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Chemical Composition and Antibacterial Activity of Essential Oil of *Lantana camara* L.

[1]*Anil Kumar Saikia and* [2]*Ranjan Kumar Sahoo*

[1]Department of Pharmaceutical Sciences, Pharmaceutical Chemistry Division,
Dibrugarh University, Dibrugarh-786004, Assam, India
[2]Department of Pharmaceutical Chemistry,
GIET School of Pharmacy, Rajahmundry-533294 Andhrapradesh, India

Abstract: The chemical constituents of essential oil from the fresh leaves of *Lantana camara* L. was examined by GC-FID and GC-MS technique. Hydro distillation method was used to extract the essential oil and the yield was 0.25%v/w. Forty-one components (93.25%) were identified, among the major components were β-caryophyllene (27.0%),α-humulene (11.8%), sabinene (9.7%), bicyclogermacrene (8.1%) and davanone (4.7%). The disc diffusion method was used for antibacterial testing. The essential oil exhibited significant antibacterial activity against Escherichia coli, Bacillus subtilis, Bacillus cereus and Staphylococcus aureus.

Key words:*Lantana camara* L. · Chemical composition · GC-FID · GC-MS · β-caryophyllene · Antibacterial activity

INTRODUCTION

Lantana camara L. (Verbenaceae) is a woody straggling plant, commonly known as red or wild sage, with various flower colors, leaves with rounded tooth edges which comprises 650 species and indigenous to tropical and subtropical America [1]. Traditionally, L.camara oil and extracts are used in herbal medicine for the treatment of various human diseases such as skin itches, leprosy, high blood pressure, chickenpox, ulcer, asthma, tetanus and rheumatism [2-4]. The extract of leaves are used as antibacterial, antifungal, insecticidal and nemacitidal activity [5, 6]. Different varieties of Lantana have been extensively studied to establish composition of essential oils [7-13]. However; there have been no reports on the constituents of the essential oil of *Lantana camara* L. from Assam, India and its antibacterial activity.

MATERIALS AND METHODS

Plant Material: The fresh leaves of *Lantana camara* L. were collected from the herbal garden of Dibrugarh University, Dibrugarh, Assam, India in March 2010. Theplant was identified and authenticated by Department of Life Sciences, Dibrugarh University. A voucher specimen no. DU/PHC/HRB-3/10 is deposited in the departmental of herbarium store.

Essential Oil Extraction: The fresh leaves of *Lantana camara* (2000gm) were cut into small pieces and hydro distilled using Clevenger apparatus for 5 hours. The essential oil (yield 0.25%v/w) was transferred into a stoppered tube, dried over anhydrous Na_2SO_4 and stored in a refrigerator at 4°C until analyzed.

GC-FID and GC-MS Analyses: The capillary gas chromatography with flame ionization detector was used as the method of choice for quantitative determination. GC analysis was carried out on a Shimadzu Gas chromatograph-2014 fitted with ZB-5 capillary column (cross linked 5% biphenyl and 95% dimethyl siloxane, 30m×0.25mm, film thickness 0.25µm). Nitrogen was used as a carrier gas at 1.11ml/min. The oven temperature was kept initially at 60°C and increased at the rate of 3°C /minute up to 250°C and holding for 10 minutes at final temperature. The injector and detector temperature were 250°C and 280°C respectively. The volume of injection was 0.5µl and split ratio 1:20. GC-MS analysis of essential oil was performed by using Thermo Finnigan

Corresponding Author: Ranjan Kumar Sahoo, Department of Pharmaceutical Chemistry, GIET School of Pharmacy, Rajahmundry-533294 Andhrapradesh, India. Tel: +91-8801301176.

