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Moses S. Owolabi
Department of Chemistry, Lagos State University P.M.B. 001, Ojo, Lagos, Nigeria.

Oladipupo A. Lawal
Department of Chemistry, Lagos State University P.M.B. 001, Ojo, Lagos, Nigeria.

Noura S. Dosoky
Department of Chemistry, University of Alabama in Huntsville, Alabama 35899, USA

Prabodh Satyal
Department of Chemistry, University of Alabama in Huntsville, Alabama 35899, USA

William N. Setzer
Department of Chemistry, University of Alabama in Huntsville, Alabama 35899, USA.

Correspondence:

Moses S. Owolabi
Department of Chemistry, Lagos State University P.M.B. 001, Ojo, Lagos, Nigeria.
E-Mail: sunnyconcept2007@yahoo.com

Chemical composition, antimicrobial, and cytotoxic assessment of *Mitracarpus scaber* Zucc. (Rubiaceae) essential oil from southwestern Nigeria

Moses S. Owolabi, Oladipupo A. Lawal, Noura S. Dosoky, Prabodh Satyal, and William N. Setzer

ABSTRACT

The leaf essential oil composition of *Mitracarpus scaber* was determined by GC-MS. The oil was dominated by pentadecanal (38.5%), methyl (7Z, 10Z, 13Z)-hexadecatrienoate (11.8%), 2,3-dimethylnaphthoquinone (6.0%), and α-pinene (5.4%). The oil was screened for antimicrobial activity using the broth dilution technique and screened for *in-vitro* cytotoxic activity against human MCF-7 breast tumor cells. *M. scaber* leaf oil showed only marginal antibacterial activity against *Bacillus cereus* (MIC = 625 μg/mL), marginal antifungal activity against *Aspergillus niger* (MIC = 313 μg/mL), and marginal cytotoxic activity on MCF-7 cells (58.7±7.6% kill at 100 μg/mL).

Keywords: MCF-7, pentadecanal, methyl (7Z, 10Z, 13Z)-hexadecatrienoate, 2, 3-dimethylnaphthoquinone.

1. Introduction

The genus *Mitracarpus* (Rubiaceae) consists of about 30 species distributed in the Neotropics [1]. *Mitracarpus scaber* Zucc. [Syn. *Mitracarpushirtus* (L.) DC], an African representative of the genus, is a perennial herbaceous shrub with rough leaves (Olorode, 1984). In Nigeria, the plant is known as '*Obuobwa*' in Igbo, '*Gududalin*' in Hausa, and '*Irawole*' in Yoruba [2]. Leaves of *M. scaber* have been widely used in traditional medicine in West Africa for the treatment of headaches, toothaches, amenorrhea, dyspepsia, hepatic diseases, skin infections, veneral diseases, as well as leprosy [3]. Leaf extracts of *M. scaber* have shown antimicrobial [3, 4, 5], antitrypanosomal [6], hepatoprotective [7], and anti-inflammatory [8] effects. Several bioactive components have been isolated from *M. scaber* extracts including azaanthraguinone [9], gallic acid, 3,4,5-trimethoxybenzoic acid, 4-methoxyacetophenone, 3,4,5-trimethoxyacetophenone [3], oleanolic acid, and ursolic acid [10]. In this work, we expand the pharmacognistic appraisal of *M. scaber* with an evaluation of the chemical composition, antimicrobial and cytotoxic properties of the leaf essential oil.

2. Materials and Methods

2.1 Plant Material

Fresh leaves of *M. scaber* were collected from a mature plants in October, 2011, from Lagos State University, Ojo Campus staff quarters, Lagos state, Nigeria. The plant was taxonomically identified and authenticated at the Herbarium of the Department of Botany of the University of Lagos. The plant was air-dried for three days, pulverized, and a sample (350 g), subjected to hydrodistillation in a Clevenger-type apparatus for 4 h, to give 0.83% yield (w/w) of essential oil. The oil was dried over anhydrous sodium sulfate and stored in a sealed vial under refrigeration prior to analysis.

2.2 Gas Chromatographic / Mass Spectral Analysis

The volatile oil sample was subjected to GC-MS analysis on an Agilent system consisting of an Agilent model 6890 gas chromatograph, an Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/sec) and an Agilent Chemstation data system.

The GC column was a HP-5ms fused silica capillary with a (5% phenyl)-methyl polysiloxane stationary phase, film thickness 0.25 µm, length 30 m, and internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and a flow rate of 1.0 mL/min. Inlet temperature was 200 °C and MSD detector temperature was 280 °C. The GC oven temperature program was used as follows: 40 °C initial temperature, hold for 10 min, increased at 3 °C/min to 200 °C, increased 2 °C/min to 220 °C. The sample was dissolved in dichloromethane and a splitless injection technique was used. Identification of the constituents of the volatile oil was achieved based on their retention data (retention indices) determined with reference to C_{10}-C_{40}*n*-alkane homologous series, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [11] and stored on the MS library [NIST database (G1036A, revision D.01.00) / ChemStation data system (G1701CA, version C.00.01.08)].

