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Chemical Composition, Antioxidant and Antimicrobial Activities of the Oil from Stem of *Hoslundia Opposita.*

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ABSTRACT

Essential oil was extracted from the stem of *Hoslundia opposita*, that was collected from Southwestern Nigeria. The extraction was done using soxhlet extraction method. Gas Chromatography mass spectroscopic technique was used to determine the chemical composition of the essential oil. The essential oil was investigated for antioxidant activities using 2,2, -Diphenyl picrylhydrazine (DPPH) radical scavenging capacity. The essential oil was investigated for antimicrobial activities using different clinical isolates of bacterial strains viz., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium and* with fungi strain *Aspergillus niger,* and *Penicillium sp*. It revealed the presence of o-cymene (0.03 %), copaene (0.47 %), caryophyllene (0.90 %), humulene (0.24 %) pinene (0.02 %) as minor constituents while sitosterol (13.03 %), 9,12 octadecanoic acid ethyl ester (5.9 %), stigmasterol (6.90 %) were identified as the major constituents. It revealed that the essential oil showed a significant percentage % radical scavenging activity at 66.80 % at concentration of 100 µg/ml when compared with the % radical scavenging activity of the standard ascorbic acid at the same concentration. The antioxidant activities gave a significant IC_{50} of 61.62 µg/ml which was not comparable with the IC_{50} value 33.28 µg/ml of the standard potent drug ascorbic acid used. At concentrations of 100 to 50 mg/ ml, the essential oil was active against all bacteria and fungi except *Aspergillus Niger*. This study revealed that *Hoslundia opposita* is a good source of bioactive compounds with antimicrobial and antioxidant activities.

Keywords: Antioxidant, Antimicrobial, Essential oil, Bioactive, Ascorbic Acid

INTRODUCTION

A medicinal plant can be described as any plant that contains substances which are precursors for drugs synthesis or can be used for therapeutic purposes (Abayomi *et al.,* 2013). Medicinal plants have essential oils in their tissues, leaves, fruits, stem that prevent bacteria, molds/microbes from growing. They are commonly used in the treatment of diseases, ailments that are harmful to humans (Schulz *et al.,* 2001). Essential oils are mixtures of volatile organic compounds biosynthesized in specialized plant cells (Sadgrove *et al.,* 2022). They are normally produced as secondary metabolites. They are highly odorous and volatiles in nature (Ayeza *et al.,* 2018).

Hoslundia opposita is a scrambling shrub like perennial. It can be found in Senegal, Eritrea, South Africa, and Nigeria. (Burkil, 2004). This plant has been used in the treatment of fevers, cold, malaria, herpes, wounds and gonorrhea (Muhammad *et al.*, 2012). *H. opposita* possess anti-bacterial, antimalarial, anti-convulsant, anti-microbial and antioxidant activities (Achenbach *et al.*,1992), Olajide *et al*., 1999), (Ojo *et al.*, 2010).

Previous study on the essential oil from the fruits and leaves of *Hoslundia opposita* revealed the presence of α- thujene, sabinene, 1-8 cineole, terpine-4-0-1B- pinene (Usman *et al.*, 2010). There is no scientific data on the chemical composition of essential oil from the stem of *Hoslundia opposita.* The aim of the present study is to determine the chemical composition of the essential oil from the stem of *Hoslundia opposita*. And also, to investigate this essential oil for antimicrobial and antioxidant activities.

Materials and Methods

Collection of plants

Hoslundia opposita was collected in south western Nigeria with [$7^0$40'33.48"N Lat, $3^0$53'29.24"E Log] in Oluyole, Ibadan in Oyo state. It was identified and authenticated at Forestry Research Institute of Nigeria with a voucher specimen number 113740 deposited.

Extraction of essential oil

Extraction of essential oil from the stem of *Hoslundia opposita* was carried out using soxhlet extraction method. The stem (100 g) were air dried, pulverized and placed in the thimble of the soxhlet chamber. Then 500 ml of hexane was placed in a round bottom flask and assembled for soxhlet extractor, then the distillation process was initiated. The filtrate obtained after exhaustive extraction was transferred to a rotary evaporator and concentrated. The essential oil obtained was preserved on a glass tubes at 4 ^{0}C.