3800 gas chromatograph equipped with multiquadrupole mass spectrometer. The column was used SolGel-1ms type (30m×i.d 0.25mm× film thickness 0.25µm) and operated in full scan between 50-650 a.m.u. The mass spectrometer was operated in the electron ionization mode at 70eV. The transfer line temperature was 275°C. Helium was used as carrier gas at 2 ml/min. The components of essential oil of L. camara were identified based on the kovat retention index. The kovat retention index was calculated with reference to the retention times of authentic hydrocarbons C_8-C_{28} n-alkanes. The individual components of essential oil were confirmed by comparing kovat retention index, co-injection with standards and was compared to mass spectra with published spectra [14] and to the NIST mass spectral library [15].

Determination of Antibacterial Activity

Test Organisms: *Bacillus subtilis* (ATCC11774), *Pseudomonas aeruginosa* (ATCC10662), *Staphyloccus aureus* (ATCC1026), *Bacillus cereus* (ATCC10876), *Escherichia coli* (ATCC10536) and *Kliebsella pneumonia* (ATCC 33495) were collected from the National Chemical Laboratory, Pune, India.

Evaluation of Antibacterial Activity: The antibacterial activity of essential oil of *Lantana camara* L. was determined by the disc diffusion method [16]. The bacterial inoculums suspension were prepared and adjusted with sterile saline (0.9%) to obtain standard turbidity visually comparable of a McFarland no. 0.5 standard (10^5-10^8 CFU/ml). 20ml of Muller-Hinton nutrient agar was poured into each Petri plates to obtain uniform depth and allowed to solidify. 500µl of standard inoculums suspension were spread over Petri plates in order to get uniform microbial growth on test plates. The essential oils were dissolved in 10% DMSO with Tween 80 (0.5%v/v). Then under aseptic conditions, sterilized disc of 6mm diameter (Whatmann filter no.1) were impregnated with essential oil and placed on the surface of Petri plate with sterile forceps. The Petri plates were kept in room temperature for 30 minute to allow the diffusion of oil and they are incubated at 37°C for 24 hour. After the incubation period, the zones of inhibition were measured and recorded. Control discs impregnated with 10µl of DMSO and reference standard Gentamycin (5µg/disc) were used along side the test discs in each experiment. The above experiment were done in triplicate and mean value was calculated.

RESULTS AND DISCUSSION

Chemical Composition of *Lantana camara.* L Essential Oil: Hydro-distillation of fresh leaves of *L.camara* L. afforded pale yellow oil and the yield was 0.25%v/w based on the dry weight of the plant. Forty-one components were identified accounting for 93.25% of the oil (Table 1). The results of the analysis show that the essential oil was rich in sesquiterpene (70.65%) comprising sesquiterpene hydrocarbons (33.35%) and oxygenated sesquiterpenes (37.30%). The main constituents were β-caryophyllene (27.0%), α-humulene (11.8%), sabinene (9.7%), bicyclogermacrene (8.1%) and davanone (4.7%). From these observations, the essential oils of *L. camara* are rich source of sesquiterpene hydrocarbons. The essential oils proportions differ depending on the origin and on the part of plant used but β-caryophyllene was observed as the versatile common component in all Lantana oils analyzed so far.

Antibacterial Activity of *Lantana camara* Essential Oil: As it can be noticed from Table 2, the essential oil of *L. camara* exhibited notable antibacterial activity against all the bacterial strains tested. Gram positive *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* were the most sensitive strains to L. camara essential oil. However, Gram negative *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were not susceptible to the essential oil at lower concentration. As observed, Gram-positive bacteria were more sensitive to the essential oils than gram-negative bacteria and this results are agree with Zaikia [17]. The significant antibacterial activity due to presence of α-pinene, β-pinene, p-cymene and 1, 8-cineole [18-26] in essential oil of L. camara. These chemical components exhibit antibacterial activity by disrupting bacterial cell membrane [27, 28]. It has been shown that α-pinene and β-pinene are able to destroy cellular integrity and therefore, inhibit ion transport process [28, 29]. This is strongly supported by the study on the effects of different essential oil components on outer membrane permeability in Gram -negative bacteria [30] which exerts membrane damaging effects to microbial strains and stimulates leakage of cellular potassium ions which is responsible for lethal action of cellular membrane damage. (Due to hydrophobic nature of the essential oils and their components, which enable them more permeable [27, 31] and exit of molecules, ions [32] will lead to cell death. Revise this sentence carefully).

