

Research article**A study of *Lantana camara* linn aromatic oil as an antibacterial agent**

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Jamadarpali, Sason, Sambalpur-768200, India**ABSTRACT**

The essential oil of *Lantana camara* Linn. (Verbenaceae) a common weed in Western region of Orissa was tested against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sp* the strains were procured from Deptt. Of Microbiology, Burla, Sambalpur. Attempts have been made to quantitate the antibacterial activity by determining the MIC. Efforts were made to study the activity of essential oil on bacterial cells. In addition to this a *in-vivo* study was done on guinea pigs regarding wound healing activity of *Lantana* oil.

Key words: Essential oils, *Lantana camara*, antibacterial, MIC, wound healing property.

INTRODUCTION

Lantana camara Linn, (Verbenaceae) is an ornamental weed with aromatic leaves, orange and bright red flowers and dark blue and black fruits (drupes). A triterpene derivative lantadene-A is the toxic principle. 155 species of these plants are found tropically and sub tropically. The constituents of essential oil of *Lantana camara* as reported by Kasali *et al.*, 2004^[1] are Sabiene (19.6-21.5%), 1,8-Cineole (12.6-14.8%), β -caryophyllene 12.7-13.4%) alpha – humulene (5.8-6.3%), two rare sesqui terpenoids humulene epoxide-III and 8 – hydroxy bicyclogermacrene. Whereas O. Oluwadayo Sonibare and I. Effiong^[2] had found presence of 1,8-cineol (15.8%), sabinene (14.7%) and caryophyllene (8.9%). There have been several reports about the microbial inhibitory impact of essential oil of *Lantana camara* vis-à-vis plant extracts against some microbes (fungicidal activity of leaf extract studied by Dabur *et al*^[3]; nematicidal activity was studied by Kumar *et al*^[4] on methanolic extract. Dena & Thoppil^[5] and Saxena & Sharma^[6] studied the effect of essential oil of *Lantana camara* against bacteria & fungi. One of the important lacuna in the field of *Lantana camara* essential oil research is the mere addition of cataloguing value of the efficacy of the oil. The present per suit aims at evaluation of *Lantana camara* essential oil as antibacterial agent and more over its way of action while killing the bacteria. In addition to this the wound healing property of *Lantana* oil was taken for in vivo study on guinea pigs.

We have studied the effect of hydro distilled essential oil of *Lantana* against strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sp.* procured from Deptt. of Microbiology, V.S.S. Medical college, Burla. The cultures were handled by following routine microbiological culture maintenance methods. For the preliminary screening of essential oils against bacterial species, a disc diffusion Pattnaik *et al.*^[7] method was followed and consequently a tube dilution method Pattnaik *et al.*^[8] was followed for the determination of Minimum Inhibitory Concentration. *Lantana camara* had bactericidal effect against *Escherichia coli*, *Staphylococcus aureus*, but had bacteriostatic effect on *Bacillus sp.* Overall a considerable

growth inhibitory property of *Lantana* essential oil was demonstrated in comparison to the standard antibiotics so tested (Table-2). In addition to this, experiments were carried out based on essential oil and bacterial cell interaction like post drug effect of oil leading cidal or static effect, correlation between killing effect of oil with temperature and pH. In addition to this microscopic observations were made in MIC as well as SIC tubes.

MATERIAL & METHODS**Essential oil**

The fresh leaves of *Lantana camara* Linn, (Verbenaceae) were hydrodistilled in the Department of Pharmacognosy, GCP, Sambalpur by using Clevenger's apparatus. Fresh leaves of *Lantana camara* were immersed in distilled water at a ratio of 1:5 w/v in a distillation flask. Then it was hydro-distilled at 100°C for three hours. Oils were collected in pre sterilized containers and were vacuum evaporated by using silica gel to reduce the water content.

Antibiotics tested:

Nalidixic acid (30mcg), Chloramphenicol (30mcg), Gentamycin (10mcg), Kanamycin (30 mcg), Penicillin (30mcg) and Ampicillin (10mcg) impregnated discs were tested against the bacterial strains by following Hi Media Discs instructions.

Bacteria:

Bacterial strains *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sp.* were taken from V.S.S. Medical College, Burla as gifts and cultures were maintained in Division of Microbiology, Gayatri College of Pharmacy, Jamadarpalli Sambalpur by following regular diagnostic methods.

