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Full Length Research Paper

Chemical composition and antimicrobial activity of the seed oil of *Entandrophragma angolense* (Welw) C.DC.

Abayomi T. Orishadipe[1], Nneka N. Ibekwe[2], Akinbo A. Adesomoju[3] and Joseph I. Okogun[2]

[1]Chemistry Advanced Laboratory, Sheda Science and Technology Complex, P. M. B. 186 Garkipo, Sheda, Abuja, Nigeria.
[2]National Institute for Pharmaceutical Research and Development, P. M. B. 21 Idu Industrial Estate, Idu, Abuja, FCT Nigeria.
[3]Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

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The seed of *Entandrophragma angolense* yielded 59% oil (w/w) on extraction with n-hexane. Methylation and gas chromatography-mass spectrometry (GC-MS) analysis of the methylated seed oil of *E. angolense* gave 11-octadecanoic acid methyl ester as major component (43.2%). Other fatty acid methyl esters (FAMEs) detected were hexadecanoic acid methyl ester, ricinoleic acid methyl ester, stearic acid methyl ester and eicosadienoic acid methyl ester. Preliminary antimicrobial evaluation of this seed oil showed activity against *Salmonella gallinallum* and *Klebseilla pneumonia*.

Key words: *Entandrophragma angolense*, octadecanoic, *Salmonella gallinallum*, *Klebseilla pneumonia*.

INTRODUCTION

Entandrophragma angolense is a large tropical forest tree that belongs to the family Meliaceae popularly called the mahogany tree (Hutchinson and Dalziel, 1958). The plant is used locally in antimalarial and antiulcer preparations (Njar et al., 1995).

Previous chemical investigations of this plant species yielded the following chemical compounds as its constituents: gedunin, methyl angolensate, B-sitosterol and entadrolide (Akinsanya et al., 1960; Okorie and Taylor, 1977). Recently, we reported the isolation of some tirucallane type triterpenoids, gallic acid, methyl gallate and pentagalloyl glucose from the leaves of the plant (Orisadipe et al., 2005; Orishadipe et al., 2008).

Biological investigation of extracts and isolates from *E. angolense* revealed that the stem bark had antiulcer activity (Njar et al., 1995) and the antiulcer agent was identified as methyl angolensate (Njar et al., 1995; Orishadipe et al., 2008). It has also been reported thatgedunin exhibit *in vitro* activities against plasmodium falsiparum (Bray et al., 1990). Moreover, gedunin was reported to exhibit antineoplastic activity (Sax, 1979). Methyl angolensate was reported recently to be active against leukemic cell lines (Chiruvella et al., 2008; Chiruvella and Raghavan, 2011; Kishore et al., 2010). Furthermore, gedunin was reported to inhibit ovarian cancer cell lines proliferation (Kamath et al., 2009).

The present investigation is directed at identifying the chemical composition of the seed oil of *E. angolense* collected from Iyere-Owo, Ondo state, Nigeria and to evaluate its antimicrobial properties and as such to find possible usage for this underutilized part of this plant.

MATERIALS AND METHODS

Plant materials

The seeds of *E. angolense* were collected from Owo in Ondo state, Nigeria and were authenticated by Mr Ohaeri of NIPRD herbarium. A voucher specimen was deposited in the herbarium with number 3606. These plants materials were crushed in a mortar.

Extraction

The crushed seed (45 g) was extracted in a soxhlet extractor for 6 h using n-hexane as solvent. The extract was concentrated on a rotary evaporator to give a colourless viscous oil (27 g) yield of 59%.

*Corresponding author. E-mail: oorisat@yahoo.com.

