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Chemical constituents and biological activities of essential oils of leaves and stem of *Homalium africanum* (Hook. F) Benth (Flacourtiaceae) and *Shorea roxburghii* G. Don (Dipterocarpaceae)

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Abstract: *Homalium africanum* (Hook. F) Benth (Flacourtiaceae) and *Shorea roxburghii* G.Don (Dipterocarpaceae) are used in the treatment of malaria, cholera, degenerative and inflammatory diseases. This study was carried out to evaluate the chemical constituents, antioxidant, antimicrobial activities and cytotoxicity of the essential oils (EOs) of leaves and stems of both plants. *H. africanum* (HA) and *S. roxburghii* (SR) were collected and authenticated at Forestry Research Institute of Nigeria. The EOs were extracted by hydro-distillation. The chemical composition, antioxidant, antimicrobial activities and cytotoxicity of the EOs were carried out using Gas Chromatography-Mass Spectrometry (GC-MS), 2, 2-diphenyl-1-phenylhydrazyl radical (DPPH), broth dilution method and brine shrimp lethality assays, respectively. Also, the minimum inhibition concentration (MIC) and minimum microbicidal concentration (MMC) were determined. GC-MS analyses of the EOs from *H. africanum* gave twenty-six compounds for the leaf (76.64%) and sixteen compounds for the stem (35.77%). Leaf and stem EOs were dominated with 2-hexanol (6.06%) and benzaldehyde (43.43%), respectively. *S. roxburghii* EOs revealed a total of forty-two compounds for leaf (73.99%) and fourteen compounds for the stem (76.64%). Leaf EO was dominated with caryophyllene oxide (8.85%) and 1H-cyclopropazulen-7-ol (8.62%) and stem EO with α- and β- phellandrene (36.02%) and limonene (26.16%). The EOs of leaves and stem of both plants showed moderate antioxidant activity with IC_{50} (mg/ml) of 1.51, 2.64 (HA) and 1.50, 1.73 (SR), respectively; moderate inhibitory (MIC µg/ml: 0.39-50) and microbicidal activity (MMC µg/ml: 0.39-25-HA, 1.56-50-SR) against all microbes used except *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Apergillus niger*. The EOs of leaves and stem of both plants were cytotoxic, with LC_{50} (µg/ml) of 5.1470, 3.465 (HA) and 4.69, 5.767 (SR), respectively. The results corroborate the plants' ethno-medicinal uses.

Key words: *Homalium africanum, Shorea roxburghii*, 2,2-diphenyl-1-picryhydrazyl, radical assay, brine shrimp lethality assay, essential oils.

Introduction

Natural products provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity.[1] According to the World Health Organization (WHO), more than 80% of the world's population rely on traditional medicine for their primary healthcare needs.[2] The use of herbal medicines in Africa represents a long history of human interactions with the environment. Plants contain a wide range of chemical compounds that can be used to treat chronic as well as infectious diseases.[3]

Natural products have also been extended for commercial purposes such as in cosmetics, dietary supplements, rubber and plastic industries, etc. Microbial resistance to the chemically synthesized drugs has compelled the move towards ethno-pharmacognosy. They found literally thousands of phytochemicals that proved beneficial and have biological reactivity such as antimicrobial, antioxidant, cytotoxicity, antidiarrheal, analgesic and wound healing activities.[4] Essential oils are specific, most common liquid products of the plant tissue and are mainly the products of higher plants. These plants are distributed across over fifty families and the most used as aromatic plants are of the family Asteraceae, Lamiaceae, Apiaceae, Rutaceae, Myrtaceae and Lauraceae.[5]

Compounds from essential oils EOs are characterized by a strong odor and they are formed by aromatic plants as secondary metabolites. They can be synthesized by all plant organs (flowers, leaves, stems, seeds, fruits, roots, wood) and can be stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes.[6] The essential oils localized in different parts of the same plant can be of a

similar composition but can also be significantly different when affected by factors such as locality of the plants, climate factors, genetic variation, plant variety, plant nutrition, fertilizers and stress during growth, thereby determining the yield and the composition of essential oils.[7,8] Manufacturers of pharmaceuticals, cosmetics and perfumes are giving greater attention to essential oils for their role in enhancing product quality and appeal. Similarly, reports indicate a growing demand for essential oils owing to their therapeutic properties and distinctive sensory attributes.

