarbons (6.84%), other compounds (1.99%) were found in the leaf while oxygenated monoterpenes (7.99%), monoterpenes hydrocarbons (33.63%), sesquiterpenes hydrocarbons (18.42%), diterpenes hydrocarbons (6.06%), other compounds (5.17%) were obtained in the stem oil (Table 1). The volatile oils of this plant are characterized by a high percentage of hydrocarbons both in the leaf and stem oil and could be a valuable alternative source of hydrocarbons.

Major compounds of leaf oil of Pericopsis laxiflora. Oxygenated monoterpenes = 28.94%, monoterpenes hydrocarbons = 22.58%, sesquiterpenes hydrocarbons = 21.63%, diterpenes hydrocarbons = 6.84% and other compounds = 1.99%.

Major compounds of stem oil of Pericopsis laxiflora. Oxygenated monoterpenes = 7.99%, monoterpenes hydrocarbons = 33.63%, sesquiterpenes hydrocarbons = 18.42%, diterpenes hydrocarbons = 6.06% and other compounds = 5.17%.



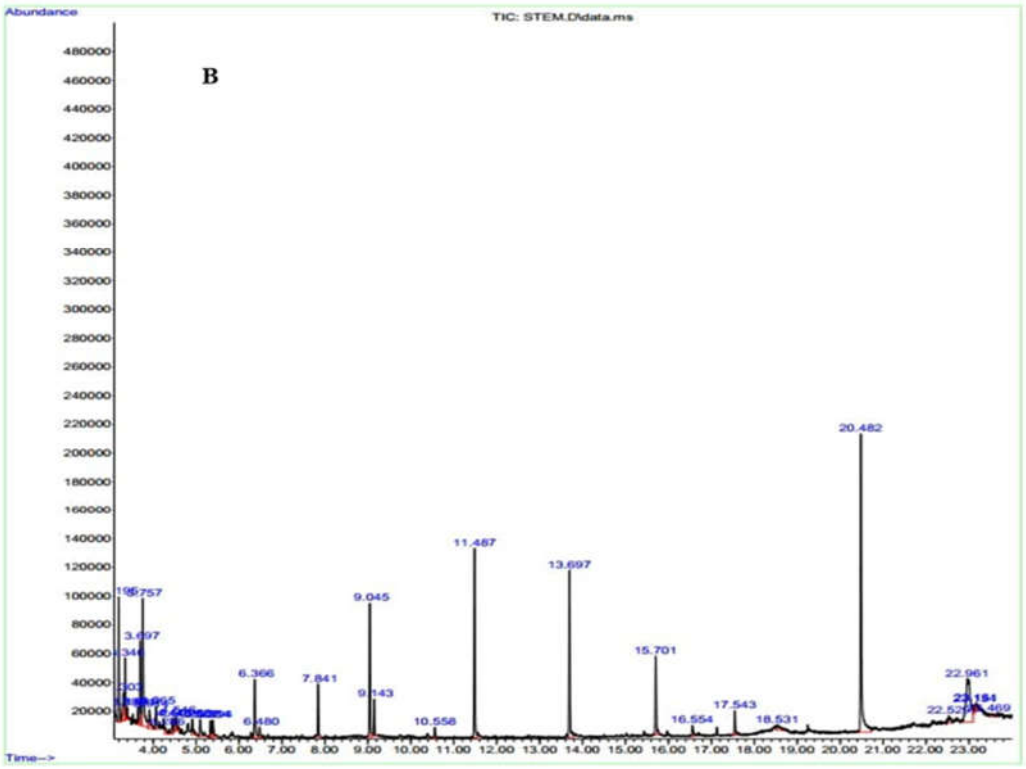


Figure 1. Chromatograms of volatile oils of *Pericopsis laxiflora* Leaf (A) and stem (B).

Table 1. Volatile compounds identified in the oil of *Pericopsis laxiflora* leaf.

