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RESEARCH ARTICLE

Evaluation of chemical profiles and biological properties of *Gliricidia sepium* (Jacq.) Walp. volatile oils from Nigeria

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Abstract

Volatile oils obtained by hydrodistillation from *Gliricidia sepium* (Jacq.) Walp. leaf and stem were examined for their chemical composition and biological activity. The oils were analyzed by gas chromatographic-mass spectrometric (GC-MS) techniques. Toxicity, antimicrobial and antioxidant activities were evaluated via brine shrimp lethality assay, agar-diffusion, and DPPH radical-scavenging methods, respectively. A total of 43 and 44 constituents were identified in the leaf and stem oils, correspondingly. The major components recognized in the leaf oil were (*E*)-hexadecatrienal (16.9%) and pentadecanal (16.0%) while humulene epoxide II (17.5%) and caryophyllene oxide (10.6%) dominated the stem oil. There was no significant activity against the bacteria but moderate inhibition zones (IZ) between 1.8±0.3 and 8.1±0.1 mm were observed against the fungi. The stem oil showed better antifungal activity than the leaf oil but not as active as the standard drug ketoconazole which inhibited the test fungi with IZ range of 10.4±0.4-21.0±1.4 mm at 200 µg. Both oils were toxic to brine shrimp (*Artemia salina*) giving LC_{50} of 79.7 µg/mL (leaf) and 38.7 µg/mL (stem). The leaf and stem oils had IC_{50} of 84.3 µg/mL and 142.2µg/mL, respectively, in the DPPH radical-scavenging assay, indicating moderate antioxidant activity relative to positive controls, butylated hydroxylanisole (IC_{50}=45.1 µg/mL) and α-tocopherol (IC_{50} =81.6 µg/mL). The results suggest that *G. sepium* volatile oil may find potential use as a natural antioxidant and antifungal agent.

Keywords: *Gliricidia sepium*; volatile oils; chemical compositions; biological activities

Introduction

Investigations of chemical and biological properties of medicinal plants have been a great help to pharmacists in dealing with the global health challenges mostly caused by microbial infections and oxidative deterioration. *Gliricidia sepium* (Jacq.) Walp. (Fabaceae), commonly known as quick stick, is a multipurpose plant that is easily propagated and drought tolerant. It provides wood fuel, animal feed, green manure, shade, plants support and used in folk medicine as fumigant, mosquito repellent, rat control, antiviral, dysentery cure, CNS depressant and wound dressing (Asolkar et al., 1992; Csurhes & Edward, 1998; Gupta, 1995; Kumar & Simon, 2016).

The leaf, which contains sufficient amounts of crude protein and minerals with its ability to thrive during dry season make the plant a good food supplement for livestock (Aye & Adegun, 2013). Reported pharmacological activities include nematicidal (Nazli et al., 2008), larvicidal (Jiby et al., 2015), antibacterial (Akharaiyi et al., 2012; Sukumar & Apama, 2014) and anti-inflammatory (Kumar et al., 2014).

Previously isolated compounds include gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, gentisic acid, β-resorcyclic acid, vanillic acid, syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, ferulic acid, sinapinic acid (*Z* and *E*), coumarin, myricetin, protocatechuic acid, stigmasterol glucoside and 7,2'' -dihydroxy-6,4''-dimethoxyisoflavone from the heartwood and leaves extracts (Ramamoorthy & Paliwal, 1993; Herath & deSilva, 2001).

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The purpose of this work is to investigate the chemical compositions and the biological properties of the volatile oils hydrodistilled from the leaves and stem of *Gliricidia sepium* from Nigeria. Biological activities, i.e., antimicrobial activity, free radical-scavenging potential, brine shrimp toxicity, and the chemical composition have not been reported before.