Homalium africanum (Flacourtiaceae) is a rainforest specie native to West Africa typically reaching a height of 24-30m.[9,10] The stem, bark and root of the plant are commonly utilized in the Northwest region of Cameroon as well as in the Democratic Republic of Congo.[11] *H. africanum* is commonly employed in traditional medicine for the management of ailments such as stomach ulcer, malaria and other inflammatory conditions.[11] The plant has also been reported to possess filaricidal and cytotoxic properties.[12] Moreover, other species found within the *Homalium* genus have diverse pharmacol-ogical effects. For instance, *Homalium letestui* demonstrates antidiabetic, anticancer, antiplasmodial, antileishmanial property and it is a cellular antioxidant.[13] Phytochemical investigations on the genus further reveal the isolation of alkaloids from *H. pronyense*,[14] lignan glycosides and flavonoids from *H. ceylanicum*[15] and amides from *H. letestui*.[16]

Shorea roxburghii (Dipterocarpaceae) is a semi-evergreen endangered tree attaining 100 m in height. It is predominantly found on hill slopes area of peninsular India, particularly in Kolli Hills of central Tamil Nadu and Alagar Hills of Madurai.[17,18] *Shorea* genus is considered a rich source for oligomeric stilbenes. Several of these stilbenoids are noted for their biological activities including cytotoxicity, antioxidant, antiplatelet aggregation and cyclooxygenase inhibitory properties.[19,20] Traditionally, the bark of *S. roxburghii* is a popular astringent and a preservative in Thai beverages, while in Indian folk medicine, it has been utilized in treating dysentery, diarrhea, and cholera.[21]

Despite the widely acclaimed ethnomedicinal uses of these two plants, there has been no published studies on the chemical profile of their EOs. In this paper, we present the chemical composition, antioxidant potential, antimicrobial properties and cytotoxic effects of the essential oils from *Homalium africanum* and *Shorea roxburghii*, to justify the scientific rationale for their traditional use in managing degenerative, inflammatory and related diseases.

Materials and methods

Collection and preparation of plant material

The fresh leaf and stem of *H*. *africanum* and *S*. *roxburghii*, were obtained from Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. Authentication were carried out at FRIN herbarium where voucher specimens were deposited (*H. africanum*: FHI 113467; *S. roxburghii* FHI 113657). Plant materials were shade-dried for 2 weeks, ground separately into fine powder and bagged.

Isolation of essential oils

The pulverized leaves (425 g) and stem (375 g) of *H*. *africanum* and *S*. *roxburghii* were subjected to hydro-distillation using a Clevenger- type all-glass apparatus for 4 hours, in accordance with the guidelines of the British Pharmacopeia.[22] The resulting volatile oils were collected in the receiver arm of the apparatus containing water and analytical grade *n*-hexane. The oils- hexane mixtures were transferred into sample bottles, after which the oils were stored in sealed airtight glass vials at 4 ºC, yields determined prior further analysis and bioassay.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The essential oils were analyzed using GC-MS on an Agilent 7809A Gas Chromatograph coupled to an Agilent 5975Mass Selective Detector equipped with a split/splitless injector and operated at 70 eV ionization energy. The ion source temperature was maintained at 200 ^{o}C, with data acquired over a m/z 50-700 at a scan rate of 1428 amu/sec. Separation was carried out on an HP-5MS capillary column (30 m x 0.25 mm i.d., 0.25 µm thickness). The oven temperature program started at 80 ^{o}C (held for 2 min), ramped at 12^{o}C/min to 24 0^{o}C and held for 6 min. Helium served as the carrier gas with a flow rate of 1 mL/min. Injection volume, linear velocity and pressure were set to 1.0 µL, 362 cm/s and 56.2 Kpa, respectively. Another oven program involved heating from 60 ^{o}C (1 min hold) to 180 ^{o}C (3 min hold) at 10 ^{o}C/min, followed by a final increase to 280 ^{o}C (2 min hold) at the same rate. Both the injector and detector were maintained at 250 ^{o}C. Identification of constituents was achieved by calculating retention indices relative to a homologous series of the n-alkanes and by matching mass spectral fragmentation patterns (W11N17main.L/Mass Hunter data system) with previously reported reference spectra.

