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Chemical composition and antibacterial properties of the essential oil and extracts of *Lantana camara* Linn. from Uttarakhand (India)

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ABSTRACT

Objective: The purpose of this study was to evaluate the essential oil composition as well as antibacterial activities of essential oil and leaves extracts of *Lantana camara* against five bacterial strains. **Methods:** Essential oil was obtained by hydro-distillation from the leaves and analyzed by GC and GC-MS. The antibacterial activities of essential oil and the leaves extracts were tested by using disk diffusion method against five bacterial strains. **Results:** Thirty seven compounds were identified representing 98.11% of the total oil, of which trans-caryophyllene (13.95%), bicyclogermacrene (9.77%), α-curcumene (8.57%), sabinene (8.28%), (E)-citral (6.90%), 1,8 cineole (5.06%), α-pinene (4.03%), γ-terpinene (3.83%) and germacrene D (3.13%) were detected as major components. In respect to the antibacterial activities, essential oil showed the high degree of sensitivity against *Micrococcus luteus*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* except *Pseudomonas aeruginosa* while extracts of leaves obtained through petroleum ether, benzene, methanol and water exhibited good to moderate antimicrobial activity against all tested bacterial strains. **Conclusions:** The present study suggested that *M. luteus* showed best zone of inhibition for the essential oil as well as aqueous extract among all the tested bacterial strains. The most active extract can be subjected to isolation of the therapeutic antimicrobials to carry out further pharmacological evaluation.

1. Introduction

Medicinal plants, which form the backbone of traditional medicine, in the last few decades, have been the subject for very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutics value and as sources of important compounds in the drug development. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problems in the treatment of infectious disease have been developed. In particular, the antimicrobial activities of extracts and plant essential oil have formed the basis of many alternative medicines and natural therapies.

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Lantana camara Linn. is a noxious weed belonging to family Verbenaceae which comprise of about 650 species spread over 60 countries. Three varieties of *L. camara* have been reported from India in which *L. camara* var. *aculeata* is the most common[1-4]. The essential oil and extracts of the plant are used in herbal medicines for the treatment of various human diseases such as skin itches, leprosy, cancer, chicken pox, measles, asthma, ulcers, tumors, high blood pressure, tetanus, rheumatism etc.[5,6]. Extracts from leaves have been reported to have antifungal[7-9], antiproliferative[10], antibacterial[11-13], nematicidal[14], termicidal[15], anthelmintic[16] and anticancer activities[6]. Beside this, the essential oil of the plants also possesses antifungal[17] and antibacterial activities[18,19]. *L. camara* whole plant and plant parts have been thoroughly studies for their chemical constituents, previously and recently[6,20-22]. All these studies revealed the presence of terpenoids, steroids and alkaloids as major constituents. However,

sesquiterpenes with β-caryophyllene, zingiberene, δ-humulene, AR-curcumene, bicyclogermacrene, germacrene D, carvone, α-phellandrene, limonene, 1,8 cineole and bisabolene were the major constitutes of leaf and flower essential oils[19,23-25]. Qualitative and quantitative variation in essential oil composition have been reported between the different daytime collection[26]. The antimicrobial activities of essential oil and extracts of *L. camara* has been previously reviewed which showed potential role against several pathogenic microorganisms as novel antimicrobial agent[10-13,16,19,27] especially against bacterial strains. Lantadenes and other secondary metabolites, such as alkaloids, terpenoids and phenolic are the responsible groups for these biological activities[18,23].

Hence, the present study was aimed to evaluate the chemical composition of the essential oil as well as antibacterial activities of the essential oil and extracts obtained from the leaves of *L. camara* collected from the Dehradun, Uttarakhand (India) against pathogenic bacterial strains which cause severe diseases in human.

2. Materials and Methods

2.1. Plant material

The leaves of *L. camara* were collected from Dehradun district in July 2011 and after identification, a voucher specimen has been deposited at the Botanical Survey of India (BSI), northern circle, Dehradun with voucher number BSD 114102.

2.2. Isolation of essential oil

The shade dried leaves of *L. camara* were subjected to hydro-distillation using a Clevenger type apparatus[28] for 3.5 hrs. The oil was dried over anhydrous sodium sulphate and stored at 4 ℃ until the GC, GC-MS and antibacterial analysis were carried out.

2.3. Preparation of extracts

The shade-dried leaves of *L. camara* were made into a coarse powder with mechanical grinder for further use. The leaves were extracted with petroleum ether (60 ℃-80 ℃) for deffating purpose in soxhlet apparatus and after complete extraction (3 to 4 hrs) the solvent removed by distillation under reduced pressure and resulting liquid was dried by evaporating the petroleum ether. The same plant material were dried and again extracted with benzene, chloroform, methanol and water, respectively. Water (aqueous) extract was prepared directly.

