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Chemical Composition and *In vitro* Antimicrobial, Anti-MRSA Activities of Essential Oil of *Clerodendrum inerme* (L.) Gaertn- grown in Western Ghats Region

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Abstract : The present study examines the chemical composition of essential oil of*Clerodendrum inerme* (L.) Gaertn leavesand belongs to the family Verbenaceae. The GC-MS analyses revealed that the presence of 18 compounds in the essential oil obtained from hydro distillation of*C. inerme* leaves. The major essential oil component was isoeugenol (35.50%), dibutyl phthalate (27.52%), eugenol (5.67%), α-pinene (5.54%),other minor components are Benzoic acid, 4-formyl-benzoic acid ethyl ester (3.44%) and 2-Nitro-1-octanol (4.08%). The *invitro* antibacterial, antifungal and anti MRSA activity against selected pathogens were evaluated, the zone of inhibition and minimum inhibitory concentrations showed that the tested essential oil has very significant antimicrobial and anti MRSA properties.These findings are very useful and provide scientific evidence that the plant is used for curing skin diseases. There are few reports available for the essential oil of *C. inerme.* There is no report on the antimicrobial activity of essential oil of *C.inerme.* This is the first kind of report of essential oil for its *invitro* antimicrobial activity.
Key words : *Clerodendrum inerme,* essential oil,antibacterial activity, antiMRSa and GC-MS analysis.

Introduction

*Clerodendrum inerme*L. is an aromatic and medicinally important plant and belongs to the family Verbenaceae. Tamil name is Chankankuppi, in Malayalam Nirnochi, and commonly known as gardern quinine is a perennial shrub found throughout India. Traditionally, whole plant parts of *C. inerme* are used as to treat coughs, scrofulous infection, Venereal infections, skin diseases and beriberi diseases[1].*C. inerme* is flowering plants found in the tropical region of the world, which usually reaches a height of 3-4 m with closely arranged, almost round, shiny, deep green leaves. The family includes some thirty five genera and around 1200 species. The verbenaceae family plants are well known for their uses in the traditional medicinal system of various countries (China, Japan, and Korea). The leaves of the plant are mucilaginous, bitter and fragrant. They have an advantage over other species that lack strategies to deal with salt in the soil and thus are excellent competitors in saline environments[2].The various extracts of *C. inerme* exerted many pharmacological effects such as anticarcinogenic, antioxidant, antibacterial,analgesic, antipyretic anti inflammatory, anti parasitic, insecticidal, anti allergic and antifungal properties[3-7]. It showed many biologically active metabolites, including anthroquinones, proteins, saponins, tannins, iridoids, diterpenes, triterpenes, sterols, steroids, carbohydrates, fixed oils,volatile oils and lignin. There is no report on the antimicrobial activity of the essential oil of the fresh leaves of .*inerme*and only few reports on essential oil of *C. inerme* in India. So the main purpose of this investigation is to determine the chemical composition of essential oil of fresh leaves and its antimicrobial and anti MRSA activity.

Materials and Methods

Plant materials

Fresh leaves of *C. inerme* were collected from home garden in Pollachi between the periods of June – July 2016. The plant material was identified and authenticated by Department of Botany, NGM College, Pollachi, Coimbatore, Tamilnadu. The voucher specimen (16CHE005) was preserved in the Chemistry department.

Isolation of essential oil

About 500g of fresh leaves was subjected to hydro distillation using Clevenger type apparatus for 4h. The oil obtained was dried over anhydrous sodium sulphate and stored in a container and kept in freezer until GC-MS analysis.

GC-MS analysis

The GC-MS analysis of the essential oil was carried out on a Agilent system consisting of model 6890N gas chromatograph, a model 5975 inert mass selective detector (EIMS, electron energy70eV, scan range 50-500 amu, and scan rate 2 scan per second), and a agilent chem station data system. The GC column was an HP-5 fused silica capillary with a (5% phenyl) – methyl polysiloxane stationary phase, filling thickness of 0.25 µm, a length of 30m, and an internal diameter of0.25 mm. The carrier gas was helium with a column head pressure of 7.07 and flow rate of1.0ml/min. Inlet temperature was 220o C and MSD detector temperature was 325 0C. The GC oven temperature program was used as follows: 85°C for 2 min, 85o – 230oC at 6oC/min, 230oC for 5min, 230o – 300oC at 4oC/min, ending with 10 min at 300oC. The sample was dissolved in 10 ml of acetone: toluene (1:1) mixture. 1 µL injections using a split less injection technique was used. Identification of oil component was achieved based on their retention indices, and by comparisonof their mass spectral fragmentation patterns with those reported in the literature and stored on theMS library [NIST database (G1036A, revision 0.01.00) / chem. station data system (0.02.275,version 2.0d)]

Invitro antibacterial activity

The bacterial strains (*S. aureus, B. cereus, B. subtilis, B. megaterium, E. Coli, A. hydrophila,, S. boydii S. typhi* were inoculated in the nutrient broth under aseptic conditions and incubated at 37°C for 18 hours [8]. After the incubation period, the test bacterial was swabbed on the nutrient agar plate using sterile cotton swab. In each of these plates, wells (10mm) were cut out using sterile cork borer. The oil was dissolved in the solvent DMSO. Controls were maintained by loading same quantity of Ampicillin in to the wells. Then the Petri dishes were incubated at 37°C for 14 hours. The antibacterial activity was evaluated by measuring the zone of inhibition in diameter (in mm).