Antioxidant inhibition assay

The antioxidant activities of the essential oils obtained from *H. africanum* and *S. roxburghii* were evaluated through the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay previously outlined by Onocha *et al.*[23] Accordingly, different concentrations of EOs (1000, 500, 250, 125, and 62.5 µg/mL) were combined with 2.0 mL 100 µM methanolic DPPH. The reaction mixture was vigorously shaken and incubated for 20 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Unico1200 & Perkin Elmer lambda 25 models) and recorded as Abs (sample). DPPH exhibits a strong absorption band in its radical form; however, the presence of antioxidant reduces this absorbance due to radical neutralization. A control test, consisting of DPPH solution without the essential oil of the plant (DPPH + methanol), was similarly prepared and its absorbance noted as Abs (control). All experiments were performed in triplicates after 5mins, with the purple-to-yellow color shift confirming the oils' hydrogen/electron-donating ability in converting the DPPH to its reduced form (DPPH-H).

The radical scavenging efficiency of the EOs was expressed as percentage inhibition using the formula:

$$\%\ \textbf{Inhibition} = \frac{\textbf{Abs}\ (\textbf{Control}) - \textbf{Abs}\ (\textbf{sample or standard})}{\textbf{Abs}\ (\textbf{Control})} \times \mathbf{100}$$

Abs (control) = the absorbance of the control (without sample)
Abs (sample) = 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.

Determination of antimicrobial activity

Leaves and stem EOs of *H. africanum* and *S. roxburghii* were tested against eight strains of microbes consisting of six bacteria: four Gram negative bacteria (*Escherichia coli*, *Salmonella typhi, Klebsiella pneumoniae and Pseudomonas aeruginosa*), and two Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*), as well as two fungi: *Candida albicans* and *Aspergillus niger*.[23]

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using broth micro dilution technique with 96-well microtiter plates.[24] Essential oil samples were first dissolved in double-strength tryptone soya broth to prepare a 50% stock solution, which was subsequently subjected to serial dilutions in sterile sample bottles to achieve concentrations of 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.3960 and 0.0625%. From each dilution, (100 µg/L) was dispensed into the corresponding wells. Gentamycin (10 µg/mL) and ketoconazole (1%) served as the positive controls for antibacterial and antifungal tests, respectively. These standard drugs were further diluted to yield concentrations of 10, 5, 2.5, 1.25, 0.625 µg/mL for gentamycin and 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125% for ketoconazole. Each well was inoculated with 10 µLl of the test microorganism and incubated at 37°C for 24hrs (bacteria) and 25°C for 48hrs (fungi). The lowest concentration of the sample or reference drug that completely inhibited visible microbial growth was considered the MIC.

Determination of Minimum Microbicidal Concentration (MMC)

After confirming growth or turbidity in the test wells for MIC determination, 10µLl of p-INT solution (piodonitrotertazolium violet) at a concentration of 0.2 mg/mLl was dispensed into each well. The plates were then re-incubated at 36°C for 30 minutes. A shift in colour from yellow to pinkish red signified the presence of microbial growth. The lowest concentration that exhibited no colour change was considered the MMC.

Brine shrimp lethality assay

The brine shrimp lethality assay was employed as a preliminary screen for bioactive compounds following the procedure of Onocha *et al*.[25] *Artemia salina* cysts (0.1 g) obtained from the Department of Pharmacognosy, University of Ibadan were hatched in natural sea water, (3.8 g/L salt) from bar beach, Ikoyi, Lagos. The nauplii were maintained for 48 h at 25°C under constant aeration and illumination. to ensure survival and maturity before use. Plant extracts were prepared as stock solutions (10 mg/mL) and serially diluted (1000–10ppm) in 10 mL test tubes. Ten nauplii were transferred into each test solution, with experiments performed in triplicate. Controls consisted of sea water alone (negative) and cyclophosphamide (positive). After the 24 h incubation at 25°C, mortality was assessed under a magnifying lens; nauplii unresponsive to probing were considered dead. Percentage mortality was calculated and LC_{50} values were determined by non-linear regression using Microsoft Excel 2013.

