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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE LEAF ESSENTIAL OIL OF *ERYTHROPHLEUM SUAVEOLENS* GUILL. AND PERR. (BRENAN) (FAMILY: FABACEAE/ CAESALPINIOIDEAE)

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ABSTRACT

The leaf of *Erythrophleum suaveolens* (*E. suaveolens*) is applied to wounds and also used to repel pest and insects. However, there is no report on the chemical composition of the leaf essential oil of the plant. This study aims to determine the chemical composition and antimicrobial activity of the essential oil of the leaf of *E. suaveolens.* The essential oil was obtained by hydrodistillation method with the aid of a Clavenger type apparatus. The GC-MS analysis of the essential oil was done using Shimadzu QP-2010plus GC-MS. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library. The antibacterial activities of the essential oil against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Candida albicans* and *Mycobacterium bovis* were evaluated using microdilution broth method using 96-well microplates. Ciprofloxacin and Fluconazole were used as the standard drugs. The minimum inhibitory concentration (MIC) value of the essential oil was also determined. The GC-MS analysis showed that the oil contained 10 compounds with α-citral, β-citral and squalene as the major compounds at 36.96%, 25.41% and 20.07% respectively. Other constituents were β-linalool (4.06 %), α-thujenal (3.73 %), hydroxylneoisolongifolane (2.91%), β-myrcene (1.85%), 2-hydroperoxyhexane (1.82%), α-farnesene (1.61%) and 1,2-epoxy-3-propoxy propane (1.59%). The antimicrobial assay showed that the essential oil possessed antimicrobial activity against *E. coli* with an MIC of 25µg/ml. The isomeric composition of citral in *E. Suaveolens* is 59.3% α-citral and 40.7% β-citral. The antimicrobial activity of the oil against *E. coli* may be due to high content of citral.

KEYWORDS: *Erythrophleum suaveolens* (*E. suaveolens*), *Staphylococcus aureus, Pseudomonas aeruginosa,* hydroxylneoisolongifolane.

INTRODUCTION

Plant essential oils are the "essence" of the plants bearing them. They are the constituents of the plant that are volatile at ambient temperature and are odoriferous. Hence, they are also referred to as volatile oils or ethereal oils.[1] Some essential oils have a broad spectrum antimicrobial activity against diverse groups of pathogens.[2] The possible mechanism for the antimicrobial activity is due to the interference of the constituents of the essential oil with the respiration and electron transport in a variety of microorganisms.[3] Essential oils are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, barks, woods and roots or rhizomes. Essential oil constituents are generally classified into monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, diterpenes, oxygenated diterpenes, triterpenes, oxygenated triterpene among others.[4] Essential oils have multiple biological activities such as antibacterial,[5] antifungal,[6] anticancer,[7] and antioxidant.[8,9] Essential oils are used for a wide variety of purposes such as flavoring, perfumery,[10] aromatherapy, food preservation,[11,12] and as raw materials for the synthesis of other compounds.[3]

Erythrophleum belonging to the family Caesalpiniaceae consists of 10 species distributed in Africa, from Senegal to Sudan in the west, Kenya in the east and from Zimbabwe to Mozambique in the south. *Erythrophleum suaveolens* has been introduced as an ornamental plant in

tropical Asia.[13] This herbaceous perennial plant with a characteristic odour is prevalent in Mozambique and its botanical characteristics had been described in the literature.[14] The leaves of *E. suaveolens* (red water tree) are poisonous to goats, horses and cattle. The leaves of *E. suaveolens* are used as pest and insect repellent; the leaves are placed in silos to keep insect and pest away. In Tanzania the leaves are pounded into a pulp and thrown into streams as fish poison. In Nigeria it is used as purgative, emetic, disinfectant, anaesthetic and as poison for execution. In Jukun (Benue state) it is used as a charm against witchcraft.[15]

The phytochemistry of the Erythrophyleum genus had been reported.[16] The anaesthetic effect of *Erythrophleum suaveolens* on clariid catfish and the effect of cold water extract of the stem bark of *E. suaveolens* on gastrointestine muscle of rabbit jejunum (oryctolague) had been reported.[17] Dongmo et al.,[18] reported the anti inflammatory and analgesic properties of the stem bark extracts of *E. suaveolens*. The in-vitro antibacterial activity of the stem bark of *E. suaveolens* had been reported.[19] Furthermore, Ngounou *et al*.,[20] reported the antimicrobial activity of diterpenoid and alkaloids isolated from *E. suaveolens.*

MATERIALS AND METHODS

Collection of plant material

The plant materials were collected from Suleja, Niger State, North Central Nigeria in the month of October 2017 by Mallam Muazzam Ibrahim. The identification and authentication of the plants were done by a taxonomist at the Institute for Pharmaceutical Research and Development, Abuja.

