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Research Article

Chemical Characterization, Antioxidant and Antimicrobial Activities of the Leaf Essential Oil of *Syzgium guineense* (Willd.) DC. var. Guineense (*Myrtaceae*) from Nigeria

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ABSTRACT

Syzgium guineense has long history of use as folklore medicine for various ailments by the people of Nigeria and other African countries. Investigation of this plant was carried out to evaluate antioxidant and antimicrobial properties of characterized essential oil of the leaf of *Syzgium guineense* (*S. guineense*). Essential oil obtained by hydrodistillation was characterized by gas chromatography coupled with mass spectrometry (GC-MS). The antioxidant potential was determined by measuring the inhibition of 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH). The inhibitory effects of the essential oil were tested against five bacteria: *Mycobacterium bovis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus*; and one fungus: *Candida albicans* using broth microdilution method. The GC-MS analysis revealed the presence of 46 components accounting for 92.63% of the essential oil constituents. Sesquiterpenoids (73.15%) and monoterpenoids (14.17%) were the main classes of the essential oil. Aromadendrene (6.98%), germacrene B (5.52%) and β-selinene (3.94%) were the predominant sesquiterpene hydrocarbons. The oxygenated sesquiterpenes were α-cadinol (6.68%), τ-cadinol (6.64%) and caryophyllene oxide (5.44%). Butylated hydroxytoluene (BHT) exhibited higher antioxidant activity compare to the essential oil. The essential oil exhibited strong antimicrobial activities against the tested microorganism with MIC ranging between 25-100µg/mL. Results indicated that the leaf essential oil of *S. guineense* had high proportion of sesquiterpenoids (73.15%) with strong antioxidant and antimicrobial activities.

Keywords: *Syzgium guineense*, essential oil, antioxidant, antimicrobial.

INTRODUCTION

Essential oils (EOs) are very interesting natural products and among other qualities they possess various biological properties, which can be harnessed for human health benefits[1]. Many plant essential oils are potent antimicrobials and are also strong antioxidant agents[2]. Antioxidants scavenge free radicals arising from chemical reactions involving reactive oxygen species (ROS), thereby preventing oxidative damage to cellular components. Scientific investigations have shown that antioxidant agents have potential to ameliorate oxidative stress and different human diseases including cancer, atherosclerosis and aging[3]. The exploration and exploitation of essential oils to combat pathogenic microorganisms and avoid the trauma of toxicity associated with the use of synthetic drugs, has open up new opportunities for new drug design and development for combating various infectious diseases[4]. Several essential oils from various medicinal and aromatic plants (MAPs) have been reported to possess a wide range of microbial inhibitory actions[5]. Essential oils are employed in aromatherapy and for the treatment of several diseases including cardiovascular disease, diabetes, Alzheimer's disease and cancer[6]. Therefore, the chemical characterization of EO is important for the understanding of its biological properties[2].

Syzygium guineense belongs to the family Myrtaceae. Myrtaceae comprises at least 133 genera and 3,800 species of woody shrubs to tall trees. The main genera are *Eucalyptus*, *Eugenia*, *Leptospermum*, *Malaleuca*, *Myrtus*, *Pimenta*, *Psidium* and *Syzygium*[7]. Several *Syzygium* species have been reported to possess antibacterial, antifungal and antiinflammatory activities[8].

Syzygium guineense is the most widely spread and abundant *Syzygium* in Nigeria. In northern Nigeria, it is called *Malmo* in Hausa, *Sumsum* in Fulfulde while in southern Nigeria it is called *Adere* in Yoruba. The plant is a leafy forest tree found in many parts of Africa with long history of use as herbal medicine. The flowers are often attacked by gall insects causing the inflorescence to develop into a densely-branched compact heads. The variety *guineense* may be distinguished by the loosely

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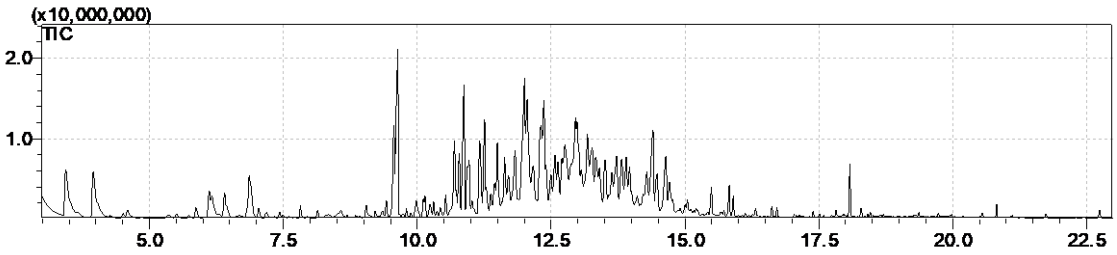

Figure 1: Chromatogram of leaf essential oil composition of *S. guineense.*

Table 1: Chemical constituents of of *S. guineense* leaf essential oil as analysed by GC-MS.

