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Full Length Research Paper

Antibacterial activity and cytotoxicity of essential oil of *Lantana Camara* L. leaves from Nigeria

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The chemical composition of essential oil obtained from air-dried leaves of *Lantana camara* L. by hydrodistillation was analyzed by GC and GC-MS. A total of thirty-two compounds representing 74.8% of the oil were identified. The main constituents were 1,8-cineol (15.8%), sabinene (14.7%) and β-caryophylene (8.9%). The essential oil was tested for antibacterial activity against 6 strains, using disc diffusion method, and for cytotoxicity using brine-shrimp lethality assay. The oil showed moderate activity against *Candida albican*, *Bacillus subtilis*, *Staphylococcus typhi*, *Pseudomonas aeruginosa* and *Bacillus aureus.* These activities support its potential use as a remedy for bacterial infectious diseases. The essential oil with LC50 value of 0.01 was found to be highly toxic.

Key words: *Lantana camara* L, antimicrobial, cytotoxicity, essential oil, 1,8-cineol, sabinene.

INTRODUCTION

Lantana camara L. is a noxious weed belonging to Verbenaceae family which comprises of about 650 species spread over 60 countries. They are native to tropical and warm regions worldwide. They are mostly cultivated for their ornamental purpose because of their flowers which can be pink, orange, yellow, white lilac depending on the variety. *L. camara* leaves have been reported to make animals ill after ingestion and its berries are toxic before they become ripe (Wolfson and Solomon, 1964; Mc Lennan and Amos, 1989; Motion, 1994). *L. camara* oil and extracts are used in herbal medicine for the treatment of various human diseases such as skin itches, leprosy, cancers, chicken pox, measles, asthma, ulcers, tumors, high blood pressure, tetanus, rheumatism, etc (Begun et al., 1995; Ghisalberti, 2000; Ross, 1999). Extracts from the leaves have been reported to have antimicrobial, fungicidal, insecticidal and nematicidal activity (Saksena and Tripathi, 1985; Begun et al., 1995; Sharma et al., 1999; Deena and Thoppil, 2000). The essential oil of *L. camara* from different regions of the world has been reported by many workers (Da Silva et al., 1999; Sefidkon, 2002; Kasali et al., 2004). The oils differ in their chemical compositions according to geographic origin of the plants. Da Silva et al. (1999) reported differrences in essential oil composition of *L. camara* collected at different places in the Amazon region of North Brazil.

Though, the essential oil composition of *L. camara* from Nigeria has been previously reported (Kasali et al., 2004) but the antibacterial and cytotoxicity of the oil have not been investigated. In this paper, we report for the first time the antibacterial and cytotoxicity of the essential oil of *L. camara* leaves from Nigeria and the newly identified compounds.

MATERIALS AND METHODS

Plant material

Fresh leaves of *L. camara* L were collected from the Botanical Garden, University of Ibadan, Nigeria. The plant was authenticated by Mr. Shasanya Olufemi of the Forest Herbarium, Forest Research Institute of Nigeria (FRIN), where voucher specimen was deposited (FHI 107914)

Isolation of essential oil

Air-dried leaves (320 g) were subjected to hydrodistillation in all-glass Clevenger type apparatus for 4 h in accordance with British Pharmacopoeia (1980) method. The essential oil was collected and dried over anhydrous sodium sulphate and stored at 4°C until analysed.

Gas chromatographic analysis

Gas Chromatographic (GC) analysis was performed on an Agilent Model 6890 Gas Chromatography equipped with a DB-5 fused silica capillary column (30 x 0.25 mm, film thickness 0.25 µm). Oven temperature was held at 60°C for 2 min and then programm-

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ed to 250°C at a rate of 4°C/min, with final hold time of 20 min; helium was used as carrier gas at a flow rate of 1 ml/min. Percentage composition of each constituent was calculated by integration of the GC peak areas.

Gas chromatography – mass spectrometric (GC-MS) analyses

GC-MS analyses were carried out on an Agilent Model 6890 GC with split/splitless injector interfaced to an Agilent 5973 Mass Selective Detector. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage, 70eV, ion source temperature, 230°C. The GC was fitted with a DB-5 fused silica capillary column (30 x 0.25 mm, film thickness 0.25 µm). The GC operating parameters were the same as above.

Identification of components

The components of the essential oil were identified based on the basis of their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra (Adams, 1989) and those of reference compounds from the Library of National Institute of Standard and Technology (NIST) database.