Materials and Methods

Sample preparation

Gliricidia sepium was collected at Saunders road, University of Ibadan (7° 23' 0.2" N/3° 54' 28.8" E) and authenticated at Forestry Research Institute of Nigeria where a voucher specimen was deposited (FHI 112496). The plant parts (leaves and stem) were air dried and pulverized. The pulverized samples were separately hydrodistilled for 3 h using an all-glass Clevenger-type apparatus, according to the British Pharmacopeia specification (1980). The oils were dried over anhydrous sodium sulfate and kept in sealed glass vials under refrigeration at 4 °C prior to analysis and bioassays.

Gas chromatography/mass spectrometry (GC-MS) analysis

The volatile oils were analysed by gas chromatography-mass spectrometry technique using a Shimadzu GC-MS-QP2010 equipped with ZB-5 fused silica capillary column (Phenomenex, Torrance, CA, USA) with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 µm. The MS detector was operated in the electron impact (EI) mode of 70 eV, scan range 40-400 atomic mass units at the rate of 3.0 scan/s. The carrier gas was helium with a column head pressure of 552 kPa and flow rate of 1.37 mL/min. The injector initial temperature was 50 °C and increased at the rate of 2 °C/min to 260 °C. A 5% w/v solution of the sample in CH_2Cl_2 was prepared and 0.1 µL was injected with a splitting mode (30:1). Identification of constituents in the volatile oils was based on comparison of their retention indices relative to the homologous series of *n*-alkanes, and comparison of their mass spectra fragmentation patterns with the ones in the literature (Adams, 2017) and those of reference compounds stored in the home database library (Satyal, 2015).

Antimicrobial assays

The antimicrobial activity of the essential oils was determined using agar-diffusion method. The oils were tested against two bacteria standard strains (*Staphylococcus aureus* ATCC 6571 and *Escherichia coli* ATCC 25925) and three clinical fungi isolates (*Candida albicans*, *Aspergillus niger* and *Fusarium solani*) from University College. Bacteria strains were maintained on Mueller-Hinton Agar and fungi on Sabouraud Dextrose Agar (SDA). Each medium was prepared following the manufacturer's instructions. Diluted overnight cultures (10^{-2} CFU/ mL) were inoculated each into sterile agar. Wells of uniform diameter were created in the seeded agar plates using 8 mm cork borer. Volatile oils of varied concentrations were allowed to diffuse into the seeded agar for 1 h before incubating for 24 h at 37 °C and 48 h at 25-32 °C for bacteria and fungi respectively after which the inhibition zones were observed and recorded. Gentamycin (10 µL/mL) was used as positive control for bacteria and ketoconazole (200 µg) for fungi.

DPPH free radical-scavenging assay

DPPH free radical-scavenging assay is one of the standard technique used to study antioxidant property of plant volatile oils (Kedare & Singh, 2011). Free radical-scavenging potential of the *G. sepium* leaf and stem oils was evaluated using the method explained by Saleh *et al.,* (2010) with some modifications. A volume of 1.5 mL of different concentrations of the oil samples (5 mg/mL, 25 mg/mL and 100 mg/mL) were separately mixed with 1.5 mL of 0.2 mM DPPH (dissolved in methanol) and incubated in the dark for 20 min at room

temperature. The absorbance at 517 nm was recorded as $A_{(sample)}$ using CE 2021, 2000 series double beam UV-Vis spectrophotometer. A blank experiment was also carried out using the same procedure but without the volatile oils and the absorbance was recorded as $A_{(blank)}$. Each experiment was carried out in triplicate and the free radical-scavenging activity of the oils was calculated as percentage inhibition using the formula;

$$\% \text{ inhibition} = A_{(blank)} - A_{(sample)} / A_{(blank)} \times 100$$

The free radical-scavenging activity of butylated hydroxylanisole (BHA) and α-tocopherol were also evaluated as positive controls for comparison. IC_{50} was calculated through Microsoft EXCEL by plotting the graph of percentage inhibition against concentration as described by Kumawat et al., (2012).