Table 1: Chemical constituents of the essential oil of *L. camara* L.

Components	KI	Peak area (%)	Method of identification
α- Thujene	930	0.3	MS, RI, Co
α-Pinene	939	2.1	MS, RI, Co
Camphene	954	t	MS, RI,
Sabinene	976	9.7	MS, RI
β-Pinene	979	2.1	MS, RI
β -Myrcene	991	0.7	MS, RI
α -Phellandrene	1003	0.2	MS, RI, Co
α -Terpinene	1017	0.4	MS, RI, Co
p-Cymene	1025	0.8	MS, RI, Co
1,8-Cineole	1018	1.0	MS, RI
(Z)-β-Ocimene	1025	t	MS, RI, Co
Δ-3-Carene	1037	1.3	Co, MS, RI
γ-Terpene	1047	0.4	MS, RI
Trans-Sabinene hydrate	1053	0.6	MS, RI
Terpinolene	1077	t	MS, RI
Linalool	1082	2.4	MS, RI
Camphor	1115	0.4	MS, RI
trans-Verbenol	1145	t	MS, RI
Pinocarvone	1156	t	MS, RI
Terpinen-4-ol	1171	0.2	MS, RI
Δ –Elemene	1325	0.3	MS, RI
α-Cubebene	1339	0.1	MS, RI
α-Copaene	1365	1.05	MS, RI
β-Cubenene	1376	0.6	MS, RI, Co
β-Caryophyllene	1403	27.0	MS, RI, Co
β-Cedrene	1413	2.0	MS, RI, Co
α-Humulene	1437	11.8	MS, RI
trans-β-Farnesene	1440	t	MS, RI
α-Muurolene	1456	1.1	MS, RI
Germacrene D	1462	3.8	MS, RI,
Gurjunene	1471	0.4	MS, RI, Co
Bicyclogermacrene	1477	8.1	MS, RI, Co
γ-Muurolene	1498	1.9	MS, RI
Spathulenol	1523	3.7	MS, RI
Δ-Cadinene	1529	0.8	MS, RI
Germacrene B	1535	1.2	MS, RI
[E]-Nerolidol	1539	1.2	MS, RI
Davanone	1551	4.7	MS, RI
Viridiflorol	1565	0.7	MS, RI
Caryophyllene oxide	1583	t	MS, RI, Co
Muurolol	1644	t	MS, RI

Monoterpene hydrocarbon 18.80
Oxygenated monoterpene 3.80
Sesquiterpene hydrocarbons 33.30
Oxygenated sesquiterpene 37.30
Total identified components 93.25

RI: Retention indices in elution order from ZB-5 capillary column; KI: Kovat's retention indices were calculated using standard hydrocarbon (relative to C_8 to C_{18} n- alkane); MS: mass spectroscopy; t: Trace amount less than 0.1%; Co: co injection

Table 2: Antibacterial activity of L.camara essential oil

		Mean zone of inhibition (mm)	
		Essential oil	Gentamycin
Test microorganism	Source	10µl/disc	5µg/disc
Escherichia coli	ATCC 10536	10.9	20.2
Pseudomonas aeruginosa	ATCC 10662	8.5	16.7
Klebsiella pneumonia	ATCC 33495	6.3	17.2
Bacillus subtilis	ATCC 11774	13.2	18.2
Bacillus cereus	ATCC 10876	14.6	17.8
Staphylococcus aureus	ATCC 1026	12.2	17.0

CONCLUSIONS

The essential oil composition of leaf oil of *L. camara* has been investigated in different parts of India. However, in present study 41 components were identified for the first time which was not reported in previous study. The chief constituents β-caryophyllene may be utilized as natural source for isolation of caryophyllene. The study further indicates that the essential oil obtained from *L.camara* has potential antibacterial activity. However, additional in vivo studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral application.

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