Materials and Methods for antifungal activity

Potato dextrose agar (PDA) plates were seeded with spore suspension of fungi. A 16 hrs broth culture of *Candida albicans, Asperigillus niger, Asperigillus flavus* and *Asperigillus terreus* was used to seed PDA plates. In each of these plates, wells (10mm) were cut out using sterile cork borer. The oil was dissolved in solvent DMSO. Using sterilized dropping pipettes, different concentrations (25 µL, 50 µL, 100 µL, 150 µL, and 200 µL) of oil was carefully added in to the wells and allowed to diffuse at room temperature for 2 hrs. The plates were then incubated at room temperature for 3 days for fungal pathogens. The antifungal activity was evaluated by measuring the zone of inhibition in diameter (in mm).

Minimum Inhibitory Concentration (MIC)

The Minimum inhibitory concentration (MIC) was determined through the dilution method [9].The Bacterial and fungal pathogens were grown in nutrient broth (NA) for 6 hrs. After this, 20 µLof 106 cells/ml were inoculated in tubes with nutrient broth supplemented with 5 different concentrations (2.5 µL, 5.0 µL, 10.0 µL, 15.0 µLand 20.0 µL) of the oils. After 24 hrs at 37°C, the MIC of each sample was measured through optical density in the spectrophotometer (620nm) through the comparison of the sample readout with the known inoculated nutrient broth andthe Ampicillin was used as a standard substance, DMSO as the negative control.

The same method was carried out for MRSA using Ampicillin as the positive control, DMSO as the negative control.

Results and Discussion

Gc-MS analysis of *C. inerme*

A total of 18 compounds were identified from the leaves of essential oil of *C.inerme* representing 90% of the oil content. The major essential oil composition was Isoeugenol (35.50%), Dibutyl phthalate (27.52%), Eugenol (5.67%), α-pinene (5.54%),other minor components are Benzoic acid, 4-formyl-benzoic acid ethyl ester (3.44%), Phthalic acid, and butyl 2-ethylbutyl ester (1.87%), and other important compounds are 2-Nitro-1-octanol (4.08%), Hexanal (2.35%), Caryophyllene (0.68%), Tau-cadinol (0.15%), 5-Heptadecene (0.14%), Cis ocimene (0.08%), 2-Hydroxy-4-methyl acetophenone (0.24%), Terpineol (0.32%), Trans-cinnamyl acetate (0.31%), Nerolidol (0.15%), e-cadinene (0.15%). The structures of the identified compounds are given in table1. The Sesquiterpenoid compounds are present (1.28%) such as cayophyllene, e-cadinene, Nerolidol, and tau-cadinol. Monoterpenoids are also present such as Terpineol, α-pinene and cis-ocimene. There are only one report is available for chemical composition of essential oil of *C.inerme* and the GC-MS analyses of the oils for leaf, twig and root parts revealed that thedibutyl phthalate was the main component of all the essential oils attaining in average 34.22%, 59.28% and 44.27% and the other kind of esters accounted for 38.30%, 17.28% and 10.89% in the twig, leaf and root parts, respectively. In addition, some pharmaceutical components such as stigmasterol, linoleic acid and ferruginol were discovered.[10)]Few literature reports could be seen describing the chemical compositions of essential oils from the genus *Clerodendrum*. The major constituent of essential oil of *C. buchholzii* grown in Cameroon was benzaldehyde (96 %), while octen- 3-ol was determined as the key odorants of the mixture.[11)]. Previously serration and lupeol were isolated from the essential oil of *C. serratum.*[12)].Whereas*C.phlomidis*has caryophyllene, Cyclohexen, 1-methyl-4- (5-methyl-1-methyleme-4-hexenyl), Phytol and 3-cyclohexen-1-ol, 4-methyl-1- (1-methyl ethyl) as the main compounds.[13)]The main constituents of the*C. polycephalum*essential oil was β-caryophyllene (28.9 %), α-muurolene (9.0 %) and β-pinene (8.6 %). There were significant amounts of 1, 5, 9, 9-tetramethyl-Z, Z, Z, Z-cycloundecatriene (7.4 %), 9-*epi*-(*E*)-caryophyllene (5.6 %) and (*E*)-nerolidol (5.5 %)[14].

