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Antibacterial Activity of *Lippia citriodora* A Folklore Plant

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The aqueous and organic extracts of *Lippia citriodora* aerial parts were tested for total phenolic content and antibacterial activity against gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*Escherichia* coli, *Klebsiella pneumoniae* and *Proteus vulgaris*) bacteria. Acetone extract of *L.citriodora* exhibited most significant activity, Methanol and ethanol extracts showed moderate activities, whereas a mild growth inhibition was observed with Chloroform, Diethyl ether and Chloroform-methanol (3:1) extracts against all the bacterial species studied. The minimum inhibitory concentration (0.01 mg mL^{-1}) of acetone extract showed that the fraction was most active against all bacterial species, especially gram positive bacteria. Total phenolic content was found to be high in methanol extract compared to other extracts.

Key words: *Lippia citriodora*, phenolic content, antibacterial activity, Gel diffusion, MIC.

Lippia citriodora (Verbenaceae) also called lemon verbena and *Alyosia triphylla* is a perennial shrub endemic to Arasavilli area of Srikakulum district, Andhra Pradesh and locally used as a spice and a medicinal plant. Its leaves are reported to possess digestive, antispasmodic, antipyretic, sedative and stomachic properties. It has traditionally been used in infusions for the treatment of asthma, cold, fever, diarrhoea and skin diseases[1-2]. The plant also possesses anti-inflammatory[3-4], analgesic[5] and mosquito repellent[6] properties.

Multiple drug resistance by various bacterial species has become a major problem in pharmaco therapeutics[7-8]. Exploring the healing potential of plants to combat this problem is receiving much attention[9-11]. Previous studies on *L.citriodora* revealed that this plant contains several flavonoids, phenolic acids and phenyl propanoids[12-15]. However information relating to the antibacterial properties of this plant is not available. The object of the present study is to evaluate the phenolic content and antibacterial activity of *L.citriodora* aqueous and organic extracts against gram positive and gram negative bacteria.

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MATERIAL AND METHODS

Plant material

L. citriodora plants were collected from Arasavilli area of Srikakulam district of North coastal Andhra Pradesh, India, with the help of local folks. The plant was identified and authenticated at herbarium of Botany department, Andhra University.

Preparation of extracts

Plant extracts were prepared according to the method of Alade and Irobi[16] with little modifications. 10 g of shade dried, powdered plant material were soaked separately in 100 mL of double distilled water, Acetone, Methanol, Diethyl ether, Chloroform, ethanol and Chloroform-methanol (3:1) for 72 h with periodical stirring and mixing. Then the extracts were separately filtered through cheesecloth. The crude extracts were evaporated to dryness under reduced pressure at 40°C. The residues were weighed and appropriate quantities were dissolved in Dimethyl sulfoxide (DMSO) to obtain a final concentration of 1mg mL^{-1}.

Microbiological studies

The following test organisms were used in this study: *Escherichia coli* (ATCC 11775), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 12600), *Klebsiella pneumoniae* (ATTC 13883) and *Proteus vulgaris* (ATTC 13315). The bacterial strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacteria were grown in nutrient broth (Himedia Pvt, Ltd., Bombay; India) at 37°C and maintained on nutrient agar slants at 4°C and stock at -20°C. Antibacterial activities of the aqueous and organic extracts of aerial parts were determined by agar well diffusion method[17]. Nutrient agar plates were prepared by pour plate method using 20 mL of nutrient medium. The molten sterile nutrient agar medium was cooled to 45°C and mixed thoroughly with 1.0 mL of growth culture of concerned test organism (1×10^8 cells) and then poured into sterile flat-bottomed petridish (9.0 × 1.5 cm diameter) and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and 40 µL of extract (1.0mg mL^{-1}) was added to each well aseptically. Then the plates were incubated at 37°C for 24 h and the diameters of the circular zones of growth inhibition were measured by using Himedia zone reader. Results presented are the average values of triplicates. DMSO served as negative control, while Penicillin and streptomycin as positive controls.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined for active acetone extract by agar diffusion method[17]. The lowest concentration of extract that inhibited the growth of microorganisms was considered as MIC.

Determination of total phenolics

The total phenolic content in different extracts was determined as per the method of Javanmardi *et al.*[18] using gallic acid as standard and expressed as milligrams of gallic acid equivalent per gram of tissue.