Essential oil isolation

500 g of air-dried leaf of *E. suaveolens* was chopped into small pieces and transferred into a 2L round bottom flask; 1000 ml of distilled water was added. The essential oil was isolated by hydrodistillation method using a Clavenger type apparatus for 4 h. Yield of the essential oil was determined. The essential oil obtained was dried with anhydrous sodium sulphate, filtered through 0.22 micron filter and used within 30 minutes for analysis.

Gas Chromatography-Mass Spectrometry Analysis

The GC-MS analysis of the essential oil of *E. suaveolens* was done by 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 Optima-5ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25µm. The carrier gas was helium with flow rate of 1.61 ml/min. The program used for GC oven temperature was 60 -180^{o}C at a rate of 10^{o}C/min, then held at 180^{o}C for 2 minute, followed by 180 -280^{o}C at a rate of 15^{o}C/min, then again held at 280^{o}C for 4 minutes. The injection port temperature was 250^{o}C while detector temperature was 280^{o}C. Diluted sample (1/100 in hexane, v/v) of 1.0 µl was injected using autosampler and in the split mode with ratio of 10:90. The analysis was performed in triplicate. Individual constituents were identified by referring to compounds known in the literature, [11] and also by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentage of each component was reported as raw percentage based on the total peak area.

Microbial Strains

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, *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 13883), *Escherichia coli* (ATCC 10798), fungi *Candida albicans* (ATCC No. 2876) and *Mycobacterium bovis* BCG (ATCC 35737).

Antimicrobial Activity

The antimicrobial activity of essential oil of *Erythrophleum suaveolens* was carried out using microdilution broth method in 96-well microplate. The oil was dissolved in dimethyl sulfoxide (DMSO) followed by addition of sterile Mueller-Hinton nutrient broth to achieve concentration of 505 v/v. The organisms adjusted to 0.5McFarland was inoculated in duplicates and incubated for 18 hours at 37^{o}C and the results were read spectrophotometrically at 450nm. 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, Lagos 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 broth, control culture (inoculum), Ciprofloxacin, Fluconazole, essential oils and DMSO were performed.

The minimum inhibitory concentration (MIC) value of the essential oil of *Erythrophleum suaveolens* was determined in triplicate by the microdilution broth method in 96-well microplates. 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 was used as a positive control for bacteria and Fluconazole was used as the standard drug for fungi at stock concentration of 50 µg/ml. Controls of sterility for the Mueller-Hinton broth, control culture (inoculum), Ciprofloxacin, Fluconazole, essential oil and DMSO were performed. The microplates were closed and incubated aerobically at 37 °C for 24 h. The MIC values were determined as the lowest concentration of essential oil capable of inhibiting the growth of the microorganisms.

RESULTS

The results of the GC-MS analysis of essential oil obtained from dried leaf of *E. suaveolens* are presented in figure 1 and Table 1. The GC-MS analysis revealed that the oil contained 10 compounds with α-citral, β-citral and squalene as the major compounds at 36.96%, 25.41% and 20.07% respectively. Other constituents are β-linalool (4.06 %), α-thujenal (3.73 %), hydroxyl-neoisolongifolane, (2.91%), β-myrcene (1.85%), 2-hydroperoxyhexane (1.82%), α-farnesene (1.61%) and 1,2-epoxy-3-propoxy Propane (1.59%).

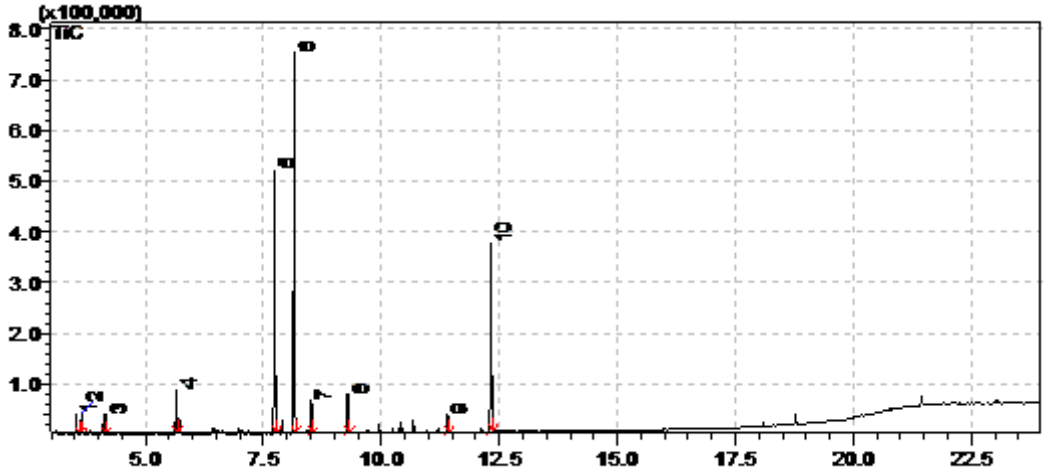

Figure 1: Chromatogram of leaf essential oil composition of *Erythrophleum suaveolens.*

