

Antimicrobial activity of *Lantana camara* L.

Ashish Saraf¹, Sadaf Quereshi², Kavita Sharma³, Noor Afshan Khan⁴

¹Faculty of Life Sciences, MATS University, Raipur- 492001(C.G.), India

²Centre for Scientific Research & Development, People Group, Bhopal (M.P.), India

³H.O.D. Botany, Arts & Commerce Girls College, Raipur (C.G.), India

⁴Quality Analysis Lab for Medicinal & Aromatic Plants, Dept. of Plant Physiology, J.N.K.V.V. (M.P.), India

Abstract

The antimicrobial activity of crude Methanolic and acetone extracts of *Lantana camara*, a traditional medicinal plant was determined against thirteen test bacteria and eight test fungal strains. Both the solvent extracted extracts inhibited the growth of *Staphylococcus aureus* to the maximum. The fungitoxic spectrum of the test plant's leaf & stem extracts indicated maximum percentage growth inhibition at 1000µgml⁻¹ concentration against *Alternaria alternata*. Thus, phytochemicals from *L. camara* have a broad antimicrobial spectrum and might be a novel source of antimicrobial drugs.

Keywords: Lantana camara, Crude extract, Methanolic extract, Acetone extract, antimicrobial activity

INTRODUCTION

L. camara is a low erect, rugged hairy, evergreen shrub (Verbenaceae) native to tropical America. Known by several common names viz., blacksage, cuasquito, angel lip, flowered sage, shrub verbena, white sage and wild sage all over the world, it is a significant weed of which there are some 650 varieties in over 60 countries or island groups. *L. camara* has several uses, mainly as a herbal medicine and in some areas as firewood and mulch. It is also used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera [1]. In some countries, it is planted as a hedge to contain or keep out livestock. Extracts from the lantana leaves exhibit antimicrobial, insecticidal and nematocidal activity and also contain verbascoside, which possesses antimicrobial, immunosuppressive and antitumor activities [2]. Lantanoside, linaroside and camarinic acid have been isolated and are being investigated as potential nematocides. Lantana oil is sometimes used for the treatment of skin itches, antiseptic for wounds, leprosy and scabies.

The biological diversity potential of plant metabolites is evident from the fact that 47-marketed drugs have been derived from 39 tropical forest plants. Phytochemical studies carried out by different group of workers, on different parts of the plant have resulted in the isolation of various terpenoids, steroids and flavonoids. In the course of investigations on the constituents of the aerial parts of *L. camara*, three new pentacyclic triterpenoids namely camaryolic acid, methylcamaralate and camangeloyl acid were isolated. The structures of these constituents were elucidated as 3, 25 – epoxy – 3

α – methoxy – 22 β - [β, - β dimethylacryloyloxy]-urs-12-en-28-oic acid, methyl 22 β -acetoxo-3, 25-epoxy-3 α -hydroxy-urs-12-en-28-oate and 3,25-epoxy-3 α -hydroxy-22 β -[(Z)-2'-methyl-2'-butenoyloxy]-11-oxoolean-12-en-28-oic acid respectively on the basis of various 2D-NMR techniques including 1H – 1H correlation spectroscopy (COSY), nuclear overhauser enhancement spectroscopy (NOESY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC). In addition six known compounds. β -sitosterol 3-O- β -b-glucopyranoside, octadecanoic acid, docosanoic acid, palmitic acid, camaric acid and lantanolic acid were isolated from this plant. *Lantana camara* afforded a novel triterpene 22 beta-acetoxylantic acid and the known triterpenes, lantic acid, 22 beta-dimethylacryloyloxylantanolic acids, a mixture of 22 beta-dimethylacryloyloxylantanolic acid and 22 beta-angeloyloxylantanolic acid and lantanolic acid. 22 beta-Acetoxylantic acid showed antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhi*. This compound and 22 beta-dimethylacryloyloxylantanolic acid also showed antimutagenic activity [3].