Statistical Analysis

All results were conveyed as means ± standard deviation. All graph/chart was drawn with Microsoft Excel 2013 package.

Results and Discussion

Physical properties of the essential oils

Leaf and stem essential oils (EOs) of *Homalium africanum* (Hook. F) Benth and *Shorea roxburghii* G. Don possessed characteristic aroma. Relatively low yields were obtained for the EOs [*H. africanum* (leaf 0.39%, stem 0.36% w/w), *S. roxburghii* (leaf 0.29%, stem 0.33% w/w)].

Chemical composition of essential oils

The phytochemical screening results showed that the methanol extract, ethyl acetate and hexane fraction from both plants contain saponins, flavonoids, terpenes, resins and steroids, which could be responsible for their pharmacological uses.[12,13] The chemical profiles of EOs from the leaves and stem of *H. africanum* and *S. roxburghii* are presented in tables 1 and 2, respectively and figures 1-4. GC-MS analysis revealed 28 and 16 compounds in the leaves and stem, respectively, for *H. africanum,* contributing 76.64% and 35.62% of its EOs, respectively. In the case of *S. roxburghii,* 42 compounds for leaf and 14 compounds for stem accounted for 73.99% and 76.64% of the EOs, respectively. The identified classes included non-terpenes/ terpenoids, monoterpenes, oxygenated mono-terpenes, sesquiterpenes, oxygenated sesqui-terpenes, diterpenes and triterpenes for both plants while *S*. *roxburghii* also contained oxygenated diterpenes.

To date, leaf EOs of *H. africanum* and *S. roxburghii* have not been characterized. The constituent of *H. africanum* leaf EO include benzaldehyde (43.43%), benzoic acid (4.67%), pentadecanal (3.02%), 3- buten-2-one (2.7%), 1,6,10-dodecatrien-3-ol (2.57%), α-ionone (2.46%) and methyl salicylate (2.33%). The oil was characterized by high content of non-terpenes/terpenoids (64.38%), sesquiterpenes (3.89%), oxygenated mono-terpenes (3.58%), monoterpenes (2.8%), diterpenes (1.24%), triterpenes (0.39%) and oxygenated sesqui-terpenes (0.38%). The stem of *H. africanum* was found to contain 2-hexanol (6.06%) as the most abundant constituent. The other major constituents were limonene (4.7%), 3-hexanol (4.6%), decane (3.94%), mesitylene (2.85%), cyclohexane (2.79%) and cyclotrisiloxane (2.16%). The oil was found to contain non-terpenes (31.06%) and monoterpenes (4.71%). The compositional profile of *H. africanum* leaf and stem EO showed that both oils contain limonene (0.24 and 4.7%, respectively).

The compounds in the leaf EO of *S. roxburghii* with high % composition include caryophyllene oxide (8.85%), 1H-cyclopropazulen-7-ol (8.62%), caryophyllene (6.61%), 1,5-cyclodecadiene (5.78%) and phytol (4.57%). The oil was characterized by hydrocarbon sesqui-terpenes (32.2%), oxygenated sesquiterpenes (25.79%), low content of non-terpenes (7.78%), oxygenated diterpenes (4.57%), diterpenes (1.38%), oxygenated monoterpenes (1.28%) and triterpenes (0.83%). The stem oil was found to contain phellandrene (36.02%) and limonene (26.16%) as its major constituents. Minor constituents identified include, 2-pinene (7.23%), β-myrcene (3.36%) and linalool (2.03%). The oil was found to comprise monoterpenes (74.38%), oxygenated monoterpenes (2.03%) and non-terpenes/terpenoids (0.23%). The compositional profile of *S. roxburghii* leaf and stem essential oils contained caryophyllene (6.61 and 0.08%), humulene (2.25 and 0.08%) and gamma-terpinene (0.16 and 0.08%), respectively.