S/N	Compound[a,b]	MF[c]	RT[d]	% Composition[e]
1.	α-pinene	$C_{10}H_{16}$	3.436	2.58
2.	β-pinene	$C_{10}H_{16}$	3.948	3.30
3.	D-Limonene	$C_{10}H_{16}$	4.504	0.18
4.	Pinocarveol	$C_{10}H_{16}O$	6.113	1.00
5.	Pinocarvone	$C_{10}H_{14}O$	6.400	1.37
6.	Myrtenal	$C_{10}H_{14}O$	6.868	2.55
7.	Verbenone	$C_{10}H_{14}O$	7.039	0.33
8.	Citral	$C_{10}H_{16}O$	7.818	0.35
9.	Undecan-2-one	$C_{11}H_{22}O$	8.139	0.23
10.	α-cubebene	$C_{15}H_{24}$	9.045	0.57
11.	(-)-β-bourbonene	$C_{15}H_{24}$	9.563	2.71
12.	Germacrene B	$C_{15}H_{24}$	9.634	5.52
13.	γ-muurolene	$C_{15}H_{24}$	9.982	0.81
14.	2-Tridecanone	$C_{13}H_{26}O$	10.786	1.78
15.	β-selinene	$C_{15}H_{24}$	10.873	3.94
16.	β-guaiene	$C_{15}H_{24}$	10.965	2.83
17.	γ-cadinene	$C_{15}H_{24}$	11.167	2.98
18.	α-calacorene	$C_{15}H_{20}$	11.444	1.19
19.	Guaiadiene	$C_{15}H_{24}$	11.634	2.63
20.	Alloaromadendrene oxide	$C_{15}H_{24}O$	11.827	3.71
21.	Aromadendrene	$C_{15}H_{24}$	12.002	6.98
22.	(-)-globulol	$C_{15}H_{26}O$	12.314	4.53
23.	Caryophyllene oxide	$C_{15}H_{24}O$	12.365	5.44
24.	α-cedrene	$C_{15}H_{24}$	12.573	2.42
25.	τ-cadinol	$C_{15}H_{26}O$	12.752	6.64
26.	α-cadinol	$C_{15}H_{26}O$	12.955	6.68
27.	1,8-cineole	$C_{10}H_{18}O$	13.508	2.51
28.	δ-cadinol	$C_{15}H_{26}O$	13.723	3.24
29.	Isoshyobunone	$C_{15}H_{24}O$	13.817	2.81
30.	Longiverbenone	$C_{15}H_{22}O$	14.217	1.18
31.	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	$C_{15}H_{24}O_2$	14.643	3.10
32.	6-(1,3-Dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0] hept-2-ene	$C_{15}H_{22}O$	14.708	2.07
33.	Aciphyllene	$C_{15}H_{24}$	15.003	0.45
34.	1-methyl-4-isopropyl-7,8-dihydroxy-,(8S), Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane	$C_{15}H_{24}O_3$	15.422	0.23
35.	Benzyl salicylate	$C_{14}H_{12}O_3$	15.828	0.84
36.	Hexadecan-1-ol	$C_{16}H_{34}O$	15.901	0.50
37.	Farnesyl acetone	$C_{18}H_{30}O$	16.319	0.22
38.	Neryl (S)-2-methylbutanoate	$C_{15}H_{26}O_3$	16.622	0.26
39.	Farnesyl acetate	$C_{17}H_{28}O_2$	17.328	0.01
40.	1-Nonadecanol	$C_{19}H_{40}O$	17.821	0.19
41.	Phytol	$C_{20}H_{40}O$	18.080	1.04

42.	Pregnanlone	$C_{21}H_{32}O_2$	18.291	0.22
43.	(Z)-9-Octadecenamide	$C_{18}H_{35}NO$	19.733	0.10
44.	2-methylhexacosane	$C_{27}H_{56}$	20.553	0.10
45.	Nonacosane	$C_{29}H_{60}$	21.743	0.09
46.	Squalene	$C_{30}H_{50}$	22.743	0.22
Non-terpenoids				4.05
Monoterpene hydrocarbons				6.06
Oxygenated monoterpenes				8.11
Sesquiterpene hydrocarbons				33.03
Oxygenated sesquiterpenes				40.12
Oxygenated diterpenes				1.04
Triterpene hydrocarbons				0.22
Total identified				92.63