Lantana has been used as medicines over hundreds of years, constitute an over choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effect or merely based on folklore. Therefore, present investigation has been focused on isolation and partial purification of bioactive molecule from *Lantana camara* and investigation of their in vitro antimicrobial activity.

MATERIALS AND METHODS

Plant material and extraction procedure

Aerial parts (stem, leaves) of *Lantana camara* L. were collected in March 2007 from university campus Jabalpur. The taxonomic identification of plant material was confirmed by Prof. Akhilesh K. Pandey, Botanist, Department of Biological Sciences, R. D. University, Jabalpur India. A voucher specimen is deposited at the herbarium of the Department of Botany, Jabalpur University, Madhya Pradesh; Jabalpur, India. Collected plant material was dried in shade and grounded in a grinder with a 2mm diameter mesh. The dried and powdered plant material (500g) was extracted successively with 1L

*Corresponding Author

Aberoumand
Behbahan Khatemolania Technology University,
Behbahan, Iran

Tel: +91-91-0771-2606677
Email: ashish.saraf22@gmail.com

of acetone, Methanol and Methanol using Soxhlet extractor for 48h at a temperature not exceeding the boiling point of the solvent [4] The aqueous extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40 °C using a Rotary evaporator (Buchi, Germany). The residues obtained were stored in a freezer at -40 °C until further analysis.

Culture media

Nutrient agar, Nutrient broth, Potato dextrose agar [5] were used throughout the study. The plant material was cleaned and decontaminated with ethylene oxide and irradiated.

Micro-organisms used

a. Bacteria and yeast

Escherichia coli (MTCC - 443), *Bacillus subtilis* (MTCC 1789), *Staphylococcus aureus**, *Streptococcus sp.**, *Pseudomonas aeruginosa** *Vibrio cholerae**, *Aeromonas faecalis*1, *Bacillus cereus**, *Klebsiella pneumoniae* (MTCC 2405), *Vibrio haemolyticus*1, *Candida albicans* (MTCC 1022) and *Candida tropicalis* FGCC# 26 (ct, *Saccharomyces cerevisiae* (MTCC 17322).

b. Fungi- All fungi mentioned below were obtained from Fungal Germplasm Collection Center, Applied Mycological research laboratory, Department of Biological Science, R. D. University, Jabalpur.

Aspergillus niger, *Aspergillus flavus*, *Penicillium spp.*, *Fusarium oxysporum*, *Alternaria alternata*, *Sclerotium rolfsii*, and *Curvularia lunata*.

Antimicrobial activity tests

The dried plant extracts were dissolved in the same solvent (acetone, Methanol or Methanol) to a final concentration of 30 mg/ml and sterilized by filtration through 0.45µm Millipore filters. Antibacterial (a set of Gram positive and Gram negative) and anti yeast test were then carried out by disk diffusion method [6], using 100 µl of suspension containing 10⁸ cfu/ml of bacteria and 10⁶ cfu/ml of yeast spread on nutrient agar and sabouraud dextrose agar respectively. The disks (6mm) were impregnated with 10µl of the extracts (100µg/ml disk) and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extract. Gentamycin sulfate (10mg/ml disk) was used as a positive reference standard to determine the sensitivity of one strain from each bacterial species. The inoculated plates were incubated at 35±10°C for 24h for bacteria and 48h for yeast.

Antifungal activity was determined by poisoned food technique [7] was adopted to evaluate the effect of essential plant extract on the growth of human and plant pathogenic fungi. 20ml of sterilized and cooled (40°C) growth media (PDA) with 10mg streptomycin were poured into pre sterilized Petri plates. Requisite amount of different concentration of plant extract like 100, 300, 500, 1000 µg were added into the plates. The assay plates were rotated carefully to ensure an even distribution of the oil in the medium. In control plates the medium was supplemented with sterilized distilled water to compensate the volume instead of plant extract. After the solidification of the agar medium, inoculums of the test organism

(disc of 6 micrometer diameter cut from periphery of a 7 day old culture with the help of sterile cork borer) was placed aseptically in the centre of each petri plates of treated and control sets. The assay plates were then incubated at 28 ± 10°C for 6 days. After the desired period of incubation diameter of the fungal colony of treated as well as control sets were measured. The experiment was conducted in the multiples of 3 triplicates.

