Biosciences, Biotech. Research Asia Vol. **2** No.(**2**) 123-126 (2004)

ANTIMICROBIAL ACTIVITY OF *Lantana camara* ROOT AND STEM EXTRACTS AGAINST *Staphylococcus aureus*

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(Received May 06, 2004; Accepted October 17, 2004)

ABSTRACT

In the present investigation an attempt has been made to experimentally test the anti-microbial efficacy of a common wild plant, *Lantana camara* against *Staphylococcus aureus*. Both root and stem extracts were tested separately using different solvents for the extraction of active soluble principles. The highest ZOI in the extract of root was found to be 25 mm under 200 mg/ml concentration of acetone followed by 23 mm under 100mg/ml concentration of the same solvent. All the concentrations of the root with ethyl acetate extract were found to be least effective. The anti-microbial efficacy of the chloroform extract was next to that of acetone extract. The acetone extract of stem (200 mg/ml) was found to be highly effective against *Staphylococcus aureus*, which gave ZOI of 24 mm, followed by 20 mm under 100 mg/ml concentration of the same solvent and that of 100 mg/ml of benzene extract. The most ineffective extract was that of petroleum ether in which only a very small zone (8 mm) of inhibition was recorded for 50 mg/ml whereas both 100 and 200 mg/ml concentrations proved ineffective. In rest of the solvents both root and stem extracts exhibited varying degree of inhibitory activity.

INTRODUCTION

The treatment of diseases caused by pathogens is carried out using antimicrobials, which may be synthetic or semi-synthetic such as penicillin's. Both narrow and broad-spectrum antibiotics are very expensive and beyond the reach of common man. Anti-microbial efficacy or activity of plant extracts means their ability to inhibit the growth of microorganisms or how effectively it reduces, inhibit or kill the microorganisms.

Antimicrobial effects of plants are known for centuries, which are attributed to the natural products called secondary metabolites that they contain. These natural products are biologically active with drug like properties and are also readily absorbed and metabolized by the body. These natural products are formed *in vivo* under natural conditions to protect the plants against spoilage due to microorganisms. Since our body rarely treat these natural products as foreign and therefore easily accepted by the body because of their association with natural biological entities like lipids, proteins and carbohydrates, as such they do not produce side or after effects and can be used for therapeutic purposes (Chaudhary *et al.,* 1994).

Dependence on plants as source of medicine is more prevalent in developing countries where traditional medicines play a major role in healthcare (Farnsworth, 1994). Rural population as such is more used to traditional ways of treatment because of its easy availability and cheaper cost. Also, since the developmental cost of producing orally active medicines from these natural products is likely to be much lower than with biotechnological products or compounds or products produced through combinatorial chemistry, therefore the plant diversity much be assessed, as also the natural products have greater structural diversity than synthetic compounds (Strohl, 2000). This diversity must be assessed efficiently and effectively (Harvey, 2000).

An attempt has been made to test antimicrobial efficacy of the noxious weed *L. camara* with following objectives:

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

Lantana camara Linn. (Family Verbenaceae) is a dangerous weed that has encroached upon large parts of pasture and forest areas in tropical and subtropical parts of the world (Sharma *et al*.,

1981; Pass, 1986). It exerts inhibitory effects on the growth of other vegetation in vicinity and has now become a major " problem weed " for agriculture, animal husbandry and forestry. A large number of scientists across the world are exploring the possibilities for its eradication. 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 and stems 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 and 160g of stems were loaded.

Soxhlet extraction was carried out using solvents with increasing polarity in sequential manner for extraction of active ingredients[10].

Solvent 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

It 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).

Use and Maintenance of microbial cultures

In the present study, *Staphylococcus aureus* is taken as the test microorganism, 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 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 at 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:

Minimum inhibitory concentration that is 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 below which no inhibition zone was considered as MIC.

Table 1 : Yield of various extracts from roots and stems of *L. camara*

S. No.	Extract	Soluble Principles in Fractions (G)	Percentage Yield (W/W)	Soluble Principles in Fractions (G)	Percentage Yield (W/W)
1.	Petroleum ether	0.92	0.84	0.27	0.17
2.	Benzene	1.2	1.10	0.10	0.06
3.	Chloroform	0.85	0.77	0.06	0.37
4.	Ethyl acetate	2.25	2.05	0.22	0.14
5.	Acetone	0.58	0.53	0.26	0.16
6.	Ethanol	0.74	0.67	-	-
7.	Distilled water	4.82	4.4	5.71	3.57
	TOTAL		**10.4**		**4.5**

Weight of root sample loaded - 110 g; Weight of Stem sample loaded - 160 g

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.

It was observed that the soluble active principles extracted in varying degree in the solvents used were effective in varying proportions against *Staphylococcus aureus*. 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 25mm to 13mm against *Staphylococcus aureus* in case of root extracts of various solvents, the acetone extract being most effective at all concentrations used. In the stem extracts ZOI was observed ranging from 24 mm to 8 mm with acetone extract being most effective and petroleum ether being least effective.