2.3 Antimicrobial Screening

M. scaber leaf oil was screened for antibacterial activity against *Bacillus cereus* (ATCC No. 14579), *Staphylococcus aureus* (ATCC No. 29213), *Pseudomonas aeruginosa* (ATCC No. 27853), and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique [12]. Dilutions of the samples were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 µL of 1% w/w solutions of samples in DMSO plus 50 µL CAMHB. The sample solutions were serially diluted (1:1) in CAMHB in 96-well plates to give concentrations of 2500, 1250, 625, 313, 156, 78, 39, and 20 µg/mL. Organisms at a concentration of approximately 1.5×10^8 colony-forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 hours; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Gentamicin was used as a positive antibiotic control; DMSO was used as a negative control. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using YM broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

2.4 Cytotoxicity Screening

Human MCF-7 breast adenocarcinoma cells (ATCC No. HTB-22) [13] were grown in a 3% CO_2 environment at 37 °C in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 100,000 units penicillin and 10.0 mg streptomycin per liter of medium, 15 mM of Hepes, and buffered with 26.7 mM $NaHCO_3$, pH 7.35. Cells were plated into 96-well cell culture plates at 2.5×10^4 cells per well. The volume in each well was 100 µL. After 48 h, supernatant fluid was removed by suction and replaced with 100 µL growth medium containing 1.0 µL of DMSO solution of the essential oil (1% w/w in DMSO). This gave a final concentration of 100 µg/mL in each well. Solutions were added to wells in four replicates. Medium controls and DMSO controls (10 µL DMSO/mL) were used. Tingenone [14] was used as a positive control. After the addition of oil, plates were incubated for 48 h at 37 °C in 5% CO_2; medium was then removed by suction, and 100 µL of fresh medium was added to each well. In order to establish percent kill rates, the MTT assay for cell viability was carried out [15]. After colorimetric readings were recorded (using a Molecular Devices Spectra MAX Plus microplate reader, 570 nm), average absorbencies, standard deviations, and percent kill ratios ($\%kill_{oil}/\%kill_{DMSO}$) were calculated.

3. Results and Discussion

The chemical composition of *M. scaber* leaf essential oil was investigated by gas chromatography – mass spectrometry (GC-MS) and is summarized in Table 1. Twenty compounds were identified in the volatile oil accounting for 95.1% of the composition. The oil was dominated by the long-chain aldehyde pentadecanal (38.5%) and the polyunsaturated fatty acid ester methyl (7*Z*,10*Z*,13*Z*)-hexadecatrienoate (11.8%), with lesser quantities of 2,3-dimethylnaphthoquinone (6.0%), and α-pinene (5.4%). The composition of *M. scaber* leaf oil in this work is notably different from a previous study [16], which showed to oil to be composed largely of saturated fatty acids (64.0%) and no monoterpene or sesquiterpene hydrocarbons.

The essential oil of *M. scaber* was screened for antibacterial activity against *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*, antifungal activity against *A. niger*, and *in-vitro* cytotoxic activity against human MCF-7 breast tumor cells. The oil showed no antimicrobial activity against *S. aureus*, *E. coli*, or *P. aeruginosa* (MIC = 1250 µg/mL) and only marginal activity against *B. cereus* (MIC = 625 µg/mL) or *A. niger* (MIC = 313 µg/mL). In addition, *M. scaber* leaf oil was only marginally cytotoxic to MCF-7 cells (58.5±7.6% kill at 100 µg/mL).

4. Conclusion

The leaf essential oil of *Mitracarpus scaber* showed only marginal antimicrobial or cytotoxic activities. Thus, the ethnobotanical uses and the bioactivity of the plant must be due to non-volatile constituents (see above) rather than essential oil components.

Table 1: Chemical composition of *Mitracarpus scaber* leaf essential oil

RI[a]	Compound	%
799	*n*-Octane	1.0
809	2-Hexanol	2.2
941	α-Pinene	5.4
978	β-Pinene	1.5
993	2-Pentylfuran	1.6
1002	Unidentified[b]	3.1
1024	*p*-Cymene	3.0
1028	Limonene	1.5
1058	γ-Terpinene	3.8

1284	(*E*)-Anethole	3.4
1418	(*E*)-Caryophyllene	0.9
1453	α-Humulene	1.2
1463	*cis*-Cadina-1(6),4-diene	0.9
1487	(*E*)-β-Ionone	3.3
1524	δ-Cadinene	4.3
1613	Tetradecanal	1.4
1614	1,10-di-*epi*-Cubenol	2.4
1628	2,3-Dimethylnaphthoquinone	6.0
1715	Pentadecanal	38.5
1798	Octadecane	1.2
1845	Unidentified[c]	1.8
1894	Methyl (7*Z*,10*Z*,13*Z*)-hexadecatrienoate	11.8
	Total Identified	95.1

[a] RI = Retention Index determined with respect to a series of *n*-alkanes on an HP-5ms column.
[b] MS, m/e(%): 136(79), 107(89), 94(73), 91(25), 81(61), 79(79), 77(66), 68(100), 53(54).
[c]MS, m/e(%): 124(2), 109(14), 95(15), 85(30), 71(67), 69(27), 59(41), 58(100), 57(61), 55(41).

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