Chemical constituents and antioxidant activities of the essential oil from stem of *Olax manii*

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Abstract

Olax manii stems were collected in Ikire, Osun State, Nigeria. Using the hydrodistillation process, essential oil was extracted from the stem of *Olax manii.* To ascertain the chemical composition of the essential oil, Gas chromatography mass spectrometry (GCMS) technique analysis was employed. Using doses ranging from 100 to 25 µg/ml, the antioxidant properties of the essential oil were examined using the 2, 2-Diphenyl-1 picryl hydroxyl (DPPH) radical scavenging activity method. The results of the Gas chromatography mass spectrometry (GC-MS) analysis indicated that the main chemical constituents were squalene (6.18 %), amyrone (4.49%), and neophytadiene (4.22 %). The minor constituents were identified as limonene (0.23 %), caryophyllene (0.31 %) and phytol (1.62 %). The essential oil radical scavenging activity at a concentration of 100 mg/ml was compared to the standard ascorbic acid at the same concentration and it revealed that the essential oil exhibited a significant percentage radical scavenging activity of 78.52 %. The percentage radical scavenging activities increased with increase in concentrations of the essential oil. The Essential oil constituents which possess antioxidant activities could be responsible for the antioxidant activities and ethnomedicinal uses of *Olax manii.*

Keywords: essential oil, antioxidant. Constituents, DPPH

1.0 Introduction

Medicinal plants have been used in traditional medicine as a form of medication for thousands of years. Their medicinal effects have been communicated through human generations for centuries (Khan, 2014).The potential health benefits and chemical composition of many species of medicinal plants have not yet been studied or still yet to be deeply investigated. (Jamshidi-kia *et al.,* 2018). Medicinal plants remain the "pillar" of traditional medicine and more than three billion people in least developed countries make use of medicinal plants as medications on a regular basis (Davidson-Hunt, 2000).

Essential oils are complex mixtures of volatile secondary metabolites or compounds, which are chiefly composed of terpenoids and phenolic compounds. They are biosynthesized in cell types in all parts of plants that produce essential oils called aromatic plants. The chemical

composition of the essential oils vary from plant to plant (Baptiste *et al,* 2020). *Olax manii* belongs to the family olacaceae and can be found in Ghana, Nigeria, Sierra leone. The fruits are about 1/2 -3/4 cm when ripe and the leaves are lanceolate to ovate (Sule *et al,* 2011). It is used in traditional medicines practice to treat fever and snake bite (Burkill, 1992).It is found in mostly forests and grows to a height of 1-2 metres. The chemical constituents of the essential oil from stem of *Olax manii* is not known. The present study focus on the investigation of the chemical constituents and antimicrobial activities of the essential oil from the stem of *Olax manii*.

2.0 Materials and Method

2.1 Gathering of plant specimens

In Agbegi Odofin village, Ikire, Osun State, in southwest Nigeria (7°23'10.54° N Lat, 4°13' 8 . 8.5 °E Lon), *Olax manii* was collected. At the Forestry Research Institute of Nigeria (FRIN), it was authenticated, and a voucher specimen with the number 113754 was placed. For five hours, the stem of *Olax manii* was extracted using the hydrodistillation process with a Clavenger-style apparatus. The extracted essential oil was stored at 4 °C in glass tubes.

2.2 Analysis of Gas Chromatography Mass Spectrometry

An Agilent 7820A gas chromatography connected to a 5975C inert mass spectrometer with an electron impact source was used to perform the mass spectrometry analysis . The stationary phase was an HP-5 capillary column (30 m length x 0.32 mm diameter × 0.25 µm film thickness) coated with 5% Phenyl methyl siloxane. Helium was employed as the carrier gas, with an average velocity of 44.22 cm/sec and a constant flow rate of 1.4871 mL/min at an initial nominal pressure of 1.4902 psi. A single 1µL injection of the sample was made in splitless mode, with a 300 °C injection temperature. The 15 ml/min purge flow to the split ventor and put in a round-bottom flask. Following a thorough extraction, the filtrate was moved. With a total flow of 16.654 ml/min, the gas saver mode was turned off after 0.75 minutes. A 400°C initial program was followed by a 120°C/min ramp up to 3000°C (10min). The run took 32.667 minutes, with a solvent delay of 5 minutes. With an ion source temperature of 230°C quadrupole temperature of 150 °C, and transfer line temperature of 280 °C, the mass spectrometer was run in electron impact-ionization mode at 70 eV. Scan mode was used to acquire the ions (scanning at a rate of 2.0 s/scan from m/z 45 to 550 amu). By comparing the oil constituents' retention times and retention indices with those of previous studies and matching their mass spectra fragmentation patterns with relevant data of

additional mass spectra that have been released, the essential constituents were identified (Wiley 275) L. library).