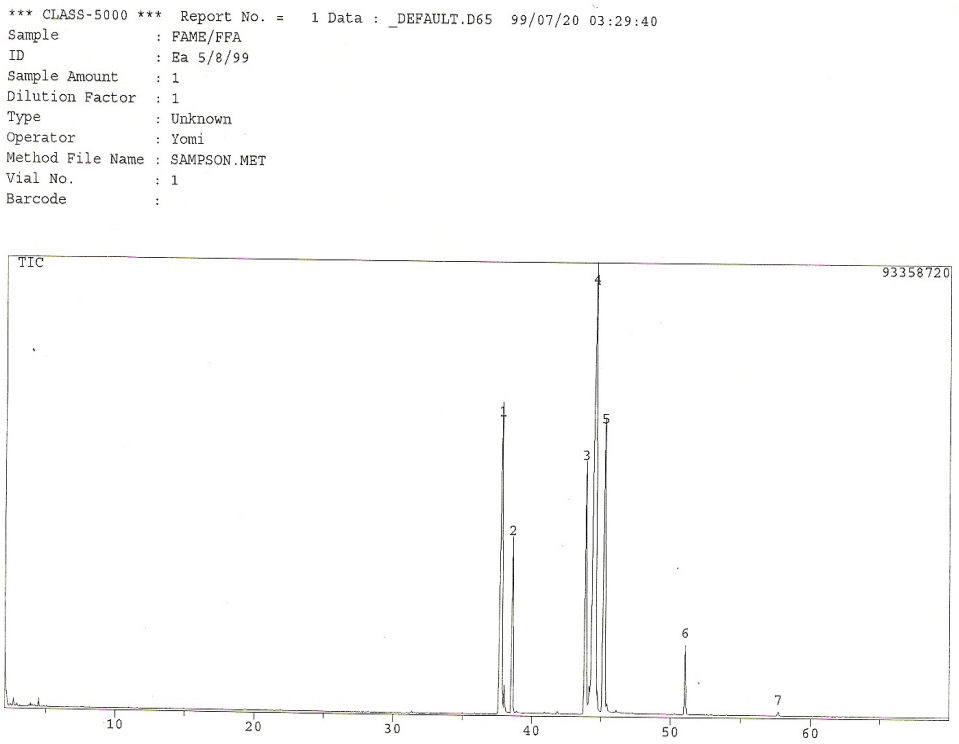

Figure 1. Chromatogram of the GC-MS analysis of the seed oil of *E. angolense*.

Methylation

The oil sample (400 mg) was dissolved in petroleum ether and to that was added 0.5 N sodium methoxide in dry methanol with few numbers of anti-bumping granules added to the mixture. The mixture was heated at 40°C for 5 min on a water bath to effect the transmethylation. The excess sodium methoxide was neutralized with 15% aqueous solution of sodium hydrogen sulphate. The fatty acid methyl esters (FAMEs) were extracted by partitioning the mixture with petroleum ether. Aliquot from the ether phase was dried using sodium sulphate and use for gas chromatography-mass spectrometry (GC-MS) analysis.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the FAMEs of the seed oil of *E. angolense* was carried out on a computerized Shidmadzu GC-17A interfaced with a quadrapole mass spectrometer QP-5000 machine. The GC was equipped with a DB-1 column (30 m, 0.32 mm I d, film thickness 0.25 µm) (J & W Scientific, Folson, CA 9630-4714, USA). The column oven temperature was programmed from 80 to 250°C at 10°C/min; the interface temperature was 230°C and detector temperature at 250°C. Carrier gas He (1.6 ml/min). Mass spectra were taken with ionization voltage of 70 eV.

Identification of components was based on comparing the MS data of individual component with those of authentic standards held in the Shidmadzu NIST 62 library.

Antimicrobial activity determination

Materials used include stock culture of standard American Type Culture Collection, molten agar, Petri dishes and MacFarland standard. The test organisms were standardized, after inoculation with freshly prepared nutrient broth from an overnight culture for 2 to 5 h before use. The standardized bacteria suspension was compared to the turbidity of half (0.5) McFarland standard (105 cfu).

Employing agar dilution method

The test extract (48 mg) was dissolved in sterile water (3 ml) to give a concentration of 16 mg/ml of the extract suspension (Perez et al., 1990). Thereafter, 1 ml of this suspension was taken and added into 15 ml of water with sterile nutrient agar. This was mixed well and poured into plates and allowed to solidify. Same procedure was repeated with 0.5 ml of the 16 mg/ml suspension and that gave a concentration of 0.5 mg/ml. The final concentrations of the media infused with the extract were 1 and 0.5 mg/ml. The plates were inoculated with standardized pure culture of each test organism (0.1 ml). The cultures were incubated at 37°C for 18 to 24 h, and the degree of inhibition was measured as a zone of inhibition (area of no growth) diameter (mm).