[a]Compounds listed in order of retention time (RT) from a HP-5ms column; [b]identification; [c]molecular formula; [d]retention time in minutes, GC-MS, gas chromatography-mass spectroscopy; [e]components percentage composition were calculated from peak areas.

branched terminal inflorescences and rather small grayish fruits, which are not edible[9]. Leaf decoction of *S. guineense* is taken as remedy for intestinal parasites and stomach-ache, used as an enema against diarrhoea, and as an embrocation to bathe and then massage into areas of sprain. Leaf decoction or pulverized leaves is given as tonic to pregnant women. The fruit is used for treating dysentery[10]. The plant is used in the treatment of inflammatory diseases and diabetes in Nigeria[11]; management of HIV/AIDS opportunistic infections particularly in the treatment of *Herpes zoster* in Namibia and used as antimalaria in Uganda[12]. In Ethiopia, the leaf extract is used in the treatment of measles, eye disease and also for wound dressing[13].

The dried leaf essential oil of *S. guineense* harvested from Benin had been reported[14]. The oil mainly contained α-humulene (39.5%), β-caryophyllene (20.1%), citronellyl pentanoate (15.2%) and *cis*-calamenen-10-ol (14%). It was also noted that none of the chemical compositions reported in Benin comes close to that reported in Gabon, in which δ-guaiene was preponderant (30%)[14]. Chemical components of the essential oil from the buds and leaves of other species and variant of *Syzygium* had been reported by several workers[15 -17]. The major components of the essential oil obtained from the buds of *Syzygium aromaticum* were eugenol (71.56 %) and eugenol acetate (8.99 %)[15]. Essential oil obtained from *Syzygium caryophyllatum* was reported to contain eugenol (74.3%), eucalyptol (5.8%) and caryophyllene (3.85%) as its main constituents[16]. Essential oils from the leaves of *Syzygium malaccense, S. samarangense* and the buds of *S. aromaticum* contained limonene (48.8%), α-cardinol (12.7%) and eugenol (78.5%) as the major constituents respectively[17]. Hydrodistilled essential oil from fresh leaves of *Syzygium malaccense* grown in Nigeria largely composed of monoterpenes (61.1%) characterized mainly by (+)-α-pinene (7.3%) and sesquiterpenes (30.8%) with (-)-β-caryophyllene (9.0%)[18]. A total of 84 compounds were identified from the fresh leaf oil of *Syzygium densiflorum* among which β-maaliene (17.43%), isoledene (12.46%) and α-gurjunene (10.44%) were the major constituents[19]. Similarly, essential oil from the leaf of *Syzygium gardneri* was reported to contain 20 compounds with caryophyllene oxide (49.6%) as the main constituent[20]. About 80 volatile compounds were identified from *Syzygium jambos* from Reunion Island, which included 12 hydrocarbons, 8 oxides and acetals, 12 aldehydes, 9 ketones, 5 esters, 31 alcohols and 3 acids[21]. The major constituents of the oil from the leaves of *Syzygium cumini* were α-pinene (32.32%), β-pinene (12.44%) and caryophyllene (11.19%)[22].

The antioxidant activities of *S. guineense* extracts had been reported[23]. Antioxidant activities were reported for some Syzygium species including *Syzygium cumini*[24], *S. aqueum*[25], *S. malaccense, S. samarangense* and *S. aromaticum*[17].

Antimicrobial activities of the essential oil of some Syzygium species had been reported[4-6,26]. Triterpene constituents from the leaves of *S. guineense* showed the most significant antibacterial activities against *Escherichia coli, Bacillus subtilis* and *Shigelli sonnei*[27]. The aqueous extracts of the leaf and stem barks of *S. guineense* markedly inhibited the growth of *Staphylococcus epidermidis, Staphylococcus aureus* and *Shigella dysentariae*, and moderately inhibited that of *Yersinia enterocolitica, Salmonella sp., Serratia marcescens, Pseudomunas aeruginosa* and *E. coli*[28]. *Syzygium aromaticum* essential oil and its main component eugenol showed inhibitory activity against *Candida, Aspergillus* and dermatophyte species. They caused a considerable reduction in the quantity of ergosterol, a specific fungal cell membrane component[29].The essential oil from *S. cumini* leaf was reported to moderately inhibit the growth of some bacterial strains[22].