2.3 Tests of antioxidants

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical Scavenging Determination

An estimate was made of the essential oil extract's impact on the DPPH radical. A 0.1 mM DPPH solution was made in methanol, and 1.0 mL of this solution was combined with 1.0 mL of extract in methanol that had varying extract concentrations (25-100 µg/ml) . After carefully vortexing the reaction mixture, it was allowed to sit at room temperature for 30 minutes in the dark. Using spectrophotometry, the mixture's absorbance was calculated at 517 nm. The standard was ascorbic acid.

The following formula was used to determine the percent DPPH scavenging effect.

(A0-A1)/A0) x 100 = DPPH Scavenging effect (%)

where A0 represented the control's absorbance and A1 the absorbance in the the standard sample or extract is present. The concentration of the compounds that resulted in a 50 % inhibition of the formation of DPPH radicals was indicated by the IC50 value.

3.0 Results and Discussion

Table 1: Chemical constituents from the essential oil from stem of *Olax manii*

Compounds	Molecular formular	%composition
D-limonene	$C_{10}H_{16}$	0.23
Citral	$C_{10}H_{16}0$	0.12
Caryophyllene	$C_{15}H_{24}$	0.31
Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	4.43
Hexadecanoic acid	$C_{16}H_{36}O_2$	0.85
Octadecanoic acid	$C_{18}H_{36}O_2$	0.25
Phytol	$C_{20}H_{40}O_2$	1.62
Squalene	$C_{30}H_{50}$	6.18
Eicosane	$C_{20}H_{42}$	0.57
B-amyrin	$C_{30}H_{50}O$	1.05
Stigmasterol	$C_{29}H_{48}O$	0.32
Amyrone	$C_{30}H_{48}O$	4.49
Neophytadiene	$C_{30}H_{38}$	4.22

Table 2: Antioxidant activities of essential oil from stem of *Olax manii*

Concentrations µg/ml	Sample % Radical Scavenging activities	Ascorbic acid
100	78.52	83.94
75	70.18	75.44
50	56.57	62.58
25	42.45	36.54

IC_{50}= 35.56 µg/ml

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

In Table 1, the gas chromatography mass spectrometry (GCMS) analysis indicated that the main ingredients were squalene (6.18 %), amyrone (4.49%), and neophytadiene (4.22 %). Additionally present were limonene (0.23 %), caryophyllene (0.31 %) and phytol (1.62 %) as minor chemical constituents. From previous researches, it was found that amyrone possess anti-inflammatory and antifungal activities (de Almeida *et al.,* 2015) . Also, caryophyllene possess antioxidant activities (Dahham *et al*., 2015). A diterpene phytol was also reported to possess antioxidant activities (Slam *et al,* 2018). And one of the major constituents squalene possess antioxidant activities (Se kwon ,2012). Terpineol (11.5 %) was reported to be the major chemical constituent in the diffirent specie of Olax , which is *Olax acuminata* though terpineol was not present in the essential oil of *Olax manii* (Chetia, 2014). When the essential oil's percentage of radical scavenging activity at a concentration of 100 mg/ml was compared to that of the standard ascorbic acid at the same concentration in Table 2, the antioxidant test showed that the essential oil exhibited a significant percentage radical scavenging activity of 78.52 % with IC_{50} of 35.56 µg/ml. The percentage radical scavenging activity increased with increase in concentrations of the essential oil.The essential oil constituents which possess antioxidant activities could be responsible for the antioxidant activities and ethnomedicinal uses of the *Olax manii.*

4.0 Conclusion

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

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Disclosure Statement

No potential conflict of interest was reported by the authors

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