2.4. Instrumentation and analytical conditions

2.4.1. GC and GC-MS Analysis

The GC analysis of essential oil was performed by using an Agilent Technology 6890 N gas chromatograph data handling system equipped with a split-splitless injector and fitted with a FID using N2 as the carrier gas. The column was HP-5 capillary column (30m x 0.32mm, 0.25 μm film thickness) and temperature program were used as follows: Initial temperature of 60 ℃ (hold: 2 min) programmed at a rate of 3 ℃ /min to a final temperature of 220 ℃ (hold: 5 min). Temperatures of the injector and FID were maintained at 210 ℃ and 250 ℃, respectively.

The GC-MS analysis of essential oil was carried out on a Perkin Elmer Clarus 500 (Shelton, CT 06484, USA) gas chromatograph equipped with a split-splitless injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 m x 0.32mm, 0.25 μm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 ml/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI_+ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as the same as described above for GC analysis. The temperatures of the injector, transfer line and ion source were maintained at 210 ℃, 210 ℃ and 200 ℃, respectively.

2.4.2 Qualitative and quantitative analysis

Identification of the individual component was made by matching their recorded mass spectra with the library (NIST/ Pfleger /Wiley) provided by the instrument software, and by comparing their calculated retention indices with literature values[29]. Relative area percentages of the individual components were obtained from GC-FID analysis.

2.5. Antimicrobial Activity

2.5.1. Test microorganisms

The test organisms used in this study were *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 87), *Micrococcus luteus* (MTCC 106), *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 741). These strains were procured from the Microbial Type Culture Collection (MTCC, Chandigarh, India). The strains were maintained on nutrient agar slants at 4 ℃. A loopful of each bacterial strain was added to a 50 ml sterile nutrient broth in a 100 ml conical flask. The flasks were incubated for 24 hrs to activate the strains.

2.5.2. Culture media and inoculums preparation

Muller Hinton Agar (Himedia, India) were used as the media for the culturing of bacterial strains. Loop full of all bacterial cultures was inoculated in the Nutrient Broth (NB) at 37 ℃ for 24 hrs.

2.5.3. Antibacterial screening of essential oil and leaves extracts

The antibacterial activities of essential oil and extracts of leaves of *L. camara* plant were evaluated by the standard disc diffusion method. 100 μl of inoculums, which contain 10^5 cells/ml were poured on agar plate and spread equally. Different dilutions of extracts and essential oil were prepared in dimethyl sulphoxide (DMSO). Different dilutions were impregnated on sterilized Whatman filter paper No. 1 disc. The discs were then aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37 ℃ for 24 h. The zones of inhibition were measured and the mean values were compared with zone of inhibition produced by antibiotic. The zones of inhibition produced by antibiotic disc were compared with Bayer and Kirby Chart.

3. Results

3.1. Chemical composition of essential oil

The essential oil, obtained by hydro-distillation of shade dried leaves of *L. camara*, analyzed by GC and GC-MS led to the identification of 37 different constituents, representing 98.11% of the total oil (Table 1). The study revealed that sesquiterpene hydrocarbons afforded a major portion of the oil (43.99%) with trans-caryophyllene (13.95%), bicyclogermacrene (9.77%), α-curcumene (8.57%) and germacrene D (3.13%) as the most abundant compounds. Monoterpene hydrocarbons, the second major class of compounds constituted 34.14% of the oil with sabinene (8.28%), α-pinene (4.03%) and γ-terpinene (3.83%) as the major components, whereas oxygenated monoterpenes comprised only 18.82%, having (E)-citral (6.90%), (Z)-citral (3.48%) and 1,8 cineole (5.06%) in appreciable amounts. The remaining chemical constituents were detected in lesser amounts.

3.2. Antibacterial activities of essential oil and extracts

In the present study, the results of antibacterial activity of leaves extracts and essential oil were investigated against five bacterial strains and are summarized in Table 2. The results revealed that the essential oil exhibited notable antimicrobial activity against all bacterial strains. Strains *M. luteus*, *E. coli*, *S. aureus*, *B. cereus* showed high degree of sensitivity to essential oil except *P. aeruginosa*. The extracts of leaves obtained through petroleum ether, benzene, methanol and water exhibited good to moderate antimicrobial activity against all tested bacterial strains. Chloroform extract showed low antimicrobial activity against *E. coli*, *P. aeruginosa* and did not show any inhibitory effect against the *M. luteus*, *B. cereus* and *S. aureus*. Petroleum ether extract showed high degree of sensitivity to *E. coli*, *P. aeruginosa* and moderate activity to *B. cereus* and *M. luteus*. Benzene and methanol extracts showed good inhibitory effect against *S. aureus* and *P. aeruginosa* while *B. cereus* and *E. coli* showed moderate activity against benzene, methanol and aqueous extracts. Among all the bacterial strain *M. luteus* showed best zone of inhibition for both the essential oil and aqueous extract. The antibacterial activities of essential oil and different extracts are shown in Figure 1.