Table 1. Antibacterial activity of *L. citriodora* extracts on different bacterial strains

Formulations (40 µg/well)	Zone of growth inhibition (mm)				
	E. coli	*K. pneumoniae*	*P. vulgaris*	*S. aureus*	*B. subtilis*
Aqueous	ND	ND	ND	ND	ND
Methanol	13.0±0.1	10.0±0.2	15.0±0.1	11.0±0.1	12.0±0.1
Ethanol	11.0±0.2	11.0±0.1	14.0±0.1	12.0±0.2	10.0±0.1
Acetone	24.0±0.1	25.0±0.2	23.9±0.2	32.0±0.1	31.8±0.1
Chloroform	7.5±0.2	7.0±0.1	7.0±0.1	8.0±0.1	8.0±0.2
Diethyl ether	6.2±0.1	7.0±0.2	7.0±0.2	7.0±0.2	7.0±0.1
Chloroform-methanol (3:1)	7.0±0.1	8.0±0.1	6.6±0.1	8.4±0.1	6.9±0.2

ND: Not detected

All the values are average of five determinations and expressed as mean ±S.D.

Table 2. Minimum inhibitory concentration of acetone extract of *L. citriodora*

Concentration (mg mL^{-1})	Zone of growth inhibition (mm)				
	E. coli	*K. pneumoniae*	*P. vulgaris*	*S. aureus*	*B. subtilis*
10	19	18	20	23	26
1.0	12	13	18	20	22
0.1	8	7	10	15	16
0.01	ND	ND	ND	8	9
0.001	ND	ND	ND	ND	ND
0.0001	ND	ND	ND	ND	ND

ND: Not detected

Table 3. Minimum growth inhibitory concentration of standard antibiotics

Antibiotic	MIC values in mg mL^{-1}				
	E. coli	*K. pneumonia*	*P. vulgaris*	*S. aureus*	*B. subtilis*
Penicillin	0.1	0.1	0.1	0.01	0.01
Streptomycin	0.001	0.01	0.001	0.01	0.01

Statistical analysis

Each value is an average of five determinants and statistical significance was evaluated by student's *t*-test and the value were expressed as mean ± SD. Level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The antibacterial activity of *L.citriodora* extracts is presented in Table 1, indicates that aqueous extract of *L.citriodora* did not exhibit any activity against the microorganisms tested, whereas all the organic extracts studied have exhibited different levels of antibacterial activity against both gram positive and gram negative bacteria. Most significant inhibitory activity was observed with acetone extract against all bacterial species studied with inhibition zones ranging from 30 mm with *K.pneumoniae* to 37 mm with *B.subtilis*. Methanol and ethanol extracts exhibited moderate activity with inhibition zones ranging from 13-17 mm and 9-15 mm respectively, while chloroform, diethyl ether, and chloroform: methanol (3:1) exhibited mild activity. These results showed that the plant under study exhibited good antibacterial activity, especially against gram positive bacteria and justifies the use of *L.citriodora* as a traditional medicinal plant for various ailments[1-2]. Pawar *et al* also showed that antibacterial activity of A*loe vera* leaf gel extract was mainly found in acetone extract[18]. Based on our experimental data, it is concluded that the antibacterial compound(s) of *L.citriodora* can be isolated from acetone extracts. The MIC values of active acetone extract presented in Table 2, confirm the antibacterial potency of acetone extract against all the bacterial species studied with MIC values ranging from

Table 4. Total phenolic content of *L. citriodora* extracts

Plant extract	Total phenolics (mg of gae/g)
Aqueous	12.0±0.1
Methanol	60.0±0.08
Ethanol	53.1±0.1
Acetone	43.6±0.08
Chloroform	29.1±0.1
Diethyl ether	25.6±0.08
Chloroform – methanol (3:1)	38.0±0.1

GAE- Gallic acid equivalents
All the values are average of five determinations and expressed as mean ±S.D.

0.01 mg mL^{-1} for gram positive to 0.1 mg mL^{-1} for gram negative bacteria. MIC values of crude acetone extract are nearly equal to that of pure standard penicillin Table 3. Further work on isolation and characterization of antibacterial agent is under progress.

The total phenolic content of *L.citriodora* extracts is presented in Table 4. The phenolic content of methanol extract was very high compare to other organic extracts but the antibacterial activity was found to be more in acetone extract. These results suggest that the total phenols in acetone fraction may function as strong antibacterial compound(s) against the bacterial species studied.

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