Brine shrimp lethality test

The toxic effect of the volatile oils against brine shrimps of *Artemia Salina* (Artemiidae) was evaluated at different concentrations. Ten shrimp nauplii were introduced into 5 mL of 1000 ppm, 100 ppm and 10 ppm solution of essential oils (dissolved in DMSO) and sea water. The experiment was performed in triplicate making a total of 30 shrimps per dilution. Blank experiment was also carried out by introducing 10 shrimps into 5 mL seawater with DMSO to serve as negative control in other to eliminate other factors contributing to the total number of dead nauplii. The number of surviving shrimps were counted and recorded after 24 hours. The median lethal concentration (LC_{50}) was analyzed at 95% confidence intervals using a probit regression analysis (Finney, 1971). High toxicity is attributed to LC_{50} values less than 100 ppm while values between 100-500 ppm is moderately toxic, 500-1000 ppm is less toxic and greater than 1000 ppm is non-toxic (Meyer et al., 1982).

Results and Discussion

Identification of *Gliricidia sepium* volatile oils constituents

The GC-MS results of the essential oil obtained from the leaf and stem of *Gliricidia sepium* are listed in Table 1 in order of their elution from a ZB-5 fused silica capillary column. A total of 43 compounds were identified in the leaf oil amounting for 97.6% of the whole volatile oil. The oil comprised mainly non-terpenes which made up 73.0% of the total oil. The most abundant constituents were *E*-hexadecatrienal (16.9%), pentadecanal (16.0%) and methylcyclohexane (8.0%). The leaf oil characterized via GC-FID and GC-MS from Costa Rica (Chaverri and Cicció, 2015) presented 96 compounds with pentadecanal (18.7%), (*Z*)-phytol (7.8%) and nonanal (5.1%) as most abundant constituents, which were also detected in the present study in quantity 16.0%, 0.9% and 1.5%, respectively.

In contrast, Kaniampady et al. (2007) gave account of propylene glycol (25.1%), coumarin (18.2%), (*Z*)-3-hexanol (17.7%), β-farnesene (14.2%) and 2*E*-hexanol (6.5%) as the major components in *G. sepium* leaf oil amid the sixteen constituents identified by GC.

Likewise, safrole (12.3%) and 2''-hydroxy-acetophenone (12.1%) were found to be main constituents among the eighty volatile compounds from Colombia leaf oil analysed by GC-FID and GC-MS (Quijano-Célis et al., 2015).

The stem oil contained 44 compounds including 64.7% sesquiterpenes, 1.2% monoterpenes and 29.3% non-terpenes equivalent to 95.2% of the whole essential oil. The main constituents were determined as (9*Z*)-docosenamide (18.0%), humulene epoxide II (17.5%), caryophyllene oxide (10.6%) and α-cadinol (5.0%). The only monoterpene hydrocarbon present was α-pinene (0.4%). Alongside were oxygenated monoterpenes (*E*)-myrtanol (0.5%) and geranyl acetone (0.3%). In contrast, Jose and Reddy (2010) reported methyl-3-(*E*)-

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pentenyl ether (11.6%), 3-methyl-2-butanol (10.7%), 1-(1-ethoxyethoxy)-2-hexene (9.7%), 2-decanol (9.0%), coumarin (8.1%) and hexadecanoic acid (5.2%) as main components among the 19 compounds identified from the stem bark oil. Although, the leaf and stem oils were extracted from the same plant, there was significant difference in their chemical compositions. All the dominant constituents in the leaf oil were absent in the stem oil and vice versa except methylcyclohexane (8.0%, 0.2%), phytone (4.5%, 0.5%) and (*Z*)-hexadecatrienal (4.9%, 0.9%) that were present in the leaf and stem volatile oils, respectively.