Table 1

Percentage composition of the essential oil of leaves of *L. camara*

Components	RI	% in oil
α-thujene	931	0.53
α-pinene	939	4.03
camphene	953	1.57
sabinene	976	8.28
β-pinene	980	2.99
β-myrcene	991	1.12
δ-3-carene	1011	2.73
α-terpinene	1018	1.84
p-cymene	1029	1.51
limonene	1031	3.39
1,8 cineole	1033	5.06
cis-ocimene	1042	1.56
γ-terpinene	1062	3.83
α-terpinolene	1088	0.76
4-nonanone	1093	0.85
linalool	1100	0.48
camphor	1142	0.20
limonene oxide	1172	0.17
piperitone	1245	0.48
(Z)-citral	1253	3.48
(E)-citral	1267	6.90
trans-geraniol	1268	1.20
δ-elemene	1340	0.38
α-cubebene	1345	1.67
geranyl acetate	1375	0.27
α-copaene	1379	0.51
β-elememe	1393	0.34
trans-caryophyllene	1420	13.95
α-humulene	1456	2.85
γ-muurolene	1475	0.64
germacrene D	1485	3.13
bicyclogermacrene	1492	9.77
δ-cadinene	1519	0.84
cis-nerolidol	1534	1.34
α-curcumene	1553	8.57
caryophyllene oxide	1581	0.52
β-eudesmol	1654	0.37
Monoterpene hydrocarbons		34.14
Oxygenated monoterpenes		18.82
Sesquiterpene hydrocarbons		43.99
Oxygenated sesquiterpenes		1.16
Total identified		98.11

Table.2

Antibacterial activities of essential oil and leaves extracts of *L. camara*

Bacterial Strains	Minimum Inhibitory Concentration (mm)					
	Extracts					Essential oil
	Petroleum ether	Benzene	Chloroform	Methanol	Aqueous	
Bacillus cereus						
MTCC 430	14	9	–	9	10	19
Staphylococcus aureus						
MTCC 87	10	16	–	17	9	20
Micrococcus luteus						
MTCC 106	14	12	–	–	27	25
Escherichia coli						
MTCC 443	20	13	11	12	13	24
Pseudomonas aeruginosa						
MTCC 741	18	17	13	15	11	9

(–) denotes absent

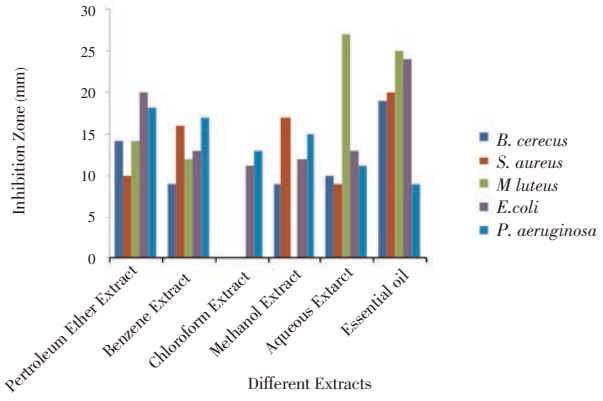

Figure 1. Bar chart showing antibacterial activities of different extracts and essential oil of *L. camara*

Earlier some investigation have reported the essential oil composition of *L. camara* from India and abroad. The dominant component was trans-caryophyllene constituted 24.40%[25], 23.79%[26] and 27.00%[27]. These findings showed similarities with the present study representing trans-caryophyllene (13.95%). The essential oil analysed in the study has been found to be different as compared to other published reports[17,22,25-27] due to the presence of one of the most valuable compound in remarkable amount (E)-citral (6.90%) and (Z)-citral (3.48%). Thus, the present study showed the qualitative and quantitative differences in the composition of essential oil from the earlier reports. These variations with other earlier studies might be occurred due to difference in habitat conditions, geographical locations, altitudes or season of sample collection.

In vitro antibacterial studies showed that the essential oil and extracts of *L. camara* inhibited bacterial growth but their effectiveness varied. In our study, essential oil revealed high degree of sensitivity against *M. luteus, E. coli, S. aureus, B. cereus except P. aeruginosa* strains even though earlier study have reported the same[25]. On the other hand, chloroform extract showed low antimicrobial activity against two strains *E. coli and P. aeruginosa* whereas, petroleum ether extract showed high degree of sensitivity against these two stains. Benzene and methanol extracts showed good inhibitory effect against *S. aureus* and *P. aeruginosa*.

5. Conclusion

The present study concluded that the monoterpenes and sesquiterpenes hydrocarbons followed by oxygenated monoterpenoids were the predominant parts of the essential oil. The extract and essential oil possess antibacterial activity against all tested microbial strains, whereas *M. luteus* showed best zone of inhibition for aqueous extract and essential oil among all the tested bacterial strains. The zone of inhibition varied suggesting the varying degree of efficacy. The antibacterial activity of essential oil and leaves extracts may be due to the presence of various active constituents in their leaves. Therefore, the most active extract can be subjected to isolation of the therapeutic antimicrobials to carry out further pharmacological evaluation, especially citral (E & Z).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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