The percentage of mycelial inhibition was calculated/ computed by mean value of colony diameter by the following formula:

$$\text{Percentage of mycelial inhibition} = \frac{dc - dt}{dc} \times 100$$

dc

dc – average diameter of fungal colony in control sets

dt - average diameter of fungal colony in treated sets.

Antimicrobial Screening Assay:

The isolated plant extract was prepared for screening of antimicrobial activity by mixing 2-5ml (100%) of respective solvents in which the plant biomolecule has been isolated shaking for 15min. and then centrifuging for 15min. at 3000 rpm. The desired aliquots of the Methanol extracts were evaporated to half of their volume in a nitrogen flow in order to increase the concentration of the solvent. The respective extract (25ml) were applied to the surface of the seeded assay plates, which were incubated at entire 28 ± 20°C (Yorco, India) or 37°C ± 20°C (bacteria). Inhibition zones around the application points were measured after 24hrs.(data not shown)

Statistical Analysis

Each experiment was performed at least three times and the results are expressed as Means±SD. Data were analyzed by Duncan's Multiple Range Test (DMRT) with P values > 0.05 considered to be significant.

FTIR Analysis: The HPLC purified sample were further subjected with IR Spectroscopy.

RESULTS

Antibacterial activities of *Lantana camara* leaves and stem extract

The study showed that the solvent extracts investigated were active against various Gram positive and Gram negative bacteria (Table 1). Alcoholic extract of *Lantana* leaves exhibited stronger antimicrobial activity in comparison with acetone extract. Among the set of test bacterial strains alcoholic extract of the test plant inhibited the growth of *Staphylococcus aureus* to the maximum followed by *B. subtilis* and *B. cereus*. Intermediate zone of inhibition was observed for *Streptococcus sp.* and *E.coli* while minimum antibacterial activity was reported with *V. haemolyticus* and *V. cholerae*. No inhibition zone was observed for any species of *Candida sp.*

Acetone extract of *Lantana* leaves showed comparatively less antibacterial activity than the alcoholic extract. Amongst the test strain maximum effect of the extracted material was observed on *B. cereus* followed by *S. aureus* and *B. subtilis*. Mild antibacterial activity was observed with *Streptococcus sp.* less activity was observed against *K. Pneumoniae* and *V. Cholerae*.

Table 1. Antibacterial activity of *Lantana camara* leaf and stem extracts.

Test organisms	Zone of Inhibition (mm)				Reference Gentamycin sulphate
	Alcoholic		Leaf	Acetone Stem	
	Leaf	Stem			
<i>Acetigenes faecalis</i>	16.0 ^c	16.0 ^b	13.80 ^b	10.0 ^b	36.0
<i>Bacillus cereus</i> *	22.0 ^b	15.1 ^b	22.5 ^a	13.0 ^a	40.0
<i>Bacillus subtilis</i> (MTCC 1789)	24.2 ^a	19.6 ^a	21.16 ^a	11.0 ^a	42.0
<i>Escherichia coli</i> (MTCC 443)	18.2 ^a	17.8 ^a	19.6 ^{ab}	9.0 ^b	36.0
<i>Klebsiella pneumoniae</i> (MTCC 2405)	10.1 ^c	7.2 ^c	9.0 ^c	8.9 ^b	38.0
<i>Pseudomonas</i> <i>aeruginosa</i> *	13.8 ^d	9.0 ^c	9.3 ^c	7.2 ^c	89.0
<i>Staphylococcus aureus</i> *	25.6 ^{bc}	20.0 ^b	20.0 ^a	11.2 ^a	33.0
<i>Streptococcus sp.</i> *	21.6 ^c	16.0 ^b	20.2 ^a	10.8 ^b	38.5
<i>Vibrio cholerae</i> *	8.2 ^d	6.8 ^d	7.0 ^d	8.9 ^b	36.0
<i>Vibrio</i> <i>hamparalyticus</i> ¹	7.0 ^e	-	-	-	32.0
<i>Candida albicans</i> (MTCC 1022)	-	-	-	-	NT
<i>Candida tropicalis</i> (ct, FCCC#26)	-	-	-	-	NT
<i>Saccharomyces</i> <i>cerevisiae</i> (MTCC 1732)	9.20 ^d	8.0 ^c	10.0 ^c	-	NT