The leaf methanol extract of *S. guineense* harvested from Ethiopia had inhibitory activities against *Neisseria gonorrhea, Streptococcus pyogenous, S. pneumonia, Staphylococcus aureus* and *Shigella dysentriea*[13]. The ethanolic and aqueous root extracts of *Syzygium guineense* inhibited *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Salmonella typhi* at 20 mg/ml. In another study, the root bark showed antimycobacterial activity with minimum inhibitory concentration ranging from 800µg/ml to 2000µg/ml[12]. The ethanol leaf extract of *S. guineense* possessed anti-inflammatory and analgesic activities[9]. The antimicrobial activities of the leaf methanolic extracts of

Table 2: Antioxidant activity of essential oil of *S. guineense* and BHT.

Concentration (µg/ml)		1	5	10	15	20	25	30	40	50	80	100
% Radical scavenging activity	Essential oil	3	7	13	18	23	27	30	36	40	50	70
	BHT	5	12	21	32	39	43	49	55	70	80	90

Syzygium forte, *S. francissi*, *S. moorei*, *S. puberulum* and *S. wilsonii* had been reported[30]. The inhibitory activities of *Syzygium australe* and *S. leuhmannii* fruit methanol extracts were tested against 14 bacteria. Both Gram-positive and Gram-negative bacteria were susceptible, although a slightly greater susceptibility of Gram-positive bacteria was noted[31]. *Syzygium alternifolium* and *S. samarangense* fruits extracts exhibited significant antimicrobial activities on certain pathogens[8].

The objective of this study was to determine the chemical composition, antioxidant and antimicrobial properties of the leaf essential oil of *Syzygium guineense* from northern Nigeria.

MATERIALS AND METHODS

Plant material

Fresh green leaves of *Syzygium guineense* were collected in August 2017 from Suleja, Niger State, Nigeria. The plant was identified and authenticated by a taxonomist at the Herbarium of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria, where a voucher specimen with number NIPRD/H/6644 was deposited. The leaves were dried at room temperature (25 ^{o}C – 30 ^{o}C).

Extraction of the essential oil

Essential oil was obtained from air-dried plant material by hydrodistillation method employing Clavenger-type apparatus. The extraction was carried out for a 4-hours period. The essential oil obtained was dried over anhydrous sodium sulphate (Sigma), filtered through membrane filter with pore size of 0.22 microns and stored in a dark sealed glass bottle at 4 °C until used for analyses.

GC-MS analysis

The essential oil was analysed by GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and Shimadzu GCMS solution data system. The GC column was HP-5MS fused silica capillary with a 5% phenyl-polymethylsiloxane stationary phase, length 30 m, internal diameter 0.25 mm and film thickness 0.25 µm. The program used for GC oven temperature was isothermal at 60 ^{o}C, increased from 60 ^{o}C to 180 ^{o}C at a rate of 10 ^{o}C/min, then held at 180 ^{o}C for 2 minutes; increased from 180 ^{o}C to 280 ^{o}C at a rate of 15 ^{o}C/min, then held at 280 ^{o}C for 4 minutes. The injection port temperature was 250 ^{o}C. The ionization of sample components was performed in the electron impact mode (70eV). Injector temperature was 250 ^{o}C while detector temperature was 280 ^{o}C. Helium was used as carrier gas at a flow rate of 1.61 ml/min. 1.0 µl of diluted essential oil (1/100 in hexane, v/v) was injected using autosampler. Split ratio was 10:90[32].

Qualitative and quantitative analysis

Components of the essential oil were identified by searching NIST Mass Spectral Library (NIST 11) and referring to compounds known in literature[33]. The percentage of each component was reported as raw percentage based on the total ion current without standardization. The essential oil constituents of *S. guineense* leaf are shown in Table 1.

Radical scavenging activity using DPPH method

The antioxidant activity of essential oil of *S. guineense* was evaluated by spectrophotometric method using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging activity assay. Fifty microlitres of various concentrations of the essential oil (1, 5, 10, 15, 20, 25, 30, 40, 50, 80 and 100 µg/mL) were added to 5 mL of a 0.004% methanol solution of DPPH. Tests were carried out with the essential oil and reference antioxidant, butylated hydroxytoluene (BHT) in concentrations ranging from 1 to 100 µg/ml. The DPPH test was based on the ability of the essential oil to donate radical hydrogen to neutralize the DPPH radical. When DPPH reacted with the essential oil, it was reduced with colour of solution changing from deep violet to light-yellow. The absorbance was measured at 517 nm on a visible light spectrophotometer. The percentage of DPPH radical scavenging capacity of the essential oil was calculated as follows:

DPPH radical scavenging capacity (%) = $[(Abs_{blank} - Abs_{essential\ oil})/Abs_{blank}]x100$

Where Abs_{blank} is the absorbance of the blank sample (time = 30 min) and $Abs_{essential\ oil}$ is the absorbance of the essential oil sample (time = 30 min)[34].