Gas Chromatography Mass Spectrometry Analysis

The Gas chromatography mass spectrometry analysis was carried out on an Agilent 7820A gas chromatography coupled to 5975 C inert mass spectrometer with electron impact source. HP-5 capillary column coated with 5 % Phenyl methyl siloxane (30 m length x 0.32 mm diameter x 0.25 µm film thickness) was used as the stationary phase. Helium was used as the carrier gas, at constant flow of 1.4871 ml/min at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. 1 µl of the sample was injected in splitless mode at an injection temperature of 300 ^{0}C. Purge flow to spilt vent was 15 ml/min at 0.75 min with a total flow of 16.654 ml/min gas saver mode was switched off. Oven was initially programmed at 40 ^{0}C for (1 min), then ramped at 12 ^{0}C/min to 300 ^{0}C (10 min). Run time was 32.667 min with a 5 min solvent delay. The mass spectrometer was operated in electron impact- ionization mode at 70 eV with an ion source temperature of 230 ^{0}C, quadrupoles temperature of 150 ^{0}C and transfer line temperature of 280 ^{0}C. Ion acquisition was via scan mode. (Scanning from m/z 45 to 550 amu at 2.0 s/scan rate). The identification of the oil constituents was based on a comparison of their retention time and retention indices, with those of literature by matching their mass spectra fragmentation patterns with corresponding data (Wiley 275 L. library) and other published mass spectra.

Antioxidant Tests

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Capacity of the essential oil fraction The effect of the essential oil extract on DPPH radical was estimated. 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of extract in methanol containing different concentrations of extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature or 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard.

The percent DPPH scavenging effect was calculated using the following equation

DPPH Scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard sample or extract. The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of DPPH radical formation.

Antimicrobial tests (Oyah *et al.*, 2015)

The antimicrobial activities of varied concentrations of the essential oil from the stem of *Hoslundia opposita* were carried out using standard bacteria strains using *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium and* with fungi strain *Aspergillus niger,* and *Penicillium sp* which were obtained from the Department of Microbiology, University of Lagos, Akoka, Nigeria. Agar well diffusion technique was used. The inocula were prepared from the bacteria and yeast cultures and were maintained in glycerol-peptone water at 4°C as a pure culture and were sub cultured into sterile peptone water in a Mccartney bottles. One hundred microliter (100 ml) of

inocula (test organisms) was introduced into the sterile, agar plates and were spread evenly with sterile swab stick, and 8 mm cork-borer was used to bore holes. The essential oil was diluted with DMSO to achieve different concentrations. One hundred micro litre of the various concentrations of the sample were introduced into the bored holes in duplicate plates. The inoculated plates were allowed to stand for 30 mins for proper diffusion of the sample into the medium. These inoculated plates were incubated at 37 ^{0}C for 24 hours and examined for zones of inhibition. Zone of inhibition was measured in millimeter with a ruler at 90^0 perpendicular to each other and the mean of the readings were then calculated. Pefloxacin and ciprofloxacin was as positive control while Dimethoxy served as negative control. Negative control was prepared using the respective solvent. Chloramphenicol was used as a positive control for bacteria, ketoconazole as a positive control for fungi and DMSO was also used as a negative control. They were incubated for 24 hours. Observations were made to check for organism growth.

Minimum Inhibitory Concentration: Petri dish plates were prepared with 20 mL of sterile Muller Hinton Agar (MDA) for bacteria and 20 mL of Potato Dextrose Agar (PDA) for fungi. The 24-hour prepared test cultures of inoculums were swabbed on top of the solidified media and allowed to dry for 10 min. The tests were conducted with a cork borer of 8.0 mm. Solutions of the essential oil were aseptically filled into the hole of agar well at the different dilutions of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. Concentrations prepared were introduced into Mueller Hinton agar (MHA) plates and Potato Dextrose Agar already swabbed with the different selected organisms in duplicates aseptically. All appropriate controls- Chloramphenicol, ketoconazole, and DMSO were also inoculated on Mueller Hinton agar in duplicates. The plates were placed on the surface bench and left for 30 minutes at room temperature for diffusion. The plates were then incubated for 24 hrs at 37 °C for bacteria and 4 days at 28 °C for fungi, respectively. The zone of inhibition was recorded by measuring the clear zone of growth inhibition on the MHA and PDA surface around the holes in millimeters.