Antioxidant assay

Tables 3 and 4 present the absorbance values from the free radical scavenging assay of the EOs of *H. africanum and S. roxburghii* at 10.0-0.0625 mg/mL and percentage inhibition values, respectively in comparison with the standard. The IC_{50} values are shown in figure 5 (supplementary data). The stem oil of *S. roxburghii* (SRS) displayed weak antioxidant activity (IC_{50} = 1.50 mg/mL), while its leaf oil (SRL) showed slightly lower activity (IC_{50} = 1.73 mg/mL) at concentration of 10mg/mL – 0.625 mg/mL. In contrast, the stem oil of *H. africanum* (HAS) exhibited the lowest activity (IC_{50} = 2.64 mg/mL) when compared to all the oil samples. The essential oil from *H. africanum* leaves (HAL) showed moderate antioxidant activity and an IC_{50} of 1.51 mg/mL at the same concentration range.

In terms of inhibition, the activity of the oils and standards decreased in the order: VIT C > BHA > HAL > HAS (Figure 4.1 (supplementary data)) and VIT C > BHA > SRS > SRL (Figure 4.2 (supplementary data)). The comparatively lower activity of the oils may be linked to the strength of their major compounds or interactions between major and minor constituents. According to Ogunlana *et al*,[26] samples rich in phenolic groups display stronger antioxidant effects than those with fewer phenolic moieties which may explain the reduced activity of the leaf and stem oils given their low phenolic content. Antioxidant capacity was also concentration dependent.

Table 1: Constituents of the leaf and stem essential oils of *Homalium africanum*

R_T (min)	Identified Compounds	% Area	
		Leaf	Stem
3.120	3-Hexanone	-	1.93
3.147	Cyclopentane	-	1.02
3.255	3-Hexanol	-	4.60
3.325	2-Hexanol	-	6.06
3.687	Cyclotrisiloxane	-	2.16
3.379	Cyclohexane	-	2.79
4.297	Octane	-	1.78
6.085	**Benzaldehyde**	43.53	-
6.415	Mesitylene	-	2.81
6.534	Decane	-	3.94
6.588	cis-2-(2-pentenyl) furan	1.06	-
6.917	3-ethyl-4-methylpentan-1-ol	1.17	-
7.009	Limonene	0.24	4.70
7.479	Benzene	-	0.77
8.133	Undecane	-	0.76
8.143	Linalool	0.80	-
9.591	methyl salicylate	2.33	-
10.245	Neral	0.10	-
10.655	Citral	0.19	-
11.039	Tridecane	-	1.26
11.757	Cubebene	0.11	-
12.119	Copaene, Naphthalene	1.25	0.22
12.303	Murrola-4(14),5-diene	0.19	-
12.363	Tetradecane	-	0.79
12.692	Caryophyllene	0.35	-
12.784	α-ionone	2.46	-
13.070	5,9-undecadien-2-one	1.98	-
13.519	3-Buten-2-one	2.70	-
13.610	Aromadendrene	-	0.18
13.670	Geranyl acetone	1.98	-
13.691	α-muurolene	0.14	-
13.751	α-farnescene	1.18	-
13.972	δ-cadiene	0.57	-
14.426	1,6,10-dodecatrien-3-ol	2.57	-
14.604	Benzoic acid	4.67	-
14.718	caryophyllene oxide	0.38	-
15.452	α-selinene	0.10	-
16.117	Pentadecanal	3.02	-
16.430	Geranyl Linalool	0.81	-
17.986	cis-7,10,13-Hexadecatriena	-	-
18.704	n-Hexadecanoic acid	-	-
19.925	Phytol	1.24	-
25.733	Squalene	0.39	-
26.197	Nonacosane	1.13	-
	Total Identified	**76.64**	**35.77**
	Hydrocarbon Monoterpenes	2.80	4.71
	Oxygenated Monoterpenes	3.58	-
	Hydrocarbon Sesquiterpenes	3.89	-
	Oxygenated Sesquiterpenes	0.38	-
	Hydrocarbon Diterpenes	1.24	-
	Triterpenes	0.39	-
	Non-terpenes/terpenoids	64.38	31.06