Table 1: Result of the GC-MS analysis of leaf essential oil of *Erythrophleum suaveolens.*

Peak#	Name	Retention Time	% Composition
1	1,2-Epoxy-3-propoxy propane	3.533	1.59
2	2-Hydroperoxyhexane	3.646	1.82
3	β-Myrcene	4.145	1.85
4	β-Linalool	5.647	4.06
5	β-Citral	7.734	25.41
6	α-Citral	8.149	36.96
7	Hydroxyl-neoisolongifolane	8.515	2.91
8	α -Thujenal	9.282	3.73
9	α-Farnesene	11.401	1.61
10	Squalene	12.320	20.07

DISCUSSION

The antimicrobial assay showed that the essential oil possessed antimicrobial activity against only *E. coli* with an minimum inhibitory concentration (MIC) of 25 µg/ml. Generally, essential oils have been reported to possess antiseptic properties and are active against a wide range of bacteria including, the antibiotic resistant strains. Moreover, they are also active against fungi and yeasts (Candida).[21] Similarly, Janssen *et al.*,[22] reported that essential oils have potent antimicrobial activities against gram-positive bacteria, yeast, and dermatophytes. The leaf essential oil of *Erythrophleum suaveolens* was composed of 62.37% citral. Citral is an isomeric mixture of α-citral/geranial (E-3,7-dimethyl- 2,6-octadienal) and β-citral/neral (Z-3,7-dimethyl-2,6-octadienal), Citral is a very significant oxygenated monoterpene aldehyde, which occurs naturally in variety of herbs and plants. It is used extensively in the food, cosmetic, and detergent industries.[23] In addition to its characteristic aroma, citral has anti-viral, antimicrobial, sedative properties;[21] anti-inflammatory properties[24] and antibacterial activity.[25] Pattnaik et al.,[26] investigated the antimicrobial activity of citral against 18 bacteria (including gram-positive cocci and rods and gram-negative rods) and 12 fungi (three yeast-like and nine filamentous) and reported that citral effectively inhibited 15 bacterial and all 12 fungi. The antimicrobial activity of the essential oil of of *E. suaveolens* in this study might be due to its high content of citral (36.96% α-citral and 25.41% β-citral). The antimicrobial activities of essential oils have been associated with high content of oxygenated constituents.[27] The result of this study was consistent with previous report.[28] Similarly, it had been reported that norerythrosauvelide, a diterpenoid from stem bark extract of *E. suaveolens*, showed potent antimicrobial activities against *E. coli*.[20] The wound healing activity of *E. Suaveolens* has also been reported.[29]

The essential oil of *Erythrophleum suaveolens* is composed of 20.07% squalene, a triterpene, which is an

intermediate in the cholesterol biosynthesis pathway. It is widely distributed in nature, with reasonable amounts found in olive oil, palm oil, wheat-germ oil, amaranth oil, and rice bran oil.[30] Squalene has different pharmacological properties including hypolipidemic, hepatoprotective, cardioprotective, antioxidant and antitoxicant activity. Squalene is effective in the treatment of diabetes mellitus type 2 and can potentiate the activity of some antitumor (antiblastoma) preparations.[31] The essential oil of *Erythrophleum suaveolens* contained β-linalool (4.06%), a naturally occurring monoterpene alcohol found in flowers and plants. β-linalool is known to exhibit biological activities such as anti-inflammatory,[32] analgesic,[33] anticonvulsant,[34] sedative[35] and anti-anxiety,[36] β-myrcene (1.85%) is an acyclic monoterpene commonly found in nature alongside other terpenes essential oils.[37] β-myrcene has been proven to possess anti-inflammatory,[38] anticonvulsant and anti-nociceptive activities.[39] Furthermore, the compounds in the essential oil of *E. Suaveolens* leaf are responsible for its ability to protect store grains against insects, pathogenic and spoilage microorganisms [40] this justify the use of *E. suaveolens* leaf as preservative for grains stored in silos.

CONCLUSION

The essential oil of *E. Suaveolens* is rich in monoterpenes and triterpene. The major compound in the oil is citral (62.37%), an oxygenated compound followed by squalene (20.07%), a triterpene. The isomeric composition of citral in *E. Suaveolens* is 59.3% α-citral and 40.7% β-citral. The antimicrobial activity of the essential oil against *E. coli* may be due to high content of citral.

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