Result

Table 1: Constituents of the essential oil from stem of *Hoslundia opposita*

Compounds	%Composition	Molecular formular
O – Cymene	0.03	$C_{10}H_{14}$
α- Copaene	0.47	$C_{15}H_{24}$
Caryophyllene	0.90	$C_{15}H_{24}$
Humulene	0.24	$C_{15}H_{24}$
Eicosane	0.08	$C_{20}H_{42}$
α- pinene	0.02	$C_{10}H_{16}$
Hexadecanoic acid	3.24	$C_{16}H_{32}O_2$
α - Cubebene	0.09	$C_{15}H_{24}$
Ascaridole	0.18	$C_{10}H_{16}O_2$
Oleic acid	2.64	$C_{18}H_{34}O_2$

α Amyrin	1.73	$C_{30}H_{50}O_2$
Neophytadiene	0.38	$C_{20}H_{38}$
Tetradecanoic acid ethyl ester	4.73	$C_{16}H_{32}O_2$
9, 12 octadecanoic acid ethyl ester	5.9	$C_{20}H_{36}O_2$
Dodecanoic acid ethyl ester	3.83	$C_{14}H_{28}O_2$
Tetradecanoic acid	6.17	$C_{14}H_{26}O_2$

Compounds	**%Composition**	**Molecular formular**
Dodecane	0.09	$C_{12}H_{26}$
Tetradecane	0.09	$C_{14}H_{30}$
Pentadecane	0.10	$C_{15}H_{32}$
Ergost-5-en-3-ol	2.81	$C_{28}H_{48}O$
Ergosterol	0.65	$C_{28}H_{44}O$
Erucic acid	0.16	$C_{22}H_{42}O_2$
Stigmasterol	6.93	$C_{29}H_{48}O$
Sitosterol	13.03	$C_{29}H_{50}O$
Tocopherol	0.64	$C_{29}H_{50}O_2$
Docosanoic acid ethyl ester	0.76	$C_{24}H_{48}O_2$
Methyl tetracosanoate	0.43	$C_{24}H_{50}O_2$
Cholesterol	0.44	$C_{27}H_{46}O_2$
9, 12-octadecadienoic acid	0.37	$C_{18}H_{32}O_2$
Hexadecanoic acid methylester	0.64	$C_{17}H_{34}O_2$
Methyl benzoate	0.03	$C_8H_8O_2$
Phenyl ethyl alcohol	0.02	$C_8H_{10}O$
Perylene	5.90	$C_{20}H_{12}$

Pyrene	9.38	$C_{16}H_{10}$
Phthalate	1.24	$C_8H_4O_4$
3- methyl Chlontherene	0.39	$C_{12}H_{16}$

Table 2: Antioxidant activities of essential oil from stem of *Hoslundia opposite*

Concentrations µg/ml	Sample	Ascorbic acid
	% radical scavenging activities	
100	66.80	83.94
75	52.38	75.44
50	44.14	62.58
25	38.02	36.54

IC_{50}= 61.6µg/ml

Table 3: Antimicrobial activities of essential oil from stem of *Hoslundia opposita*

Microorganisms	Concentrations (mg/ml) 100	50	25	12.5	DMSO	CHL	KET	MIC
	Zone of inhibition of Pathogens in mm							
E. coli.	22.0	15.0	0.0	0.0	0.0	20.0	60	100
S. aureus.	18.0	12.0	5.0	0.0	0.0	20.0	60	100
P. aeruginosa	21.0	16.0	0.0	0.0	0.0	0.0	80.0	80
S. typhimurium	17.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0
A. niger	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium sp	45.0	15.0	0.0	0.0	0.0	0.0	50.0	70.0

NOTE: CHL-Chloramphenicol, KET- ketoconazole, 0 – No zone of inhibition, MIC- minimum inhibitory concentration, minimum bactericidal concentration, Diameter of Zone of inhibition -8mm.