- Values are the mean of triplicates.
- (*) Chandrakar Pathology Laboratory, Jabalpur (India)
- (1) Culture obtained from Bacteriology Laboratory, Dept. of Biological Sciences, R.D. University, Jabalpur (India).
- Values followed by the same letters along each vertical column are not significantly different (P=0.50)

The antibacterial study of stem extract of *Lantana camara* showed its effectiveness against various Gram Positive and Gram Negative bacteria as depicted in (Table-1). Alcoholic extract of *Lantana* stem exhibited stronger antimicrobial activity in comparison to acetone extract. Among the Gram negative group of bacteria, acetone extract of the test plant inhibited the growth of *E. coli* to the maximum, followed by inhibition zones of *Pseudomonas aeruginosa*. Whereas in Gram positive bacteria maximum antibacterial activity was exhibited by plant extract against *S. aureus* followed by *B. subtilis*. Two Gram positive bacteria i.e. *Streptococcus sp.* And *B. cereus* also exhibited significant inhibition due to antibacterial activity of alcoholic extract. Similarly acetone extract of *Lantana* stem showed antibacterial activity. Among the Gram negative group of bacteria, maximum effect of the extract was observed on *E. coli* followed by *V. cholerae* and *K. pneumoniae*. Good inhibition zones were observed for Gram positive bacteria which was maximum against *B. cereus* followed by *B. subtilis* and *Staphylococcus aureus* with alcoholic leaf extract.

The study showed that the extracts investigated were active against various Gram positive and Gram negative bacteria (Table1). As we have seen in earlier study the alcoholic extract of *Lantana camara* leaves and stem exhibit stronger antimicrobial activity. So in further study we use only the alcoholic extract of the stem and leaves of *Lantana camara*. Yeast like *Candida albicans* and *Candida tropicalis* were also tested against the acetone and alcoholic extract of *Lantana* leaves and stem but they showed negative results by exhibiting no inhibition zone except one species of yeast i.e. *Saccharomyces cerevisiae* which gave mild inhibition zone. There was no antimicrobial activity of these extracts against yeast. The active concentration of extract i.e. titer used for antibacterial activity determination was 100µg/ml. Various mixture of plant leaves and stem extracts have also been tested against the Gram positive and Gram negative group of bacteria which did not show any significant

difference from which they have been individually tested. Reference taken was the antibiotic Gentamycin sulfate at 10mg/ml concentration.

Antifungal activity of *Lantana* leaf and stem extract

As shown in (Table 2) that various extract of *Lantana* leaves and stem was subjected to antifungal activity with different fungal strains at desired period of incubation. The fungitoxic spectrum of the plant leaves extract was measured by poisoned food technique as represented in (Table 2), The Ethanolic *Lantana* leaves extract was used in different concentration Viz., 100µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml to assess the antifungal activity against various fungal strains. Among all the fungal isolates tested interestingly all the isolates exhibited maximum percentage growth inhibition at 1000µg/ml concentration of the Ethanolic extract.