Media:

The media used for culture of bacteria were Nutrient Agar, Nutrient Broth, Mac Conkey Agar and broth (as selective media) and Sodium Taurocholate salt as diluent. These were purchased from Hi-Media, Mumbai.

Screening of the essential oil for the anti-bacterial activity:

For sensitivity test of bacteria towards the oil was screened by following the paper-disc method Pattnaik *et.al*^[7]. The oil was tested in neat form as well as in 1:4 dilutions with Sodium taurocholate salt solution, which was used as a solvent. The sodium taurocholate salt was prepared in distilled water at a concentration of 250mg/ ml. Bacterial broth cultures (10⁶cells/ml), O/N cultures were seeded on Mac Conkey Agar plates by using sterile cotton swabs. The paper discs were made from Whatman No.1 filter paper (3 mm in diameter). The discs were put in triplicates on to swabbed Mac Conkey plates to get three concordant readings. The oils were also put onto each discs at a volume of 3 µl for both neat and diluted form. The plates were incubated in 37⁰ C for 14 hrs. The clear zones around each disc were measured by disc measuring scale prescribed by Hi-Media, Mumbai. The zone of inhibition (in triplicates) were measured, and the mean values were compared with the zone of inhibition produced by anti-biotic discs of Nalidixic acid, Chloramphenicol, Kanamycin, Gentamycin , Penicillin and Ampicillin (Table 2). The zones of inhibition produced by antibiotic discs were compared with Bayer & Kirby Chart.

Determination of M.I.C. value:

The Minimum Inhibitory Concentration (MIC) was determined by tube dilution technique. The O/N cultures of bacterial were added to each tube to make 100fold dilution. Various concentrations of essential oils diluted with sodium taurocholate salt (1: 4) were taken to determine the inhibitory concentration ranging from 0.01µl-5µl/ml (final concentration). Sodium taurocholate salt was prepared at a concentration of 250mg/ml (w/v) as solvent for the oil. The lowest concentration of the drug preventing the development of visible turbidity was determined as M.I.C. value.

Dilution effect of the Drug:

Dilution is an important factor of a drug for its antimicrobial efficacy. So a dilution test was done with the MIC value of the oil. The MIC tubes were diluted in double fold with fresh Nutrient broth & were kept in incubator at 37⁰C for 24 hours. Then subcultures by streaking were made into Nutrient Agar plates to check the viability of the treated bacterial cells.

Bactericidal / Bacteriostatic effect of the drug:

The cidal / static effect of the drug was determined by making sub-cultures on Nutrient agar plates by streaking. The plates were then incubated for 24 hours at 37⁰ C and growth or no growth was observed.

In vivo study of Lantana oil on wound healing activity:

Four groups (two in number in each group) guinea pigs were taken for clinical experiment. One group was treated as control group and other the three groups of guinea pigs were taken as for experimentation. The animals with age group of six months old (appx.) and weight 800 gms each (appx.) were selected.

Pellets procured from Hindustan Lever Ltd. India, were given as nutrition. The housing was done by the animal house division of Gayatri College of Pharmacy. The control group was meant for treatment with Antibiotic Surfaz-SN cream and the other three groups were for essential oil treatment. The hairs of each guinea pig were removed by using sterile razor and incisions and scratches were made on the surface near neck portion. The control group was applied with the Surfaz-SN (Franco-India pharmaceuticals Pvt. Ltd) cream and the test groups were applied with essential oil after testing the counter irritant effect if any. The guinea pigs were applied with drugs twice daily at 6 hrs of intervals. Besides routine observation, the final observation was made after 7 days regarding the wound healing activity of essential oil. The temperature was also recorded before and after each application of the drugs.

RESULTS AND DISCUSSION

In this study the essential oil of *Lantana camara* had considerable antimicrobial effect. It was observed that the strains of *E.coli* and *Staphylococcus aureus* were more susceptible towards essential oil in comparison to the antibiotics. The strains of *Bacillus* were also inhibited by the action of Lantana oil. In contrast, this *Bacillus* strain was found to be resistant towards Nalidixic acid, Chloramphenicol, Kanamycin and Gentamycin.