Antimicrobial Activity

Bacterial and fungal strains, culture conditions and preparation of sample

The following microorganisms were used in the evaluation of the antibacterial activity of the essential oil: Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923); Gram-negative bacteria, *Escherichia coli* (ATCC 10798), *Klebsiella pneumonia* (ATCC13883), *Pseudomonas aeruginosa* (ATCC 27853); fungi: *Candida albicans* (ATCC 2876); and *Mycobacterium bovis* BCG (ATCC 35737). These strains were procured from the American Type Culture Collection (ATCC, USA).

Overnight broth cultures of the test organisms were diluted to10^{7} cfu/ml and monitored by spectrophotometric methods as described by Dominguez et al. (2001)[35]. Briefly, two to three colonies of 20 h growth of the organisms to be studied grown on Mueller-Hinton Agar were suspended in 50 ml pre-warmed (37°C) Mueller-Hinton broth. The suspension was incubated overnight at 37°C, diluted to 1/2500 in the same pre-warmed medium and incubated in water bath with agitation (50 rpm). The absorbance of the culture was monitored with a

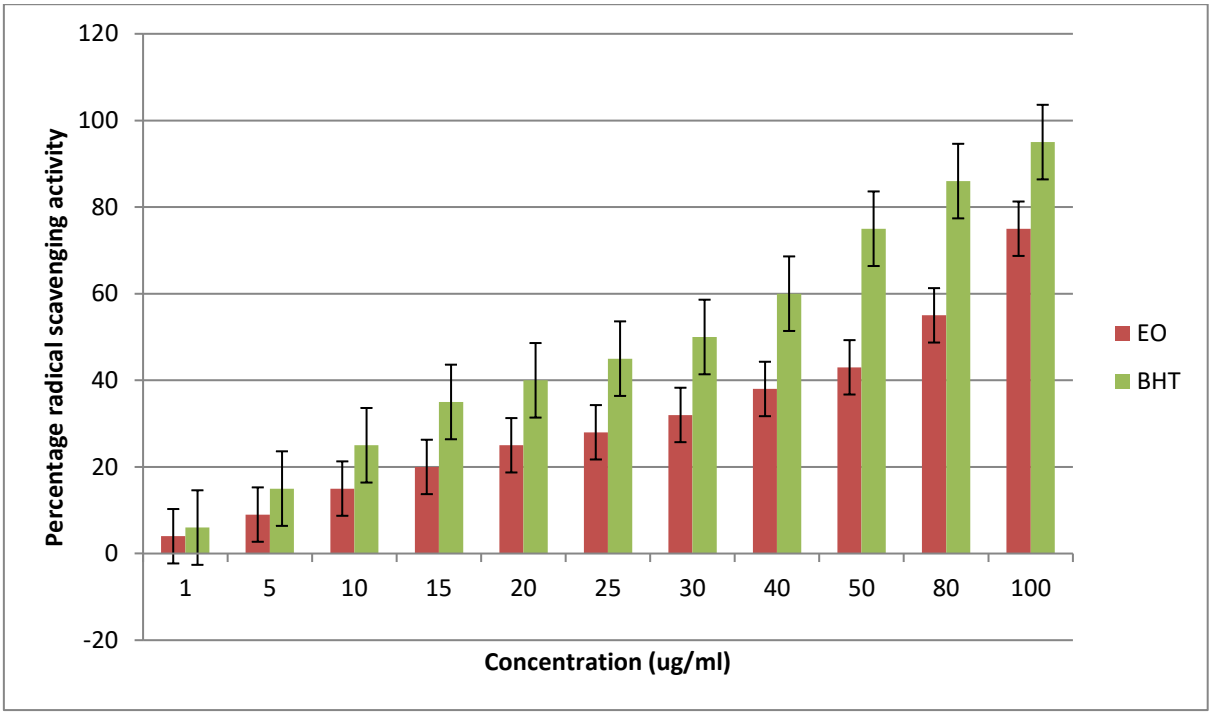

Figure 2: A graphical representation of the percentage radical scavenging activity of the leaf essential oil of *S. guineense* (EO) and BHT.

spectrophotometer (6405 Jenway, Barloworld Scientific Ltd. Dunmow, Essex CMB 3LB), using 450 nm wavelength and 1 cm cuvette until absorbance of 0.1 was reached (equivalent to 2.5-3.0 × 10^7cfu/ml for *E. coli* and 1.8-2.0 × 10^7cfu/ml for *S. aureus* and *B. subtilis*, respectively). The experiment was done in duplicates.