Maximum percentage growth inhibition was recorded in case of *A. alternata* followed by *F. moniliformae* and *C. lunata* at 1000µg/ml concentration of solvent extracted active moiety. Good percentage growth inhibition was observed with *F. oxysporum* followed by *A. flavus* and *S. rolfsii*. Minimum percentage growth inhibition was observed in *A. niger*. Similarly studies on percentage growth inhibition observed were also done with acetone extract of *Lantana* leaves against different fungal isolates. As shown in (Table 2) the fungitoxic spectrum of the plant leaves extract show similar results as of Methanolic extract of test plant leaves with mild variation. Maximum percentage inhibition was seen in *C. lunata* followed by *F. oxysporum* and *F. moniliforme*. Good percentage of inhibition was observed in *S. rolfsii* while less percentage growth inhibition was reported in *Penicillium sp.* The fungitoxic spectrum of plant leaves acetone extract showed greater antifungal potential then the ethanolic extract thus this may lead to conclude the possibility of the active moiety in acetone leaves extract of the test plant then ethanolic extract.

Table 2. Fungitoxic spectrum of Lantana leaves and stem extract at 1000 µg/ml.

Fungal Test Organisms	% of growth inhibition			
	Leaves		Stem	
	Ethanol	Acetone	Ethanol	Acetone
<i>Aspergillus niger</i> (FGCC# 492)	46.8 ^{3a}	61.0 ^b	30.4 ^a	39.2 ^b
<i>Aspergillus flavus</i> (FGCC# 133)	58.0 ^c	67.0 ^b	36.3 ^b	28.6 ^c
<i>Penicillium</i> sp. (FGCC# 124)	32.0 ^a	46.3 ^c	32.8 ^b	41.1 ^b
<i>Fusarium oxysporum</i> (FGCC# 503)	77.7 ^b	92.0 ^a	45.0 ^a	40.0 ^a
<i>Fusarium moniliforme</i> (FGCC# 193)	91.0 ^a	91.8 ^a	18.6 ^c	30.0 ^c
<i>Alternaria alternata</i> (FGCC# 418)	92.0 ^a	89.0 ^a	53.8 ^a	40.5 ^a
<i>Curvularia lunata</i> (FGCC# 102)	89.0 ^a	93.0 ^a	20.6 ^d	28.2 ^c
<i>Sclerotium rolfsii</i> (FGCC# 406)	50.0 ^c	74.2 ^{ab}	48.2 ^a	59.5 ^a

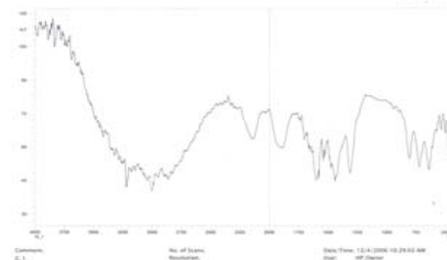
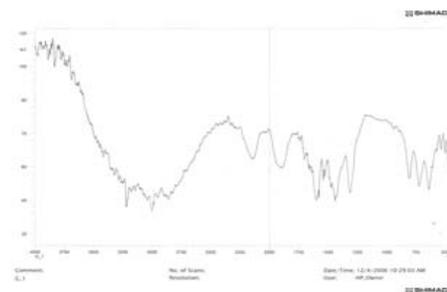
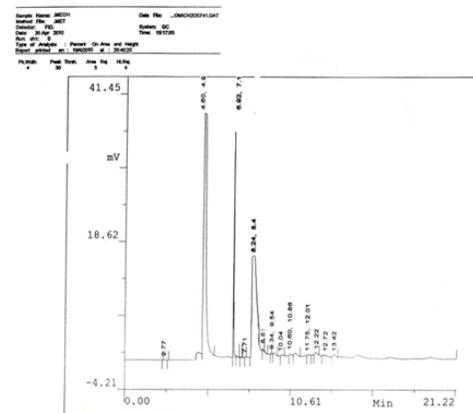
- Values are the multiple of three replicates.
- Fungi were obtained from Fungal Germplasm Collection Center, Applied Mycological research laboratory, Department of Biological Sciences, R. D. University, Jabalpur.
- Values followed by the same letters along each vertical column are not significantly different (P=0.50)