Discussion

Monoterpenes cymene (0.03%), α – pinene (0.02%), three sesquiterpenes –copaene (0.49%), humulene (0.24%), caryophyllene (0.90%) were identified as minor constituents in the essential oil fraction from the stem of *Hoslundia opposita* while sterols sitosteol (13.03%), stigmasterol (6.9%) and fatty acid ester 9,12, octadecanoic acid ethyl ester (5.9%), were identified as the major constituents in Table 1. Polycyclic aromatic hydrocarbon (PAHs) perylene (5.9%), pyrene (9.38%), neophytadiene (0.38%), phthalate (1.24%) and 3-methyl chlolathrene (0.39%) were unusually identified in the essential oil. PAH(s) are persistent organic pollutants that endangers soil ecosystems and human health. Cultivating aromatic plants in (PAH) contaminated soils is considered a

safe & sustainable photoremediation strategy to mitigate (PAH) soil pollution. The PAH(s) were present in the soil & habitant of the collection of *Hoslundia opposita* and can be found in the essential oil fraction through a photoremediation strategy of cultivating aromatic plants in PAH(s) polluted habitat e.g. phthalate can cause several human & environment hazards and can be released from waste disposal to the water & soil, leading to absorption & accumulation by medicinal or aromatic plants. Previous study on the essential oil from the fruits and leaves of *Hoslundia opposita* revealed the presence of α – thujene, sabinene, 1-8 cineole, terpineol and pinene in both parts of the plants (Usman *et al.,* 2010). Moreover, α – pinene, sabinene, B-pinene, 3-carene and germacrene D were identified from the essential oil from the *Plectranthus ornatus* (Soares *et al,* 2017), which belong to the family Lamiaceae. Also, thymol, cymene, carvacrol were also present in essential oil from *T. Vulgaris*, also a species from family of lamiaceae (*et al.,* 2018). The total constituents are sterol 24.06 %, fatty acid ester 17.13 %, fatty acid 13.23%, polycyclic aromatic hydrocarbon 17.17%, sesquiterpene 1.65 %, alkane 0.77 %, triterpenoid 1.73 %, monoterpene 0.23 %, diterpene 0.38 % and alcohol 0.02 %, which make up to 76.37 % of the essential oil constituents. This is the first time the chemical composition of the essential oil from stem of *Hoslundia opposita* is reported.

Table 2 showed the antioxidant activities of essential oil from the stem of *Hoslundia opposita*. At concentrations of 100 µg/ml, % the radical scavenging activities were significant and comparable to the % radical scavenging activities of the standard drug used ascorbic acid. Also, the % radical scavenging activities at 75 µg/ml to 25 µg/ml were moderate but not comparable to the % radical scavenging activities of standard drug ascorbic acid. The antioxidant activities of the essential oil maybe due to the presence of o-cymene, a natural antioxidant (Oliveira *et al.*, 2015), and caryophyllene, known for its antioxidant activity (Gushiken *et al.,* 2022). The antioxidant activities gave a significant IC_{50} of 61.62 µg/ml which was not comparable with the IC_{50} value 33.28 µg/ml of the standard potent drug ascorbic acid used. The essential oil was investigated for antimicrobial activities using different clinical isolates of bacterial strains viz., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and* with fungi strain *Aspergillus niger,* and *Penicillium sp.* At concentrations of 100 to 50 mg/ml, the essential oil was active against all bacteria and fungi except *Aspergillus niger*. There was a moderate activity against *S.aureus* at 25 mg//ml but no activity at 12. 5 mg/ml against all microorganisms used.

Conclusion

This is the first time the chemical composition of the essential oil from stem of *Hoslundia opposita* is reported. This study revealed that *Hoslundia opposita* is a good source of bioactive compounds with antimicrobial and antioxidant activities.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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