Antibacterial and antifungal screening

The minimum inhibitory concentration (MIC) values of the essential oil was determined by

Micro-broth dilution method in 96-well microplates[36]. The essential oil was dissolved in dimethyl sulfoxide (DMSO) followed by addition of sterile Mueller-Hinton nutrient broth for bacteria and Sabouraud-Dextrose nutrient broth for fungi, to achieve concentration of 200 µg/ml. The final DMSO concentration was 20% (v/v) and this solution was used as a negative control. The inoculum was adjusted for each organism to yield a cell concentration of 2 × 10^7 colony forming units (cfu) per ml. Ciprofloxacin (Fidson, Nigeria) was used as a positive control for bacteria and Fluconazole (Pfizer, UK) was used as the standard drug for fungi at stock concentration of 50 µg/ml. Controls of sterility for the Mueller-Hinton nutrient broth, control culture (inoculum), Ciprofloxacin, Fluconazole, essential oil and DMSO were performed. The microwell plates were closed and incubated aerobically at 37°C for 24 h. Post-incubation, to the plates were added 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and incubated for another 2 h to indicate colour change in wells where there was no activity. Minimum inhibitory concentration (MIC) was defined as lowest concentration of the essential oil at which reddish coloration was not observed. All assays were carried out in triplicate[32]. Results are shown in Table 2.

Antimycobacterial Assay

Determination of antitubercular activity was carried out on *Mycobacterium bovis* BCG (ATCC 35737) using the broth dilution method previously described[32]. *Mycobacterium bovis* BCG cells were grown to an optical density of 0.2-0.3 at 650 nm in 7H9/ADC/Tween consisting of Middlebrook 7H9 broth supplemented with 0.5% bovine serum albumin fraction V, 0.08% NaCl, 0.2% glucose, 0.2% glycerol and 0.05% Tween 80. The essential oil sample was dissolved in dimethyl sulfoxide (DMSO), centrifuged for 20 minutes at 13,000 rpm, followed by addition of sterile 7H9/ADC/Tween to achieve concentration of 200 µg/ml solution. The final DMSO concentration was 4% (v/v) and this solution was used as a negative control. 50 µl of media was introduced into wells 2 to 12 of a 96-well micro-titre plate, while 100 µl of sample (200 µg/ml) was delivered into the first well of the 96-well plate. Two-fold dilutions were performed by sequential transfer of 50 µl of each sample from well 1 to 2; after thorough mixing, 50 µl was transferred from well 2 to 3. The process was repeated through to well 11 where 50 µl was discarded. 50 µl of inoculum prepared by diluting a 5-7 day old culture of *Mycobacterium bovis* BCG (OD 0.2-0.3) 1:1000 (by adding 50 µl of cell culture into 50 ml 7H9/ADC medium) was added to all the wells and incubated for 14 days at 37°C, after which the growth or inhibition of growth was read by direct recording of visual growth. All of the minimum inhibitory concentration (MIC) determinations were done in duplicates. Post incubation was done to indicate respiratory activity. To the plates were added 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and incubated for another 2 h to indicate colour change in wells where there was no activity. MICs were defined as lowest concentration of essential oil at which the red formazan of

Table 3: MIC of essential oil of *S. guineense.*

S/N	Microorganisms	Minimum inhibitory concentrations	
		Essential oil (µg/ml)	Standard drug (µg/ml)
1*	*Pseudomonas aeruginosa* (ATCC 27853)	50	0.05
2*	*Klebsiella pneumonia* (ATCC 13883)	100	0.39
3*	*Escherichia coli* (ATCC 10798)	100	0.39
4*	*Staphylococcus aureus* (ATCC 25923)	100	0.10
5**	*Candida albicans* (ATCC 2876)	25.5	6.25
7***	*Mycobacterium bovis* BCG (ATCC 35737)	100	0.07

**Bacterial strain; **fungal strain; ***mycobacterium strain. Ciprofloxacin (standard drug for bacterial strains); fluconazole (standard drug for fungal strain); isoniazid (standard drug for mycobacterium strain)*

MTT was not observed. Isoniazid was used as positive control. Results are shown in Table 2.