S/N	Compounds	Retention time (min)	% Composition
1	2-Pentanol, 4-methyl	3.201	7.35
2	Mesitylene	3.303	5.11
3	Cyclopentanol	3.352	4.33
4	13-Octadecenal, (Z)	3.649	2.56
5	Decane	3.757	6.08
6	beta-Ocimene	3.914	0.84
7	Cyclohexane, 1,3-butadienylidene	4.508	0.83
8	3-Hexanol,1,5-dimethoxy-2,4-dimethyl	4.589	2.51
9	1-Dodecene	6.366	2.6
10	Tridecane	7.841	2
11	1-Tetradecene	9.046	4.85
12	Tetradecane	9.143	1.26
13	alpha-Farnesene	10.51	0.72
14	beta-Myrcene	11.352	1.12
15	Cetene	11.482	12.66
16	Cyclohexadecane	15.701	3.14
17	1-(3-ethylcyclobutyl)-ethanone	16.835	2.89
18	Octacosyl acetate	17.543	4.12
19	2(-tetradecyloxy)-,Ethanol	19.245	0.48

20	Cholesterol	19.407	8.31
21	Dodecane, 1,2-dibromo	22.988	3
22	gamma.-Sitosterol	23.377	6.84
23	2-Propenamide	15.274	0.37
24	1-Octadecanal	16.56	0.61
Total %			81.98%

Table 2. Volatile compounds identified in the oil of *Pericopsis laxiflora* stem.

S/N	Compounds	Retention time (min)	% Composition
1	2-Hexanal	3.195	6.92
2	Cyclopentane, pentyl	3.649	0.72
3	Decane	3.757	8.39
4	Trans-beta-ocimene	3.914	3.56
5	2,4-Nonadiyne	4.065	3.37
6	2-Nonyn-1-ol	4.286	0.47
7	Cyclohexane,1,3-butadienylidene	4.502	1.9
8	Undecane	5.097	3.14
9	1-Dodecene	6.366	4.13
10	Tridecane	6.48	2.97
11	1-Tetradecene	9.045	6.88
12	Tetradecane	9.143	2.66
13	Butyl Benzene Acetate	10.558	0.6
14	Cyclohexadecane	11.487	8.39
15	1-Octadecane	13.697	8.18
16	9-Encosese,-(E)	15.701	3.87
17	1-Hexadecene	16.554	1.85
18	Bromo acetic acid hexadecyl ester	17.543	3.27
Total%			71.27

Antioxidant activity of Pericopsis laxiflora leaves and stem volatile oils. The percentage inhibition of *Pericopsis laxiflora* leaves and stem oil and the standards (ascorbic acid and butylated hydroxyl anisole - BHA) was calculated at different concentrations (0.25 – 1 mL) in the antioxidant assay. Ascorbic acid standard showed highest % inhibition (98.14%) at all concentrations, followed by BHA (Table 3). At 1.0 mL, the leaves volatile oil had higher percentage inhibition (60.56%) when compared with the stem oil (46.58%) thus having better antioxidant activity when compared to the leaves' oil. Antioxidants have been extensively used in the therapy of diseases related to oxidative stress [23]. The plant showed moderate activity and therefore could be source of antioxidant agent.

Table 3. Antioxidant activity of *Pericopsis laxiflora* leaves and stem volatile oils.

Concentration (mL)	% inhibition leaves	% inhibition stem	% inhibition ascorbic acid	% inhibition BHA
1.0	60.56	46.58	98.14	97.83
0.5	43.32	46.27	97.89	97.67
0.25	35.71	43.94	97.14	97.14