RESULTS AND DISCUSSION

The result of GC-MS analysis of the methylated seed oil of *E. angolense* is shown in Figure 1, and Tables 1 and 2. The antimicrobial profile of the seed oil is shown in Table 3.

The GC-MS analyses of the FAMEs of the seed oil revealed the presence of 7 fatty acids methyl esters of which 5 were identified (Figure 1, and Tables 1 and 2). The identified FAMES are hexadecanoic acids methyl

Table 1. The yield and retention time of both the known and unknown.

Name	Retention time	Percent yield
Hexadecanoic acid ME	37.85	19.53
Unknown	38.64	6.52
Eicosadienoic acid ME	43.93	11.81
11-Octadecenoic acid ME	44.61	43.16
Stearic acid ME	45.27	16.72
Ricinoleic acid ME	51.07	2.10
Unknown	57.53	0.16

Table 2. The yields of methylated oil from Ghana and Nigeria.

Name	Percent yield (Nigeria)	Percent yield (Ghana)
Hexadecanoic acid ME	19.5	6.4
Hexadecenoic acid	ND	16.5
Eicosadienoic acid	11.81	ND
11-Octadecenoic acid ME	43.16	39.4
Stearic acid ME	16.72	15.4
Ricinoleic acid ME	2.1	2.6
Unknown	0.16	

Table 3. Results of the preliminary antimicrobial assay.

Concentration of extract	Organisms					
	E. coli	***S. aureus***	***S. gallinallum***	***K. pneumonia***	***P. aeruginosa***	***B. substilis***
1000 mg/ml	-	-	++	++	-	-
500 mg/ml	-	-	-	-	-	-
OVC	++	++	++	++	++	++
MSC	-	-	-	-	-	-

Key: ++, Activity observed; -, no activity observed.

ester, eicosadienoic acid methyl ester, 11-octadecenoic acid methyl ester, stearic acid methyl ester, and ricinoleic acid methyl ester and two of the components yet to be identified. This result was in agreement with the work of Robert and Kathleen (1984) who reported $\Delta 11$ unsaturation in the fatty acids of the oils of *E. angolense* from Ghana and Ascelpias.

They also reported cis-vaccenic acid as a chemotaxonomic marker in *Entandrophragma* between 30 to 50% in yield. The biological effects of the n-6 fatty acids are largely mediated by their conversion to n-6 eicosanoids that bind to diverse receptors found in every tissue of the body. The conversion of tissue arachidonic acid (20:4n-6) to n-6 prostaglandin and n-6 leukotriene hormones provides many targets for pharmaceutical drug development and treatment to diminish excessive n-6 actions in atherosclerosis, asthma, arthritis, vascular disease, thrombosis, immune-inflammatory processes, and tumor proliferation. Nevertheless, a proper balance of omega-6 and omega-3 fatty acids is essential for health benefits (Franzen-Castle and Ritter-Gooder, 2010).

The oil from *Entandrophragma* could be important industrially. Undecadionic acid produced from the cleavage of 11-octadecanoic acid at the double bond could be used for the production of nylon II. Besides, the undecadioic acid from this reaction also would produce heptanoic acid, which is potentially useful in the production of lubricants. The preliminary evaluation of the oil for antimicrobial activity showed activity against *Salmonella gallinallum* and *Klebseilla pneumonia* (Table 3).

Conclusion

The fatty acid profile of the seed oil of *E. angolense* from Southwestern Nigeria has been established; the major difference in composition of the methylated seed oil of *E. angolense* from Nigeria and Ghana environments were

also identified. The presence of omega-6 fatty acid in this oil indicates it is of potential health benefit, since omega 6-fatty acid has been reported to play an important role in brain and heart function, and in normal growth and development (Franzen-Castle and Ritter-Gooder, 2010). Moreover, it can be of great industrial use in the production of nylon and lubricants.

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