MIC values were also tested against the pathogens of only selected extracts with good to high ZOI and was focused to near 3.125 mg/ml. At this MIC value 15.625 mg of the soluble fraction was present in each disc.

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). In stems 4.5% of total yield was recorded with 3.5% in case of aqueous fraction whereas ethanol was unable to extract. 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 gave a good ZOI against *Staphylococcus aureus* at a concentration of 200 mg/ml with acetone extract (photoplate 1) showing maximum ZOI of 25mm and ethanol 12mm. Similarly at 100 and 50 mg/ml acetone extract gave maximum ZOI of 23mm and 20mm respectively whereas ethanol gave minimum ZOI of 10mm each at 100 and 50 mg/ml concentration, with other extracts (photoplate 2, 3, 4) showing high to moderate ZOI ranging from 22mm to 13mm at various concentrations. Table - 3 shows that *Lantana camara* stems possess very good inhibitory potential. Here, three concentrations were used as above in case of roots.

A moderate to good ZOI was observed using various extracts. At 200mg/ml acetone (photoplate 5) gave maximum ZOI of 24 mm and ethyl acetate minimum ZOI of 15 mm whereas petroleum ether gave no activity, also benzene, chloroform were not tested at 200 mg/ml and alcohol extract was not tested at any of the concentration being scarce. Similarly at 100 mg/ml concentration acetone and benzene (photoplate 6) gave maximum ZOI of 20 mm each whereas petroleum ether gave no activity, with ethyl acetate giving minimum ZOI of 12 mm. At 50 mg/ml concentration benzene gave maximum ZOI of 17 mm and petroleum ether gave minimum ZOI of 8 mm with other extracts showing moderate activity in between.

MIC Determination

MIC is the minimum concentration of the active soluble principles in the extract, which inhibit the growth of organism. This study was carried out by Disc Diffusion Assay Method. Here 5 decreasing concentrations of the extracts were prepared and tested, which were 50, 25, 12.5,6.25 and 3.125 mg/

Table - 2 : Inhibitory activity of root extract of *L. camara* against *S. aureus* showing varying degree of Zone of inhibition (ZOI) in different solvents

S. No.		Extract																					
		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.	*S. aureus*	20	19	17	20	13	16	22	19	13	18	15	16	25	23	20	12	10	10	17	15	13	

Table - 3 : Antibacterial potential of stem extract of *L. camara* against *S. aureus*

S. No.		Extract																					
		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.	*S. aureus*	-	-	8	Nt	20	17	Nt	18	12	15	12	09	24	20	16	Not tested			16	14	12	

Note: Concentrations I: 200 mg/ml, II: 100 mg/ml, III: 50 mg/ml;
Zone of Inhibition (ZOI) measured in mm; Nt: Not tested

Table - 4 : MIC mesaurements of *Lantana camara* root extracts

S. No.	Extracts	Pathogen	Concentration of extract in mg/ml					MIC (mg/ml)
			I	II	III	IV	V	
1.	P.E.	*S. aureus*	17	13	11	10	9	<3.125
2.	Benzene	*S. aureus*	15	13	11	10	9	<3.125

Table - 5 : MIC mesaurements of *Lantana camara* stem extracts

S. No.	Extracts	Pathogen	Concentration of extract in mg/ml					MIC (mg/ml)
			I	II	III	IV	V	
1.	Benzene	*S. aureus*	10	9	8	6	0	<6.250
2.	Acetone	*S. aureus*	14	12	10	9	8	<3.125

Note: *Concentrations I: 50.0; II: 25.0; III: 12.50; IV: 6.250; and V: 3.125; (ZOI) measured in mm;*

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 petroleum ether and benzene were selected for MIC determination against *Staphylococcus aureus* as shown in Table 4. MIC was below 3.125 mg/ml concentration with ZOI using petroleum ether extracts as 17, 13, 11, 10, 9 mm respectively according to the above concentrations and ZOI using benzene extracts as 15, 13, 11, 10, 9mm respectively. MIC of *Lantana camara* Stem extracts: - Only 2 extracts benzene and acetone were selected for MIC determination against *Staphylococcus aureus* as shown in Table 5. ZOI using these extracts were 10, 9, 8, 6, 0 (zero) mm and 14, 12, 10, 9, 8 mm respectively. Therefore MIC values obtained were below 6.250 mg/ml and 3.125 mg/ml against benzene and acetone extracts respectively.

Conclusion

From above study it is very clear that *Lantana camara* root and stem extracts are very effective against *Staphylococcus aureus* and other organisms. A lot of anti-microbial work is being carried out throughout the world. Only few anti-microbial 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 vivo* conditions. Here, an attempt has been made to isolate the crude soluble principles from stems and 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 and stem extracts are highly effective and active against *Staphylococcus aureus* and can be used to cure diseases.

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