Antimicrobial activity. Volatile oil of *Pericopsis laxiflora* leaves and stem were tested against 10 microorganisms (six bacteria and four fungi), *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiellae pneumonia*, *Candida albicans*, *Aspergillus niger*, *Penicillum notatum* and *Rhizopus stolonifer* and the results are presented in Tables 4 and 5. It was observed that *P. laxiflora* leaves and stem oil inhibited the growth of all the test organisms at all concentrations. Both oils showed better activity against the bacteria than

the fungi at all concentrations. At 100 µg/mL, the leaves oil was most effective on *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiellae pneumonia* while at 6.25 µg/mL, the oil was least active against *Rhizopus stolonifer*. The volatile oil of *P. laxiflora* stem showed highest activity against *K. pneumonia* and *E. coli* at 100 µg/mL and 50 µg/mL, respectively. At the least concentration of 6.25 µg/mL, the oil was least active against *Aspergillus niger*, *Penicillum notatum* and *Rhizopus stolonifer*. Generally, the leaves volatile oil showed better activity against all the tested microorganisms at all concentrations than the stem volatile oil. The difference in the leaves and stem volatile oils chemical composition and antimicrobial activity may have been due to variation in metabolism process of the plants parts. This is also supported by [24] while reporting that the antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depend on location and seasonal variations and [25, 26] on the effect of seasonal and geographical variation of *Heteropyxis natalensis* essential oil on the antimicrobial activity. Therefore, the oil from this plant parts could serve as an important source of antimicrobial agent.

Table 4. Antimicrobial activity of volatile oil of *Pericopsis laxiflora* leaves.

Test organism conc. (µg/mL)	100	50	25	12.5	6.25	Negative control	Gentamicin	Tioconazole
S. a	29	25	22.5	19.5	15.5	-	38	NT
E. c	31	28	23.5	19.5	15	-	40	NT
B. s	29	25	22.5	19	15.5	-	38	NT
P. a	31	27	22.5	18.5	11.5	-	40	NT
S. t	29	25	20.5	16.5	13.5	-	38	NT
K.b	31	26	21.5	18	13.5	-	38	NT
C. a	20	18	17	14	11	-	NT	28
A. n	19	17	14	13	11	-	NT	28
P. n	19	18	16	14	11	-	NT	28
R. s	19	17	15	13	10	-	NT	28

E. c = *Escherichia coli*; S. t = *Salmonella typhi*; P. a = *Pseudomonas aeruginosa;*K. b = *Klebsiellae pneumonia*; B. s = *Bacillus subtilis*; S. a = *Staphylococcus aureus*; C. a = *Candida albicans*; A. n = *Aspergillus niger*; P. n = *Penicillum notatum*; R. s = *Rhizopus stolonifer*; (-) = -ve control: NT = not tested.

Table 5. Antimicrobial activity of volatile oil of *Pericopsis laxiflora* stem.

Test organism conc. (µg/mL)	100	50	25	12.5	6.25	Negative control	Gentamicin	Tioconazole
S. a	20	16	14	13	11	-	38	NT
E. c	25	26	17	14	11	-	40	NT
B. s	24	21.5	17.5	14	12	-	38	NT
P. a	25	21.5	17.5	14	10	-	40	NT
S. t	25	23.5	18.5	14	11	-	38	NT
K .b	27	23	20	14	11	-	38	NT
C. a	18	16	14	12	11	-	NT	28
A. n	16	13	12	10	10	-	NT	28
P. n	18	14	14	12	10	-	NT	28
R. s	17	14	14	12	10	-	NT	28

E. c. = *Escherichia coli*; S. t. = *Salmonella typhi*; P. a. = *Pseudomonas aeruginosa;* K. b = *Klebsiellae pneumonia*; B. s = *Bacillus subtilis*; S. a = *Staphylococcus aureus*; C. a = *Candida albicans*; A. n = *Aspergillus niger*; P. n = *Penicillum notatum*; R. s = *Rhizopus stolonifer*; (-) = -ve control: NT = not tested.

CONCLUSION

The colourless volatile oils extracted from the leaves and stem of *Pericopsis laxiflora* and analysed by gas-chromatography-mass spectrometry contained majorly oxygenated monoterpenes and monoterpene hydrocarbons, respectively. Variability in the chemical composition may have been due to environmental factors. The leaves' oil was most effective in inhibiting *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiellae pneumonia* while stem showed highest activity against *K. pneumonia* and *E. coli* in the antimicrobial assay. The leaves' volatile oil also showed better antioxidant activity than the stem oil when activity was compared to ascorbic acid and BHA.

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