Table 2: Constituents of the leaf and stem essential oils of *Shorea roxburghii*

R_T (min)	Identified Compounds	% Area	
		Leaf	Stem
3.202	2-pinene	-	7.23
3.485	α-pinene	-	0.57
3.715	β –pinene	-	0.61
3.867	β –myrcene	-	3.36
4.085	**Phellandrene**	-	36.02
4.402	**Limonene**	-	26.16
5.335	Linalool	-	2.03
5.902	3-carene	-	0.05
6.442	γ-terpinene	-	0.09
6.622	2-carene	-	0.19
7.954	Fenchone	0.16	-
8.788	α-cubebene	-	0.09
11.595	Copaene	0.50	0.08
12.119	Credene	0.60	-
12.611	Funebrene	0.18	-
12.708	Caryophyllene	6.61	0.08
12.778	α-Ionone	2.17	-
12.865	γ –Elemene	4.76	-
12.994	Guaia-3,9-diene	0.59	-
13.065	Geranylacetone	1.12	-
13.135	Humulene	2.25	0.08
13.173	α-Patchoulene	1.54	-
13.221	Alloaromadendrene	0.64	-
13.400	γ –Muurolene	0.68	-
13.589	α-Gurjuene	0.22	-
13.664	Bicyclogermacrene	2.51	-
13.805	α-Guaiene	0.33	-
13.972	δ-Cadiene	1.63	-
14.129	γ –Selinene	0.54	-
14.350	Longifolene	0.58	-
14.215	α-Calacorene	0.73	-
14.410	1,5-cyclodecadiene	5.78	-
14.523	Aromadendrene	1.70	-
14.669	**1H-cyclopropazulen-7-ol**	8.62	-
14.734	**Caryophyllene oxide**	8.85	-
15.220	Alismol	1.48	-
15.274	Isocaryophyllene	0.43	-
15.323	(-)-spathulenol	1.75	-
15.409	α-vetivol	0.48	-
15.550	caryophylla-3,8(13)-dien-5β	0.16	-
15.652	δ-Selinene	0.31	-
15.695	Isoaromadendrene epoxide	1.59	-
16.203	Valerenol	1.24	-
16.765	Nootkatol	0.61	-
16.873	Guaiene	0.19	-
16.495	Vulgarol B	0.22	-
16.733	2-cyclohexen-1-one	1.00	-
17.408	Neophytadiene	1.38	-
17.478	2-pentadecanone	1.73	-
18.650	n-hexadecanoic acid	1.61	-
20.071	Phytol	4.57	-
25.732	Supraene	0.83	-
31.075	Friedeline	1.12	-

Total Identified	**73.99**	**76.64**
Hydrocarbon Monoterpenes	-	74.38
Oygenated Monoterpenes	1.28	2.03
Hydrocarbon Sesquiterpenes	32.20	0.42
Oxygenated Sesquiterpenes	25.79	-
Hydrocarbon Diterpenes	1.38	-
Oxygenated Diterpenes	4.57	-
Triterpene	0.83	-
Non-terpene derivatives	7.78	-

Table 3: DPPH scavenging activity of leaf and stem essential oils of *H. africanum* and *S. roxburghii*

Sample	Mean Absorbance at each concentration				
	10.000	5.000	2.500	1.250	0.625 mg/mL
HAL	0.692±0.003	0.707±0.002	0.735±0.001	0.728±0.002	0.71±0.000
HAS	0.810±0.001	0.860±0.002	0.876±0.003	0.88±0.002	0.88±0.002
SRL	0.727±0.009	0.749±0.004	0.775±0.009	0.783±0.002	0.797±0.001
SRS	0.693±0.009	0.736±0.004	0.755±0.009	0.767±0.001	0.78±0.002
VIT C	0.099±0.002	0.124±0.002	0.138±0.002	0.140±0.000	0.143±0.002
BHA	0.133±0.026	0.14±0.021	0.147±0.023	0.147±0.001	0.158±0.002

Absorbance values in mean±standard error; BHA = Butylated hydroxyl anisole, HAL = *Homalium africanum* (leaf), HAS = *Homalium africanum* (stem), SRL = *Shorea roxburghii* (leaf), SRS = *Shorea roxburghii* (stem), DDPH = 2,2-Diphenyl-1-picrylhydrazyl, VIT C = Vitamin C, BHA = Butylated hydroxyanisole

Table 4: Inhibition profile of essential oils of leaves and stem of *H. africanum* and *S. roxburghii*

Sample	% Inhibition at each concentration					IC_{50} mg/mL
	10.000	5.000	2.500	1.250	0.625	
HAL	31.300	29.800	27.000	27.700	30.000	1.510
HAS	19.600	14.600	13.000	13.300	13.300	2.640
SRL	27.800	26.300	23.000	22.900	21.600	1.730
SRS	31.900	27.000	25.000	24.500	23.200	1.500
VIT C	90.200	87.700	87.000	86.800	86.500	0.220
BHA	86.800	86.100	85.400	86.100	85.000	0.230