Table 1. Chemical Compositions of *G. sepium* Volatile oils

Constituents	**RI**	**Percentage composition (%)**	
		Leaf	**Stem**
Z-1,2-Dimethylcyclopentane	716	1.3	-
Methylcyclohexane	718	8.0	0.2
2-Methyl-2-pentanol	723	0.7	-
Ethylcyclopentane	726	0.5	-
3-Methyl-2-pentanol	745	0.4	-
2-Methylheptane	754	0.3	-
Toluene	760	2.7	0.5
3-Methylheptane	763	0.2	-
Z-1,3-Dimethylcyclohexane	777	0.6	-
E-1,3-Dimethylcyclohexane	780	0.3	-
2-Hexanone	786	0.6	-
1-Methylcyclopentanol	792	0.7	-
Octane	798	0.5	-
Hexanal	800	0.8	0.5
Z-1,4-Dimethylcyclohexane	807	0.4	-
Ethylcyclohexane	832	0.3	-
Ethylbenzene	856S	1.0	-
1-Hexanol	864	0.4	-
o-Xylene	866	1.0	-
p-Xylene	867	0.4	-
Nonane	903	-	0.4
α-Pinene	930	-	0.4
1-Octen-3-ol	977	0.8	-
2-Pentylfuran	987	-	0.8
2-Ethylhexanol	1025	-	0.4
Limonene	1027	0.8	-
Linalool	1098	1.0	-
Nonanal	1103	1.5	0.7
Decanal	1204	0.5	-
E-*p*-Menthan-2-one	1208	2.0	-
E-Myrtanol	1260	-	0.5
β-Bourbonene	1381	-	1.4
β-Elemene	1386	-	0.3
β-Caryophyllene	1416	-	1.8
Geranyl acetone	1444	0.7	0.3

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α-Humulene	1452	-	3.9
Z-Muurola-4(14),5-diene	1471	-	0.4
(*E*)- *β*-Ionone	1475	0.5	-
Germacrene D	1478	-	0.3
β-Selinene	1486	-	0.7
α-Selinene	1492	-	0.7
α-Muurolene	1495	-	0.3
Tridecanal	1508	0.5	-
δ-Cadinene	1514	-	0.6
(3*Z*)-Hexenyl benzoate	1568	0.5	-
Dendrolasin	1570	0.7	-
Spathulenol	1573	-	1.9
Hexyl benzoate	1576	0.3	-
Caryophyllene oxide	1579	-	10.6
Salvial-4(14)-en-1-one	1589	-	1.9
allo-Cedrol	1592	-	0.6
Humulene epoxide II	1606	-	17.5
Tetradecanal	1609	-	0.3
Pentadecanal	1611	16.0	-
Muurola-4,10(14)-dien-1*α*-ol	1622	-	0.4
Caryophylla-4(12),8(13)-dien-5α-ol	1629	-	3.8
Caryophylla-4(12),8(13)-dien-5β-ol	1633	-	1.4
τ-Cadinol	1638	-	0.7
τ-Muurolol	1640	-	1.7
δ-Cadinol	1643	-	1.1
α-Cadinol	1652	-	5.0
Selin-11-en-4α-ol	1655	-	2.8
14-Hydroxy-9-*epi*-(*E*)-Caryophyllene	1667	-	1.5
Cadalene	1669	-	0.5
Germacra-4(15),5,10(14)-trien-1α-ol	1686	-	1.9
Pentadecanal	1711	-	4.1
Hexadecanal	1813	0.5	-
Phytone	1837	4.5	0.5
(*Z*)-Hexadecatrienal	1882	4.9	0.9
(*E*)-Hexadecatrienal	1887	16.9	-
Heptadecanal	1915	0.9	-
Palmitic acid	1953	0.8	-
Phytol	2102	0.9	-
(9Z)-Octadecenamide	2349	-	0.6
1-Docosanol	2484	0.6	0.5
Heptacosane	2700	1.0	-
(9Z)-Docosenamide	2754	-	18.0
Monoterpene hydrocarbons		0.8	0.4
Oxygenated monoterpenes		3.7	0.8
Sesquiterpene hydrocarbons		0.7	10.9
Oxygenated sesquiterpenes		-	52.8

Diterpene	0.9	-
Apocarotenoids	0.5	-
Non-terpene derivatives	91.0	29.4
Total compounds identified	43	44
Percentage identified	**97.6**	**95.2**

RI- Retention indices determined with respect to a series of *n*-alkanes on a ZB-5 column.

