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ANTIMICROBIAL ACTIVITY OF *Lantana camara* Linn. ROOT EXTRACTS AGAINST *Klebsiella pneumoniae*

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ABSTRACT

Lantana camara Linn., a wild plant was checked for its antimicrobial efficacy against *Klebsiella pnuemoniae*. Root extracts were tested separately using different solvents for the extraction of active soluble principles. In case of root, chloroform extract was found to be most effective at 200mg/ml concentration with ZOI of 24 mm whereas, petroleum ether extract was most effective at 100 and 50mg/ml concentrations with ZOI of 22 and 19 mm respectively. Ethanol extract was found to be least effective with ZOI of 12, 10 and 09 mm at 200, 100 and 50 mg/ml concentrations respectively, whereas other extracts exhibited varying degree of ZOI ranging between 22 and 14 mm.

Keywords: Antimicrobial efficacy, *Lantana camara, Klebsiella pneumoniae* and MIC.

INTRUDOCTION

The treatment of diseases caused in humans in varying degrees by the pathogens is carried out using antimicrobial substances of various origins. These antimicrobial substances may be natural, synthetic or semi-synthetic such as Penicillin's. Antibiotics (both narrow and broad-spectrum) are beyond the reach of common man being quite expensive and moreover microbes are becoming resistant against them. The ability of the plant extract to reduce or inhibit the growth of microorganisms or kill the pathogenic microorganism is known as antimicrobial activity or efficacy.

Dependence on plants as a source of medicine is prevalent since time immemorial. Plants have provided mankind with a source of medicinal agents, with natural products once serving, as a source of all drugs and also traditional ways of treatment prevalent in developing countries because of easy availability and cheaper cost. These plants contain secondary metabolites, which are anti-microbial in nature, produced by plants naturally in *in vivo* to protect them from infections. These secondary metabolites can be used therapeutically to treat diseases being readily absorbed and metabolized by the body as also our body does accept them and they can easily associate with natural biological molecules (Chaudhary *et al.,* 1994).

Natural products called secondary metabolites continue to be the major source of drugs and that too with greater structural diversity than drugs or compounds from standard combinatorial chemistry. In 1984, at least 25% of the prescription drugs used in USA and Canada were derived from or modeled after plant natural products (Farnsworth, 1994). Of the 520 new drugs approved between 1983 and 1994, 39% were natural products or derived from them and 60-80% of anti-bacterials and anti-cancer drugs were derived from natural products (Cragg *et al*., 1997). Since the numbers of resistant strains of pathogens are increasing and the drugs obtained using combinatorial chemistry are being rendered ineffective or are becoming ineffective against most pathogens, there is a great need to access the natural heritage for antimicrobial agents efficiently and effectively (Harvey, 2000).

The present investigation deals with the study of antimicrobial efficacy of an obnoxious weed *Lantana camara* Linn. with the following objectives:

1) Extraction of various solvent fractions of *Lantana camara* from roots.
2) Screening of various solvent fractions for anti-microbial activity.
3) Determination of MIC (minimum inhibitory concentration).

Lantana camara Linn. of the family Verbenaceae, is a dangerous weed that grows luxuriantly and has encroached upon large parts of pasture and forest areas in tropical and subtropical parts of the world (Sharma *et al*., 1981; Pass, 1986). The attendant losses in rural economy are due to its fast growth, allelochemic effects, toxicity to grazing animals and adverse effects on human and it also exerts inhibitory effects on the growth of other vegetation in vicinity and has now become a major "problem weed". However, the plant holds potential for production of bio-energy, development of bio pesticides, bio-herbicides and for medicinal purposes (Sharma, 1988).

MATERIAL AND METHODS

Fresh roots of *Lantana camara* were collected from the plants growing in and around Bhopal city. Plant samples were washed, shade dried at room temperature, pulverized and weighed before loading in the soxhlet apparatus, 110g of roots were loaded.

Soxhlet extraction was carried out using solvents with increasing polarity in sequential manner for extraction of active ingredients (Vogel, 1998).

Solvents used	Boiling point
Petroleum ether	60-80 °C
Benzene	80 °C
Chloroform	61 °C
Ethyl acetate	77 °C
Acetone	56 °C
Ethanol	78 °C
Distilled water	100 °C

Distillation

Distillation was carried out to separate the residues and solvent from liquid solvent extract. Thus the semisolid extracts were placed over a water bath for further drying. The dried residues were preserved in sealed vials for further use.

DMSO

Dimethyl sulphoxide, a colourless hygroscopic liquid (boiling point 189°C) was used for dissolving various extracts for testing anti microbial efficacy, being a very good solvent for experimental purposes (Beyer H, *et al.*, 1997).

Maintenance of microbial cultures

Various microbial cultures were used, but in the present study *Klebsiella pneumoniae* is used, the cultures were maintained on Nutrient Agar slants and stored at 4°C. Stock cultures were sub cultured at regular intervals (Aneja, 1996).

Testing of inhibitory efficacy of extracts

Agar wells were prepared in plates with the help of cork borer, 100ml (0.1 ml) of DMSO was added in wells and kept overnight in an incubator to test diffusibility and it did well. Variable concentrations of extracts were prepared by dissolving dried residues in DMSO for testing. Cup (well) Assay Method (Agar Diffusion Method) was used, with one well as control having 0.1 ml (100ml) of pure solvent (DMSO).

Microbial growth inhibition measurement

It was determined as diameter of inhibition zone around wells as an average of 4 measurements per well at 4 different directions. ZOI (Zone of Inhibition) was measured in mm.