BHA= Butylated hydroxyl anisole, HAL = *Homalium africanum* (leaf), HAS = *Homalium africanum* (stem), SRL = *Shorea roxburghii* (leaf), SRS = *Shorea roxburghii* (stem), VIT C = Vitamin C, BHA = Butylated hydroxyanisole

Antimicrobial Assay

The antimicrobial profiles of the four essential oils (EOs) are presented in Tables 5 and 6. The results revealed that the leaf essential oil (EO) from *H. africanum* has a high inhibitory effect on the growth of some of the organism: *S. typhimurium* (MIC 0.39), *C. albicans* (MIC 0.78), *E. coli* (MIC 3.125) and *B. subtilis* (MIC 12.25 µg/mL) at lower concentrations except *S. aureus* which was inhibited at a very high concentration (50 µg/mL) when compared with the standard. The microbicidal effect of the leaf EO was active at lower concentration on *C. albicans* (MMC 1.56) and MMC 25 µg/mL on *E. coli*, *S. typhimurium* and *B. subtilis*.

Stem EO of *H. africanum* also showed a good inhibitory effect on *S. typhimurium* (MIC 0.39), *B. subtilis* (MIC 0.39), *C. albicans* (MIC 1.56) and *E. coli* (MIC 12.5 µg/mL) at a lower concentration except *S. aureus* which was inhibited at a very high concentration (50 µg/mL) when compared with the standard. The microbicidal effect of the stem EO was active on *B. Subtilis* (MMC 0.39),

C. albicans (MMC 3.125) and *S. typhimurium* (MMC 6.25 µg/mL) at lower concentrations. No inhibitory effect was observed on *P. aeruginosa*, *K. pneumonia* and *A. niger* by both leaf and stem oils of *H. africanum.*

The leaf EO from *S. roxburghii* possessed the highest inhibitory effect on the growth of all the organism tested, both bacterial and fungi except *A. niger* which showed no activity. The microbicidal effect of the leaf oil was observed at a very high concentration (25 – 50) µg/mL. This is not as active when compared with the standard. At a lower concentration 0.39 µg/mL, the stem EO from *S. roxburghii* showed a maximal inhibitory effect on the growth of *E. coli, S. typhimurium* and *B. subtilis*, while a high microbicidal effect was observed on *B. subtilis* at a lower concentration of 1.56 µg/mL. No inhibitory effect was observed on *P. aeruginosa*, *K. pneumonia* and *A. niger* by the stem oils of *S. roxburghii.*

From the obtained result, it can be noticed that all the EO showed a varied inhibitory and microbicidal effect against organism species tested. Highest inhibition was observed in leaf EO of *S. roxburghii* with most of the organism and no inhibitory and microbicidal effect was observed on the fungi *A. niger.*

Table 5: Minimum Inhibitory Concentration of the essential oils from *Homalium africanum* and *Shorea roxburghii*

Test Organisms	Negative Control	Positive Control		Concentration (µg/mL)			
		Gentamicin	Ketoconazole	HAL	HAS	SRL	SRS
Escherichia coli	-	5.000	NA	3.125	12.500	50.000	0.390
Pseudomonas aeruginosa	-	>10.000	NA	NA	NA	50.000	NA
Staphylococcus aureus	-	1.250	NA	50.000	50.000	25.000	50.000
Klebsiella pneumonia	-	10.000	NA	NA	NA	50.000	NA
Salmonella typhimurium	-	>10.000	NA	0.390	0.390	50.000	0.390
Bacillus subtilis	-	5.000	NA	12.250	0.390	3.125	0.390
Candida albicans	-	NA	0.500	0.780	1.560	0.780	25.000
Aspergillus niger	-	NA	0.500	NA	NA	NA	NA

NA: Not Active, DMSO = Dimethylsulfoxide, HAL = *Homalium africanum* (leaf), HAS = *Homalium africanum* stem, SRL = *Shorea roxburghii* (leaf), SRS = *Shorea roxburghii* (stem)

