ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *LANTANA CAMARA.L* OF MEKELLE, ETHIOPIA

Shilashi Badasa*[1], G.S.Undrala[2], C.R.Unnithan[2]
[1]Department of Chemistry, Mekelle University, Ethiopia.
Email: crunnithan@yahoo.co.in

Received on 20-12-2012 *Accepted on 08-01-2013*

Abstract

The constituents of essential oil isolated by hydrodistillation of the aerial parts of *Lantana Camara.L* Verbenaceae family, from Mekelle, Northern Ethiopia was examined by GC-MS. A total of 22 chemical constituents representing 97.5% in the essential oil of *L. camara.*were identified by GC-MS analyziz. Copaene with contribution of 25.7% was found to be the principal constituent. Other important components identified were himachalene(13.1%),β-caryophyllene(9.6%),p-cymene(8.4%),β-gujunene(6.1%),ledenealcohol(5.3%),anethol(4.4%),bicyclogermacrene(3.7%) 1,8-cineole(3.3%) epizonarene (3.4%) and thujopsene (3.2%).The essential oil of *Lantana camara* showed antibacterial activities against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria.

Keywords: *Lantana camara*, verbenaceae, essential oil, antibacterial activity.

Introduction

Lantana camara. L is a hardy, evergreen perennial shrub found growing in tropical, subtropical and temperate parts of the world**.**The essential oil of *L. camara* 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[1-3].Extracts from the leaves have been reported to have antimicrobial, fungicidal, insecticidal and nematicidal activity [4-6].The leaf extract also possessed larvicidal activity [7] while extract from flowers of the plant showed repellent activity against mosquitoes [8-9]. In Africa, an infusion of leaves is used to diagnose, rheumatism, asthma, coughs and colds [10].

The essential oil of *L. camara* from different regions of the world has been reported by many workers [11-14]. The oils differ in their chemical compositions according to geographic origin of the plants.

Materials and Methods

Plant material

The aerial parts of *Lantana camara* plant were collected during the month of December 2011 from Mekelle, Northern Ethiopia and **was** identified by the authors and a herbarium sheet was deposited at the Chemistry department, Mekelle University, Mekelle, Ethiopia.

Chemical reagents

All chemicals used in the present study were of analytical grade and obtained from Sigma Co. (St. Louis, MO, USA).

Essential oil extraction

The shade dried aerial parts of *Lantana camara.L* plant collected (1Kg) was subjected to hydro distillation in a Clevenger apparatus for 3h. The essential oil was separated from aqueous layer using a 100 mL capacity separatory funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was pale yellow liquid in appearance which was stored at 4°C in dark brown 5-mL capacity sample bottle until analysis. The yield of the oil was found to be 0.355±0.01 % (w/w) in relation to the dry weight basis.

GC and GC-MS analysis

GC analyses were carried out in Agilent Technology 6890N gas Chromatograph data handling system equipped with a split-split less injector and fitted with a FID using N2 as carrier gas. The column was HP-5capillary column (30m x 0.32mm, 0.25µm film thickness) and temperature program was used as follows: initial temperature of 60 ^{0}C (hold: 2 min) programmed at a rate of 3^{0}C/min to a final temperature of 220^{0}C (hold: 5 min).Both the temperature of injector and FID were maintained at 210^{0}C.

The GC-MS was performed by Perkin Elmer Clarus 500 gas chromatograph equipped with a split-split less injector (spli rtatio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 min x 0.32 mm, 0.25µm film thickness).Helium was used as carrier gas at a flow rate of 1.0ml/min.The GC was interfaced with Perkin Elmer 500

mass detector operating in EI^+ mode.The mass spectra was recorded over 40-500 amu and revealed the Total Ion Current chromatograms.The temperature program remained the same as in GC.The temperatures of injector and transfer line were kept at 210 ^{0}C and that of ion source at 200^{0}C.

Identification of the oil components was done by comparison of their mass spectra with the NIST/Wiley library as well as by comparing them with those reported in literature. The identification of each compound was also confirmed by comparison of its retention index with those of authentic compounds[15].

Antibacterial activity

The study was conducted by using standard disc diffusion method. In each experiment, microorganisms were cultured at 37 °C for 24 hours and prepared to turbidity which is equivalent to 0.5 McFarland standards (National Committee of Clinical Laboratory Standards) [16-18].

Mueller-Hinton (MH) agar 38g was dissolved in 1000 ml of distilled water. Then it was boiled on heating mantle to dissolve the media completely and then sterilized by autoclaving at 15 lbs. and 121^{0}C for 15 min. After it was autoclaved at indicated conditions, it was poured to the sterilized petridishes and allowed to set at room temperature until the agar has solidified. It was then incubated at 37 ^{0}C for 24 hours to be ready for susceptibility test.

The stock solution of the crude *Lantana camara.* oil in Chloroform(20mg/ml) and test discs were prepared from Whatman filter paper .

A 0.5 McFarland standard was prepared as described in National Committee of Clinical Laboratory Standards (NCCLS)[19-20]. One percent V/V solution of sulfuric acid and 1.175% W/V solution of barium chloride were prepared and made it turbidity standard. A small volume of this turbid solution was transferred to a screw capped tube and vigorously shaken on a mechanical vortex mixer to have a uniform turbid appearance and stored in the dark at room temperature.