Selection of Potent Inhibitory Extracts and MIC Determination

MIC - Minimum inhibitory concentration is the minimum concentration of active soluble principles in the extracts, which inhibit the growth of microorganisms. Potent extracts with larger ZOI were selected for MIC studies. Decreasing concentrations of potent extracts were prepared for testing. Lowest concentration of extracts, below which no inhibition zone was observed, was considered as MIC.

Disc Diffusion Method was used for MIC testing. Disc of 4mm were soaked and transferred to Petriplates. The amounts of extracts per disc were computed in mg.

OBSERVATIONS

It was observed that the soluble active principles extracted in varying degree in the solvents used were effective in varying proportions against *Klebsiella pneumoniae*. Three different concentrations of the extracts (200, 100, 50 mg/ml) were used for testing anti-microbial efficacy. Very high to low ZOI was observed ranging from 24mm to 09mm against *Klebsiella pneumoniae* in case of root extracts of various solvents used,

with chloroform extract being most effective at 200 mg/ml and petroleum ether extract at 100 and 50 mg/ml concentration. MIC values were also tested against the pathogen of only selected extracts (benzene and chloroform) and good to high ZOI was focussed to near 3.125 mg/ml. At this MIC value of 3.125 mg/ml, 15.625 mg of the soluble fraction was present in each disc respectively.

RESULTS AND DISCUSSION

Table - 1 shows the amount extracted and percentage yield of soluble principles in various solvents. In roots highest yield (4.38 %) was recorded in distilled water with minimum yield in acetone (0.53%), with total amount approximately 10.5% (w/w). Table - 2 shows that *Lantana camara* roots possess very good inhibitory potential. Three different concentrations (200, 100, 50 mg/ml) were prepared in DMSO for testing.

Almost all the extracts exhibited fair ZOI against *Klebsiella pneumoniae* at a concentration of 200 mg/ml with chloroform extract showing maximum ZOI of 24mm and ethanol extract 12mm, with ethyl acetate extract showing a good activity, giving ZOI of 23mm at the same concentration. Similarly at 100 and 50 mg/ml petroleum ether extract (photoplate 2) gave maximum ZOI of 22mm and 19mm respectively whereas ethanol extract gave minimum ZOI of 10mm and 09mm respectively, with other extracts (benzene extract) showing high to moderate ZOI ranging from 24mm to 09mm at various concentrations.

MIC Determination

MIC is the minimum concentration of the active soluble principles in the extract, which inhibits the growth of microorganisms. Disc Diffusion Assay Method was employed to carry out this study. In the present investigation, 5 decreasing concentrations of the extracts were prepared and tested which were 50, 25, 12.5,6.25 and 3.125 mg/ml and these concentrations correspond to 250, 125, 62.5, 31.25, and 15.625 mg of soluble principles in each disc respectively.

MIC of *Lantana camara* Root extracts

Only 2 extracts, benzene and chloroform were selected for MIC determination against

Table - 1: Yield of various extracts from roots of *Lantana camara*

S.No.	Solvents	Roots	
		Soluble principles in fractions (g)	Percentage yield (W/W)
1	Petroleum ether	0.92	0.84
2	Benzene	1.20	1.10
3	Chloroform	0.85	0.77
4	Ethyl acetate	2.25	2.05
5	Acetone	0.58	0.53
6	Ethanol	0.74	0.67
7	Distilled water	4.82	4.40
	Total	**11.36**	**10.36**

Weight of root sample loaded – 110g

Table - 2: Antibacterial potential of *Lantana camara* root extracts

S. No.	Bacteria	Extracts																					
		P.E.			BEN.			CHL.			E.A.			ACET.			ETH.			D.W.			
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	
1.	*K. pneumoniae*	22	22	19	20	19	17	24	19	16	23	19	17		NT		12	10	09	17	16	14	

Note: 1) Roman numerals I, II, III stand for concentrations 200 mg/ml, 100 mg/ml, 50 mg/ml respectively.
2) ZOI (Zone of Inhibition) was measured in mm.
3) NT stands for Not tested.

Table - 3: MIC measurements of *Lantana camara* root extracts

S. No.	Solvents	Organisms	Concentrations of extracts in mg/ml					MIC
			I	II	III	IV	V	(mg/ml)
1.	Benzene	*K. pneumoniae*	13	10	08	07	06	< 3.125
2.	Chloroform	*K. pneumoniae*	09	08	07	07	06	< 3.125

Note:

1) Roman numerals I, II, III, IV, V stands for concentrations 50.0, 25.0, 12.50, 6.250 and 3.125 mg/ml respectively.
2) ZOI (Zone of Inhibition) was measured in mm.
3) MIC stands for minimum inhibitory concentration.

Klebsiella pneumoniae as shown in Table -3. MIC was below 3.125 mg/ml concentration of the extracts used, and ZOI observed with respect to the above 5 concentrations using benzene and chloroform extracts were 12, 10, 09, 08, 07 mm and 13, 11, 11, 10, 09 mm respectively.

CONCLUSION

The present investigation reveals that *Lantana camara* root extracts are very effective against *Klebsiella pneumoniae*. A lot of antimicrobial work is being carried out throughout the world but only few antimicrobial substances found in the natural environment are finally translated into pharmaceutical products, which are used for therapeutic purposes. Further work with respect to anti-microbial efficacy of *Lantana camara* extracts is still required, to test the anti-microbial potential in *in vitro* conditions. Here, an attempt has been made to isolate the crude soluble principles from roots of *Lantana camara.* The result of inhibitory potential reflects the activity of crude drug. In future further purification of crude extracts is required to get pure chemical entities or active principles, which would be required in low concentration for therapeutic purposes.

On the whole it can be concluded from the results obtained that *Lantana camara* root extracts are highly effective and active against *Klebsiella pneumoniae* and can be used to cure diseases.

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