Table 6: Minimum Microbicidal Concentration (MMC) of the Essential oils from *H. africanum* and *S. roxburghii*

Test Organisms	Negative Control	Positive Control		Concentration (µg/mL)			
		Gentamicin	Ketoconazole	HAL	HAS	SRL	SRS
Escherichia coli	-	10.0	NA	25.0	25.0	50.0	12.5
Pseudomonas aeruginosa	-	>10.0	NA	NA	NA	50.0	NA
Staphylococcus aureus	-	5.0	NA	NA	NA	NA	NA
Klebsiella pneumonia	-	10.0	NA	NA	NA	NA	NA
Salmonella typhimurium	-	>10.0	NA	25.0	6.3	NA	25.0
Bacillus subtilis	-	5.0	NA	25.0	0.4	50.0	1.6
Candida albicans	-	NA	0.5	1.6	3.1	25.0	50.0
Aspergillus niger	-	NA	1.0	NA	NA	NA	NA

NA: Not Active, DMSO = Dimethyl sulfoxide, HAL = *Homalium africanum* (leaf), HAS = *Homalium africanum stem*, SRL = *Shorea roxburghii* (leaf), SRS = *Shorea roxburghii* (stem)

Brine shrimp lethality assay of essential oils

Toxicity was assessed using LC_{50} values based on Meyer's toxicity index which classifies extracts with LC_{50} < 1000 µg/mL as toxic and those with LC_{50} > 1000 µg/ml as non-toxic.[26] Consequently, both leaf and stem EOs exhibited high toxicity (LC_{50} values consistently below 1000 µg/ml).[27]

Toxicity level as determined by Finney computer programme, gave the following lethal concentrations: *H. africanum* (leaf), LC_{50} = 5.147 µg/mL, *H. africanum* (stem), LC_{50} = 3.465, *S. roxburghii* (leaf), LC_{50} = 4.693 µg/mL, and *S. roxburghii* (stem), LC_{50} = 5.767 µg/mL. The higher toxicity of EO of the stem of *H. africanum* and leaves of *S*. *roxburghii* could be due to the presence of limonene.[28]

Conclusion

This study, as the first comparative report on *H. africanum and S. roxburghii,* examined the essential oils profiles, antioxidant and antimicrobial properties of their leaves and stems. GC-MS analysis showed distinct qualitative and quantitative differences between plant parts. In *S. roxburghii,* terpenoids dominated with the hydrocarbon and oxygenated sesquiterpenes prevailing in the leaves, while the stem oil was rich in phellandrene and limonene. Conversely, *H. africanum* oils were mainly non-terpenoid in nature with benzaldehyde as the major leaf constituent, and limonene occurring in both leaf and stem. The EOs from the leaf of *S. roxburghii* recorded the highest inhibitory effect on the growth of all the organism tested, both bacterial and fungal, except *A. niger* while the stem essential oil showed a moderate inhibitory effect against some of the organism. The EOs from the leaves and stem of *H. africanum* recorded a moderate inhibitory activity against some bacterial and fungal strains: *E. coli, S. typhimurium*, *C. albicans* and *B. subtilis.* These moderate antimicrobial activitivies against some of the tested organisms supported their uses in ethno-medicine. The essential oils from both plants (leaf and stem) showed moderate antioxidant activity when compared to the IC_{50} of the standards (Vitamin C and Butylated hydroxyl anisole). Cytotoxicity result revealed that the essential oils from both plants are very toxic to brine shrimp larvae which support their use in treating degenerative diseases and could give some important lead in drug design.

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Conflict of interest

The authors declared no conflict of interest.

Authors' contributions

GKO was involved in the antioxidant assay, result interpretation, and writing (editing); PAO was involved in the conceptualization, all the laboratory work, result interpretation, and writing (editing); MAO carried out the sample collection, extraction of the essential oil, cytotoxicity, antioxidant assays, results interpretation, and manuscript write-up; MGI was involved in sample collection, extraction of the essential oil, cytotoxicity and manuscript write up; AAF, MAA, COO and OOO were involved in sample collection, extraction of the essential oil, all the laboratory work and result interpretation. All authors read and approved the final manuscript.

Supplementary data

Figures 1 – 6 are provided as supplementary data

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