Antimicrobial screening

The essential oil was screened for antimicrobial activities against 6 standard strains of bacteria representing both Gram +ve and Gram –ve (*Candida albican* MTTC 227, *Bacillus subtilis* ATTC 33923, *Staphylococcus typhi* ATTC 2785, *Pseudomonas aeruginosa* ATTC 27856, *Bacillus aureus* ATTC 14579 and *Proteus mirabilis* ATTC 21784). Disc diffusion method was used to determine minimal inhibitory concentration (MIC). The bacteria were grown on nutrient agar (Mueller Hinton) which was prepared by dissolving agar (28 g) in distilled water (1000 ml). The mixture was heated to dissolve and autoclaved at 121°C for 15 min. The nutrient agar was poured into sterile petri dishes at uniform depth of 5 mm and allowed to solidify. The microbial suspensions were streaked over the surface of the agar media using a sterile cotton swab to ensure uniform inoculation. Different concentrations of the oil (10, 100, 1000, 10000 ppm) were prepared in dimethyl sulphoxide (DMSO). Different concentrations (0.01 ml) were impregnated on Whatman filter paper No 2 disc. The discs were then aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37°C for 24 h. The zones of inhibition as well as the minimum inhibitory concentration (MIC) were measured. Disc impregnated with gentamicin (0.01 ml) dissolved in DMSO (5 µg/ml) was used as control.

Brine shrimp lethality test

The shrimps were hatched in sea water for 48 h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vessel with a light source. The essential oil were prepared at 1000, 100 and 10 µg/ml (each test in triplicates) in dimethyl sulphoxide (DMSO). The essential oil (0.5 ml) was introduced in a test-tube and and sea water (4 ml) added. 10 Shrimps per test tube were added for each concentration and made up to 5 ml with sea water. The number of dead shrimps was counted after 24 h. The LC50 was then calculated using the Finney Computer Programme.

RESULTS AND DISCUSSION

Hydrodistillation of the air dried leaves of *L. camara* afforded oil yield of 0.2% (v/w). Thirty-one constituents, representing 74.8% of the oil were identified (Table 1). The main constituents were 1,8-cineole (15.8%), sabinene (14.6%) and β-caryophyllene (8.9%). Other minor constituents were E-nerolidol (5.9%), bicyclogermacrene (2.8%) and β-pinene (2.1%). The composition of the studied *Lanatana camara* specie is similar to those previously reported in Nigeria and Iran (Sefidkon, 2002; Kasali et al., 2004) but differ from species from North Brazil , in which limonene, α-phellandrene germacrene, curcumene α-zingiberen and α-humulene are major constituents (da Silva et al., 1999). However, some new compounds not previously reported in the Nigerian species, such as spathulenol (3.4%), β-gurjunene (0.5%), β-elemene (0.2%) and α-himachalene (0.2%) were detec-

Table 1. Chemical constituents of essential oil of *Lanatana camara* L. from Nigeria.

Constituents	RI	Percentage (%)
α-Thujene	926	0.4
α -Pinene	934	1.9
Camphene	947	1.0
Sabinene	971	14.7
β-Pinene	978	2.1
Octen-3-ol	994	0.3
α -Phellandrene	1006	0.3
3-Carene	1010	1.7
α -Terpinene	1016	0.3
O-cymene	1019	0.2
1,8- Cineole	1030	15.8
(Z)- β - Ocimene	1035	0.9
(E)- β - Ocimene	1045	1.2
δ-Terpinene	1056	0.6
Camphor	1140	1.6
(E)- β - Terpineol	1141	1.4
(Z)- β - Terpineol	1160	1.1
Borneol	1162	1.0
4-Terpineol	1175	1.7
α -Terpineol	1188	1.3
α -Copaene	1376	0.3
β -Elemene	1393	0.2
β -Caryophyllene	1418	8.9
β -Gurjunene	1430	0.5
α -Himachalene	1447	0.2
D-Germacrene	1480	1.4
Bicyclogermacrene	1484	2.8
γ-Cadinene	1513	1.0
(E)- Nerolidol	1568	5.9
Spathulenol	1576	3.4
α -Cadinol	1652	0.4
8-Cedren-13-ol	1688	0.3

[a]Retention indices relative to *n*-alkanes on DB-5 capillary column.

Table 2. Antimicrobial activity of *Lanatana camara* L. from Nigeria.

Microorganism	Minimum inhibitory concentration (ppm)	Zone of inhibition (mm)
P. aeruginosa	10000	11
P. mirabilis	1000	10
B. subtilis	1000	12
C. albican	10000	14
S. typhi	10000	12
B. aureus	10000	11

ted in the present study.

The high percentage of 1,8-cineole (15.8%) may likely be responsible for the traditional use of *L. camara* leave extract in the treatment of rheumatism. 1,8-cineole have been reported to have anti-inflammatory and antinociceptive effects (Santos and Rao, 2000).

The results of the antibacterial test of the essential oil are shown in Table 2. The essential oil shows activity against *P. mirabilis* and *B. subtilis* at minimum inhibitory concentration (MIC) value of 1000 ppm. It shows activity against *P. aeruginosa*, *C. albican*, *S. typhi*, and *B. aureus* at MIC value of 10000 ppm. The antimicrobial activities of the essential oil suggest its usefulness in the treatment of various infectious diseases cause by bacteria.

The essential oil has LC50 value of 0.01 in the Brine Shrimps Lethality assays with upper limit of 0.0496 and lower limit of 0.6606. These values indicate that the oil is highly toxic, confirming the earlier work which showed that *L. camara* is highly toxic to grazing animals and has also caused death in children when unripe berries were eaten (Wolfson and Solomon, 1964; Mc Lennan and Amos, 1989; Motion, 1994).

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