Statistical analysis

All data were reported as mean of independent replicates. Data were analyzed by an analysis of variance (ANOVA). $P<0.05$ was considered as significant level.

RESULTS AND DISCUSSION

Chemical composition of essential oil

Hydrodistillation of *S. guineense* leaf produced light yellow essential oil with yield of 1.7% (w/w), based on the dried weight. The gas chromatography profile of essential oil is shown in Figure 1.

The qualitative and quantitative composition of the essential oil analyzed by GC-MS resulted in the identification of 46 different compounds, representing 92.63% of the total essential oil constituents. The essential oil components consisted of non-terpenoids (4.05%), monoterpene hydrocarbons (6.06%), oxygenated monoterpenes (8.11%), sesquiterpene hydrocarbones (33.03%), oxygenated sesquiterpenes (40.12%), oxygenated diterpenes (1.04%) and triterpenes hydrocarbon (0.22%). The identified components are listed with their retention time; molecular formula and percentage composition (Table 1).

The *S. guineense* essential oil consisted mainly of sesquiterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated monoterpenes and monoterpene hydrocarbons. Aromadendrene (6.98%), germacrene B (5.52%) and β-selinene (3.94%) were the predominant sesquiterpenes hydrocarbons. Moderate quantities of γ-cadinene (2.98%), β-guaiene (2.83%) and (-)-β-bourbonene (2.71%) were also identified. The oxygenated sesquiterpenes were characterized as α-cadinol (6.68%), τ-cadinol (6.64%) and caryophyllene oxide (5.44%). Alloaromadendrene oxide (3.71%) and δ-cadinol (3.24%) were also present. Myrtenal, 1,8-cineole and pinocarvone dominated the oxygenated monoterpenes in the essential oil. β-pinene (3.30%) and α-pinene (2.58%) were the identified monoterpene hydrocarbons. Phytol (1.04%) was the only oxygenated diterpenes identified in the oil. Other compounds, including triterpenoids and non-isoprenoids were detected in lower quantities (<1.8%), as shown in Table 1.

Antioxidant activity

The antioxidant activity of *S. guineense* was tested using DPPH free radical scavenging method by comparing with the efficacy of an established antioxidant agent BHT. The result of the scavenging capacity of the essential oil and BHT are shown in Table 2.

DPPH antioxidant assay involves the abstraction of hydrogen by the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) from the antioxidant molecule, leading to decolorization of the DPPH. The degree of decolorization of DPPH is a measure of the free radical scavenging capacity of the sample under study. In this study, BHT exhibited higher antioxidant activity compared to the essential oil. However, the scavenging potential of *S. guineense* oil was concentration dependent. The essential oil can play a vital role in alleviating oxidative stress conditions in different diseases such as liver, renal, neurodegenerative and cardiovascular diseases, cancer, diabetes as well as ageing processes[37].

Antimicrobial effect

The inhibitory activities of the leaf essential oil of *S.guineense* against bacteria and fungi were evaluated using broth micro-dilution method. The oil showed significant activities against the microorganisms as shown in Table 3.

The *S. guineense* leaf essential oil consisted of monoterpenes, sesquiterpenes, non-terpenes, diterpenes and triterpenes. Sesquiterpenoids (73.15%) consisting of oxygenated sesquiterpenes (40.12%) and sesquiterpene hydrocarbons (33.03%) were the predominant classes among the identified components. Other classes of the essential oil constituents were monoterpenoid (14.17%), oxygenated monoterpenes (8.11%) and monoterpene hydrocarbons (6.06%). Among the essential oils obtained from Syzygium species, *S. samarangense* resembled the present report based on the pattern of oxygenated sesquiterpenes (40.2%), sequiterpene hydrocarbons (27.2%), oxygenated monoterpenes (11.1%), monoterpene hydrocarbons (6.6%) and oxygenated diterpenes (0.7%)[38]. The monoterpenes found in *S. guineense* essential oil included acyclic monoterpenes (citral), monocyclic monoterpenes (D-limonene) and bicyclic monoterpenes (α-pinene, β-pinene, myrtenal and 1,8-cineole). The sequiterpenes being the most abundant constituent comprised of acyclic sesquiterpenes (Neryl-(S)-2-methyl butanoate, fernesyl acetone and fernesyl acetate); monocyclic sesquiterpenes (germacrene B and isoshybunone), bicyclic sesquiterpenes (α-cadinol, τ-cadinol, δ-cadinol, caryophyllene oxide, β-selinene and 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-

octahydronaphthalene-2,3-diol) and tricyclic sesquiterpenes (aromadendrene, (-)-globulol, allo-aromadendrene, (-)-β-bourbonene and α-cedrene). Acyclic diterpene alcohol (phytol) and acyclic triterpene (squalene) were among the terpene constituents present in the essential oil.