Antimicrobial activity

The result of the antimicrobial assay is given in Table 2. The activity of the volatile oil was compared with gentamycin (10 µL/mL) and ketoconazole (200 µg) used as positive control in the antibacterial and antifungal assays respectively. DMSO used as solvent was used as negative control while the inhibition zones diameter values were reported as 1 decimal place mean±SD. There was no activity against the *E. coli* and *S. aureus* but moderate zones of inhibition were observed on the fungi. Only the stem oil gave significant antifungal activity with inhibition zones varying from 2.1±0.1 to 8.1±0.1 mm but not as active as the referenced antifungal drug (Figure 1). On the other hand, Jose and Reddy (2010) reported significant activity of leaf and flower essential oils of *G. sepium* against *E. coli* and *S. aureus* using agar-diffusion method. Similarly, good activity was documented for leaf extracts and (Chevian & Thambi, 2019; Kumar & Simon, 2016; Sukumar & Aparna, 2014; Akharaiyi et al., 2012). Variation in chemical compositions due to factors like geographical location, plant maturity, season harvested and extraction procedure might have contributed to their different activity.

Previous researches showed that most essential oils with good antimicrobial activities were made up of high percentage of monoterpenes and sesquiterpenes as well as their related alcohols, phenols and other oxygenated compounds (Griffin et al., 1999) due to the hydrophilic character of their functional groups and lipophilic nature of their hydrocarbon chains (Daferera et al., 2000; Kalemba and Kunicka 2003). The above information probably explains antimicrobial activity presented by *G. sepium* stem oil, since it was dominated by terpenes, i.e., 10.9% sesquiterpene hydrocarbons and 52.8% oxygenated sesquiterpenes. Spathulenol, *α*-selinene and *δ*-cadinene found in the stem oil but absent in the leaf oil have been reported to possess antifungal and antibacterial activity (Cakir et al., 2005; Cheng et al., 2005). Besides, synergistic effects of the oil constituents can also contribute to their activity.

Table 2. Zones of Inhibition (mm) observed in antimicrobial assay of *G. sepium* volatile oils

Essential oils	**Conc (µL/mL)**	**Inhibition zones diameter (mm)**				
		E. coli (ATCC 25925)	*S. aureus* (ATCC 6571)	*C. albicans* (clinical)	*A. niger* (clinical)	*F. solani* (clinical)
Leaf	100	0.0	1.8±.0.3	0.0	0.0	6.0±0.1
	10	0.0	0.0	0.0	0.0	3.9±0.1
	1	0.0	0.0	0.0	0.0	2.0±0.1
Stem	100	0.0	0.0	8.1±0.1	1.9±0.1	6.1±0.1
	10	0.0	0.0	4.3±0.4	0.0	3.8±0.4
	1	0.0	0.0	2.1±0.1	0.0	0.0
Positive control		9.0±1.4	11.5±0.7	21.0±1.4	10.5±0.7	10.3±0.4
Negative control		0.0	0.0	0.0	0.0	0.0