Table: 1Chemical composition of essential oil of *C. inerme*

S.No	Name of the Compound	RT	(%)
1.	2-Nitro-1-octanol	3.07	4.08
2.	Hexanal	4.65	2.35
3.	Terpineol	6.49	0.32
4.	3,3-Diethyldiaziridine	7.55	0.09
5.	5-Heptadecene	8.37	0.14
6.	Cis ocimene	9.15	0.08
7.	Caryophyllene	11.40	0.68
8.	Isoeugenol	11.77	35.50
9.	2-Hydroxy-4-methyl acetophenone	12.82	0.24
10.	Trans-cinnamyl acetate	14.07	0.31
11.	Nerolidol	14.51	0.15
12.	Eugenol	15.96	5.67
13.	Tau-cadinol	17.24	0.15
14.	e-cadinene	19.52	0.15
15.	α-pinene	23.5	5.54
16.	Dibutyl phthalate	25.02	27.52
17.	4-formyl-benzoic acid ethyl ester	25.90	3.44
18.	Phthalic acid, butyl 2-ethylbutyl ester	28.48	1.87

Invitro antibacterial activity

Many essential oils are known for their antimicrobial activity[15,16]. Since it was reported that the fresh leaf juice was used externally for treating skin diseaseand*C. inerme* was used as a febrifugal and uterine stimulant, a pest control agent and antiseptic, to arrest bleeding, treatment of asthma, hepatitis, ringworm and stomach pains [1,17]. So it is worth to test the essential oil for its antibacterial and anti MRSA activity.Theantibacterial activity was tested against the clinical isolates of four gram positive bacteria such as *S. aureusB. megaterium, B. subtitles,B. cereus* and four gram negative bacteria such as *E. coli, A. hydrophila,A. hydrophila, S. boydii*and *S. typhi*by disc diffusion method. The antifungal property was tested against *C. albicans,A. niger,A. flavus* and *A. terreus.*The result of the antibacterial and antifungal and anti MRSA activity of the essential oil is shown in Table 2-4.The zone of inhibition and the Minimum inhibitory concentration (MIC) were calculated and compared with the corresponding control values. The essential oil of *C.inerme* exhibited significant antimicrobial activity against *S. aureus, B. megaterium, E. coli*and *A. hydrophila* with zone of inhibition 17, 19 and 18 mm and 17mm respectively and also the oil exhibit good anti fungal activity against all tested microorganisms. The minimum inhibitory concentration for all the tested organisms were of 2- to10 µg/ml for bacterial strains and for anti fungal activity the MIC range of 0.003-1.5µg/ml. The results of the antibacterial activity for the essential oil from *C. inerme* were comparable with the various extract of the *C.inerme*evaluated by other workers[18-21].*C.inerme*is available in several parts of the world, but the essential oil has not reported for its antibacterial, anti fungal and medicinal uses against Anti MRSA. The results of the MRSA activity of oil showed with a zone of inhibition 15 mm and the Minimum inhibitory concentration (MIC) 2.5 µg/ml and comparable with the corresponding control values of 17 mm and 8 µg respectively. To our knowledge, this is the first kind of report of the antibacterial activity against MRSA using the essential oil from *C.inerme*.

Table 2. Antibacterial activity of essential oil from *C.inerme*fresh leaves

Bacteria	**Zone of inhibition (mm)**		**MIC (µg/ml)**	
Gram positive	*C.inerme*	Ampicillin	*C.inerme*	*Ampicillin*
S. aureus	17.0	20.0	5.0	5.0
B. subtitles	16.0	-	5.0	15.0
B. megaterium	19.0	12.0	2.5	5.0
B. cereus	14.0	15.0	5.0	5.0
E. coli	18.0	26.0	2.5	5.0
A. hydrophila	17.0	-	5.0	-
S. boydii	16.0	15.0	10.0	5.0
S. typhi	13.0	14.0	2.5	5.0

Table 3. Antifungal activity of essential oil from *C.inerme*fresh leaves

Fungi	**Zone of inhibition (mm)**		**MIC (µg/ml)**	
	C.inerme	Nystatin	*C.inerme*	Nystatin
C. albicans	12.0	24.0	2.5	0.007
A. niger	10.0	20.3	5.0	1.5
A. flavus	6.0	18.0	10	0.003
A. terreus	7.0	16.3	5.0	0.5
P. notatum.	9.0	13.0	-	0.1

Table 4. Antibacterial activity of essential oil from *C.inerme*leaves against Methicillin -Resistant *staphylococcus aurous* (MRSA).

Organism	**Zone of inhibition (mm)**		**MIC (µg)**	
	C.inerme	Ampicillin	*C.inerme*	Ampicillin
S. aureus	15	17	2.5	8

Conclusion

The chemical composition from essential oil of *C. inerme* was analysed by GC-MS method. A total of 18 components were identified, Isoeugenol, Dibutyl phthalate, Eugenol & α-pinene were the major components in of *C. inerme.* The antimicrobial properties of the essential oil showed significant activity against all tested microorganisms as well as Anti MRSA activity.this is the first kind of activity for essential oil of *C.inerme*fresh leaves and this provide scientific evidence for the plant is used traditionally for curing lot of disease.

No Conflict of interest

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