Aromadendrene, a tricyclic sesquiterpene hydrocarbon (6.98%) was the main essential oil constituent of *S. guineense*, followed by α-cadinol (6.68%), τ-cadinol (6.64%) and germacrene B (5.54%). The predominant monoterpenes included β-pinene (3.3%), α-pinene (2.58%) and myrtenal (2.55%). The predominant essential oil constituent reported in the Republic of Benin *S.* guineense included α-humulene, β-caryophyllene, citronellyl pentanoate and cis-calamenen-10-ol. These were not detected in the present study. Guaiene (30%), the prominent constituent in Gabon sample essential oil was not identified in the present study[39]. The reasons for these variabilities may be the different geographical sources, the harvesting seasons, the genotype, the climate, the drying procedure and the developmental stages. These variables influence the relative composition of essential oil[40]. The essential oil in sample from Benin contained 46 compounds accounting for 91.8% of the total constituent, which is very similar to 92.63% obtained in the present study. Other constituents of the oil from Republic of Benin, such as α-cadinol (12.7%) and caryophyllene oxide (5.5%), were in agreement with the present result[39]. The leaf essential oil chemical constituents of *S. guineense* from Nigeria varied from a large number of other *Syzygium* species collected from Nigeria, in which the major components were limonene (48.8%) and γ-terpiene (26.2%) from *S. malaccense*; α-cedrol (12.7%) and juniper camphor (12.5%) from *S. samarangense*; eugenol (78.5%) and eugenyl acetate (13.3%) from *S. aromaticum*[38]; β-maaliene (17.43%) and isoledene (12.46%) from *S. densiflorum*[41]; and caryophyllene oxide (94.7%) obtained from *S. gardneri*[42].

The antioxidant activity of the essential oil evaluated using DPPH method revealed a moderate scavenging potential ranging from 3-70%. The result of this study is in agreement with previous report which showed that the hydro-ethanol extract of the leaves of *S. guineense* moderately scavenge DPPH radicals[43]. Oxygenated monoterpenes have been found to have good antioxidant activity[44]. Sesquiterpene hydrocarbons and their oxygenated derivatives have very low antioxidant activity[45]. The oxygenated monoterpenes such as 1,8-cineole, myrtenal, citral and pinocarvone may have contributed to the total antioxidant activities of the essential oil[46]. The oxygenated bicyclic monoterpene myrtenal was reported in a similar research to have an excellent free radical scavenging activity and anticancer activity[47]. D-limonene exhibited remarkable antioxidant potential[48].

Antimicrobial activity of plant based products can be classified according to their MIC results as strong inhibitors (MIC below 500 μg/mL); moderate inhibitors (MIC between 600 and 1,500 μg/mL); weak inhibitors (MIC above 1,600 μg/mL)[40]. As shown in Table 3, *S. guineense* essential oil exhibited strong antimicrobial activities against the tested microorganisms with MIC range of 25-100μg/mL. The oil showed the most potent antimicrobial activity against *Candida albicans* with MIC value of 25.5μg/ml. *Candida* infections are very common causing oral, vaginal and/or systemic candidiasis. Oropharyngeal candidiasis is frequently encountered in AIDS patients who do not have access to highly active antiretroviral therapy (HAART), whereas oral candidiasis often affects cancer patients undergoing chemotherapy and/or radiotherapy[49].

The observed bioactivities of the essential oil may be attributable to possible synergistic interactions between components[50]. The observed antimicrobial activities might due to the oxygenated terpenes (49.27%) and aromadendrene, which is in agreement with previous report[51], in which aromadendrene inhibited the growth of methicillin-resistant staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) *Enterococcus faecalis*. The possible synergic interactions among some of the essential oil components have been reported[52 - 54]. The anti-candidiasis activity of the oil may be due to the presence of α-pinene which was reported previously to be active against *Candida albicans*[55].

CONCLUSION

The GC-MS analysis of *S. guineense* leaf essential oil revealed the presence of 46 components accounting for 92.63% of the essential oil constituents many of which are biologically active. Some of the compounds have antioxidant, anti-inflammatory, antifungal and antimicrobial properties. *S. guineense* essential oil exhibited strong antimicrobial activities against the tested microorganisms with MIC range of 25-100μg/mL.

Conflict of interest statement

The authors declare no conflict of interest.

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