Antioxidant activity

DPPH radical-scavenging assay is one of the standard technique commonly used to evaluate the antioxidant potential of essential oils or other phytochemicals extracted from plant. The DPPH has a characteristic absorption at 517 nm. It reduces when exposed to an antioxidant agent that can scavenge its free radical either by donating an electron or a hydrogen atom. At the concentrations observed (5, 25 and 100 µg/ mL), the percentage inhibition of the DPPH• by the leaf and stem volatile oils of *Gliricidia sepium* were between 40-55 %. The leaf and stem oils demonstrated a concentration dependent scavenging ability and the IC_{50} values obtained from the graph of percentage inhibition against the concentration were 84.26 and 142.15 µg/ mL respectively (Table 3), indicating lower scavenging ability than BHT (IC_{50} = 45.11 µg/ mL) that was used as positive control while only the leaf volatile oil showed higher scavenging activity than α-tocopherol (IC_{50}= 81.58 µg/ mL). The lower the IC_{50} value the higher the free radical-scavenging activity. In the previous studies, the leaf extracts especially the ethanoic fractions showed high antioxidant property (Ang et al., 2019; Akharaiyi et al., 2012).

Table 3. IC_{50} values obtained from DPPH radical-scavenging activity of the volatile oils

Tested Samples	Leaf oil	Stem oil	α-Tocopherol	BHA
IC_{50} (µg/mL)	84.26	142.15	81.58	45.11

IC_{50} was obtained by plotting the values of % inhibition against concentration using Microsoft EXCEL

Toxicity

The data from the toxicity study of plant volatile oils is important in order to ascertain their safety when used by human as a source of drug. The brine shrimp lethality assay result was given in Table 4. The stem oil has higher toxicity (LC_{50}= 38.7081 µg/mL) than the leaf oil (LC_{50}= 79.6717 µg/mL). β-elemene (0.3 %), β-caryophyllene (1.8 %) and α-humulene (3.9 %) present in the stem oil have shown high toxicity although not against brine shrimps but tumour cells (Hanusova et al., 2017; Jiang et al., 2017). These compounds are not the major constituents but their presence might have added up to the toxic effect of the stem oil in addition to the synergistic effect of other components of the oil. Several studies have shown good correlation between lethal concentrations (LC_{50}) obtained from the brine shrimps lethality assay using *Artemia salina* and the acute oral toxicity assay using mice (Arlsanyolu and Erdemgil, 2006). This implies the volatile oils from the leaf and stem of *G. sepium* may possess antitumor potential. The toxicity of *G. sepium* leaf extract against brine shrimps have been reported (Ang et al., 2019).

Table 4. The Brine shrimp lethality assay result

Tested samples	LC_{50}	LC limit	UC limit	CL
Leaf oil	79.6717	128.0301	203.9742	0.2275
Stem oil	38.7081	20.5002	66.9387	0.1200

CL-Confidence limit, LC-Lower confidence, UC-Upper confidence, LC_{50}- Conc at which there was 50 % mortality

Conclusion

Chemical compositions, antimicrobial, antioxidant and toxicity of the volatile oils from the leaf and stem of *Gliricidia sepium* from Nigeria are reported for the first time. There were differences in the biological activities of the studied essential oils and the ones from the literatures due to variation in their geographical locations, extraction procedure and chemical compositions. The leaf and stem oils showed poor antibacterial

activity but moderate antifungal activity. The leaf oil is largely non-terpenes: dominated by (*E*)-hexadecatriene (16.9 %) and pentadecanal (16.0 %) while the stem oil composed principally terpenes mainly humulene epoxide II (17.5 %) and caryophyllene oxide (10.6 %). The marked difference between the chemical compositions of the two essential oils was because they were extracted from different plant organs. Therefore, the antifungal activity exerted by the stem oil may be as a result of the synergic effect of several sesquiterpenes present in the oil but absent in the leaf oil. The leaf oil showed better antioxidant activity than the stem oil while both oils were toxic to *Artemia salina* larvae. The results suggest that the stem essential oil from *G. sepium* may find potential use as a natural antiseptic agent. The lower LC_{50} values of the oils signify they are biologically active. Thus several other bioassays will be helpful to search for more activities of these volatile oils whether they possess the same activities like the extract or support their traditional use.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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