Purely cultured Mueller-Hinton agar petridishes were labeled with different names of bacteria. Then 5 ml of sterile Normal Saline Solution (NSS) was pipetted out into a three different sterile screw-cap tubes. These tubes were labeled according to the type and number of bacteria used to test (*E. coli* and *S. aureus).* To prepare inoculums, 3 well isolated colonies of the same morphological types were selected from an agar plate culture. The top of each colony is touched

with a loop, and growth was transferred into a tube containing 5 ml of NSS that corresponds to each bacterium names. These inoculums containing tubes were mixed by using mechanical vortex mixer and their turbidity was compared accurately.

The sterile discs which were prepared by office perforator were inserted in to different concentrations of *Lantana camara* oil with stock solution of 20mg/ml. It was impregnated in to negative and positive controls petroleum ether and chloroform, and amoxicillin respectively. After that, discs with different concentrations were placed on the inoculated plates using a pair of sterile forceps. Seven discs were placed on a 90 cm diameter petridish plate and the space between each disc was given as 24 mm gap from center of the disk to the center of petridish. The pressed discs were completely stacked the agar surface, plates were inverted and placed in an incubator at 37 C for 24 hour. After overnight incubation, the diameter of each zone (including the diameter of the disc) were measured and recorded.

Table-1: Chemical composition of essential oil of *Lantana camara.*

S.No	RT	Compounds Identified	MF	Component (%)
1	7.090	3-carene	$C_{10}H_{16}$	1.6
2	10.311	p-cymene	$C_{10}H_{14}$	8.4
3	10.992	Ocimene	$C_{10}H_{16}$	0.7
4	11.523	Terpinolene	$C_{10}H_{16}$	0.5
5	12.065	1,8-cineole	$C_{10}H_{18}O$	3.3
6	12.642	Borneol	$C_{10}H_{18}O$	0.2
7	13.236	Linalyl isobutyrate	$C_{14}H_{24}O_2$	1.3
8	16.814	4-terpineol	$C_{10}H_{18}O$	1.0
9	17.598	Anethol	$C_{10}H_{12}O$	4.4
10	19.533	β- caryophyllene	$C_{15}H_{24}$	9.6
11	21.265	Bornyl acetate	$C_{10}H_{20}O_2$	0.1
12	21.540	3-p-menthene	$C_{10}H_{18}$	0.1
13	22.831	γ-elemene	$C_{15}H_{24}$	0.6
14	23.426	Thujopsene	$C_{15}H_{24}$	3.2
15	23.930	α-cubebene	$C_{15}H_{24}$	0.9
16	25.305	β-copaene	$C_{15}H_{24}$	25.7
17	25.567	β-bourbonene	$C_{15}H_{24}$	4.3

18	27.663	Himachalene	$C_{15}H_{24}$	13.1
19	28.678	Bicyclogermacrene	$C_{15}H_{24}$	3.7
20	32.764	β -gurjunene	$C_{15}H_{24}$	6.1
21	35.740	Ledene alcohol	$C_{15}H_{24}O$	5.3
22	36.193	Epizonarene	$C_{15}H_{24}$	3.4
Totalcompounds				97.5

Note: RT=Retention Time MF=Molecular Formula

Table-2: *In vitro* antibacterial test of *Lantana camara* oil.

Micro-organism	Zone of inhibition						
	Concentration of crude essential oil(%v/v)					Positive control	Negative control
	25	50	75	100	Rresult	Amoxicillin.	Chloroform
E.coli	9.29±0.28	9.89±0.15	10.6±0.19	11.14±0.1	sensitive	16.1±0.23	-
S.aureus	9.96±0.08	11.1±0.24	12.00±0.0	13±0.00	sensitive	18.8±0.10	-

Results and Discussion

The composition of *Lanthana camara* oil of is shown in the table 1.A total of 97.5% was identified. The major components identified were copaene(25.7%), himachalene(13.1%), β-caryophyllene(9.6%), p-cymene(8.4%), β-gujunene(6.1%), ledenealcohol(5.3%), anethol(4.4%), bicyclogermacrene(3.7%), 1,8-cineole(3.3%), epizonarene (3.4%)and thujopsene (3.2%). Our result of analysis of *L.camara* is in agreement with a report from Nigeria and Iran[12-14]. But differ from species from North Brazil [11], in which limonene, α-phellandrene germacrene,curcumene, α-zingiberen and α-humulene are major constituents . The above study revealed that the variation of essential oil contents in similar chemo types of *Lanthana camara* may be attributed to different climatic and geographical condition of the regions.

Antibacterial zone of inhibitionfor *L.camara* oil against *E.coli* and *S.aureus* are shown in table2. The essential oil of *L.camara* exihibited moderate antibacterial activity against gram negative (*E.coli*) and gram positive bacteria(*S.aureus*)which is in agreement with previous studies[14,21] .

Acknowledgement

The authors wish to thank Higher Education of Ethiopia for providing financial support to conduct the study. The authors also acknowledge the moral support and lab facilities given by the Dean, College of Natural and Computational Sciences, Mekelle University, Ethiopia.

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Corresponding Author:
C.R.Unnithan[2],
Email: *crunnithan@yahoo.co.in*