Table 1 Effectiveness antibiotics towards bacteria (Compared with Kirby Bayer chart)

S.N.	Names of Bacteria	Names of antibiotics					
		Kn	Chl	Gen	Pen	Amp	Na
1.	<i>Escherichia coli</i>	S	MS	S	R	R	R
2.	<i>Staphylococcus aureus</i>	S	S	R	S	S	S
3.	<i>Bacillus sp</i>	R	R	R	S	S	R

Illustration 1



Effect of Antibiotics on *Escherichia coli* . 1: Kanamycin (S),

2: Penicillin.(R), 3: Ampicillin (R), 4: Kanamycin (S), 5: Gentamycin (S), 6: Nalidixic acid (R)

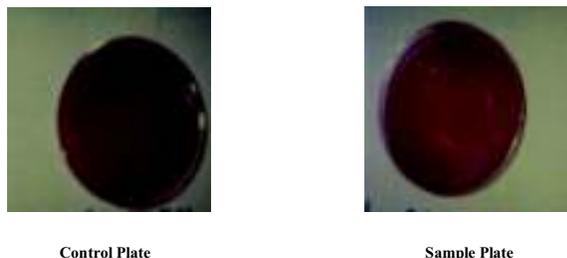
The results are being interpreted as 5mm-15 mm as resistant, 15-25 mm as moderately sensitive and beyond 25mm zones of inhibitions are regarded as very sensitive. Based on the above mentioned interpretation, strains of *E.coli* and *Staphylococcus aureus* were very sensitive towards the essential oil of *Lantana*,

whereas the stain of Bacillus was found to be moderately sensitive. In a comparison study, with Antibiotics, it had been

Table 2 Effectiveness of *Lantana oil* towards bacteria.

S.N.	Names of Bacteria	Zone of Inhibition (in mm)	Interpretation
1	<i>Escherichia coli</i>	25	Sensitive
2	<i>Staphylococcus aureus</i>	29	Sensitive
3	<i>Bacillus sp</i>	19	Moderate

Illustration 2



(Sample Plate Left showing the zone of inhibition is in triplicate Effect of *Lantana* essential oil on *Escherichia coli*)

seen that Gram positive bacterial strains like *Staphylococcus aureus* and *Bacillus sp.* were resistant towards Nalidixic acid, Gentamycin, Chloramphenicol, Kanamycin but were inhibited by effect of Ampicillin and Penicillin. The data was compared with Kirby Bayer chart and interpreted as R-Resistant, S-sensitive and Int S- Intermediately sensitive. And more over *Escherichia coli* was found to be resistant towards Nalidixic acid (Illustration # 1), the DNA gyrase inhibitor of *E coli* J. R. Johnson et al^[9]. In contrast it was observed that *Escherichia coli* were sensitive towards *Lantana* oil (Illustration 2).

Attempts were made to perform the quantitative assay of the essential oil by determining the minimum inhibitory concentration (M.I.C.) by following the tube dilution method adopted by Pattnaik et al^[7]. The minimum inhibitory concentrations of the essential oil were determined to be 1.25µl/ml, 1.0µl/ml and 1.75µl/ml for *Escherichia coli*, *Staphylococcus aureus* and *Bacillus sp.* respectively (Table 3).

Bacteriostatic / Bactericidal:- Efforts were also made to study the interaction between the essential oil and the bacterial cultures. The following observations were made for the observation regarding the way of action of essential oil while inhibiting the bacterial strains. The drug *Lantana camara* had cidal effect against *E.coli* at the Minimum Inhibitory Concentration (MIC) depicting no post drug recovery of cultures in subcultured plates.

Table 3: The MIC values (in µl/ml, final concentration w/v)

S.No	Names of Bacteria	MIC Values (in µl/ml)
1	<i>Escherichia coli</i>	1.25
2	<i>Staphylococcus aureus</i>	1.0
3	<i>Bacillus sp</i>	1.75

In -vivo study:

In the experiment to evaluate the efficacy of the *Lantana* oil towards topical application on wounded skin, of mosaic guinea pigs it was observed that the oil had tremendous wound healing property which was comparable with Surfaz-SN cream. (Illustration # 3)

Illustration # 3



Upper part showing wound before topical application of *Lantana* oil

Lower part showing diminished wound after topical application of *Lantana* oil

CONCLUSION

On the basis of above studies on *Lantana camara* Linn leaves oil, we conclude that the plant leaves oil having sufficient bactericidal activity on human pathogenic strains in addition to the oil having wound healing properties resemble to standard.

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