The fungitoxic spectrum of the plant stem extract measured by poisoned food technique is depicted in (Table 2). Ethonolic and acetone extract were assessed for antifungal activity against different fungi. A common feature observed in case of all fungal strains was that maximum percentage growth inhibition was reported 1000µg ml-1 concentration of the solvent extract of plant stem. Ethanolic extract of stem of target plant at 1000µg ml-1 effective concentration exhibited maximum antifungal activity against *Alternaria alternata*. This was followed by *Sclerotium rolfsii*, *Fusarium oxysporum* and *Penicillium* sp. at 1000µg ml-1 concentration. Considerable antifungal activity was exhibited against *Aspergillus flavus* and *Aspergillus niger* at 1000µg ml-1 concentration of the extract. Similarly percentage growth inhibition was also determined with different concentrations of the test plant acetone stem extract against different fungal strains as depicted in (Table 2). The test titers for determination of antifungal activity included 100 µg ml-1, 250µg ml-1, 500 µg ml-1 and 1000 µg ml-1.

The growth of *Sclerotium rolfsii* got inhibited to maximum extent with 1000 µg ml-1 concentration of acetone stem extract of Lantana. Significant percentage growth inhibition was noted in other fungal strains like *Alternaria alternata*, *Pencilium* sp., *Fusarium oxysporum* and *Aspergillus niger*. Less percentage growth inhibition was recorded in *Aspergillus flavus* and *Curvularia lunata* at 1000 µg ml-1 concentration of stem acetone extract.

Out of the two extract of Lantana stem i.e. ethonolic and acetone, results shows that acetone extract elicits more antifungal potential and efficacy then its ethanolic counterpart. This suggests the presence of antifungal ingredient in acetone extract, which is thus proved significantly and experimentally.

Highly effective sample were further subjected with HPLC and FTIR. Total 11 peaks were obtained for fraction of ethanolic extract by subjecting to HPLC out of which 2 peaks were very sharp with retention time of 4.60 and 6.92. The effective fraction were subjected to FTIR for detection of active functional group which contain amino and hydroxyl function group with substituted benzene.



DISCUSSION

Similar results with other plant extract against Gram positive and Gram negative bacteria were reported by others [8]. 20µl acetone extract of other plants like *Glycyrrhiza glabra*, *Cinnamomum cassia*, *Juniperus oxydurus* elicited maximum antibacterial activity against *B.brevis*, *B.cereus*, *B.megaterium*, *B.subtilis*, *P.aeruginosa* and *S.aureus* [9]. The antimicrobial activity may be due to the presence

of triterpene secondary metabolite in the extract. Similarly Barre et al., 1997 have reported a bioactive triterpene- 22 beta aceto xylantic acid and other triterpenes which showed antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhi*. Antibacterial activity of different plant extract on phytopathogenic *Xanthomonas campestris* pathovars was studied and reported by other workers [10]. Using Soxhlet methodology of solvent extraction, excellent antifungal activity of Methanolic extract of *Senna alata*, a Thai medicinal plant was obtained against certain dermatophytes [11]. Some pharmacological properties of root extracts of *Terminalia sericea* exhibiting antifungal activity against *Candida albicans* and *Aspergillus niger* was also reported by other scientists [12]. Methanol extract of various parts of *Lagerstroemia parviflora* Roxb exhibited antifungal activity against *Aspergillus* and *Penicillium* species and yielded good zones of inhibition. Thus, the extract was found to be fungistatic in its action [13]. Antifungal activity of Methanolic leaf extract of *Indigofera suffruticosa* was also determined against *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. [14].

Therefore, present work highlights the use of solvent extracted stem and leaves extracts of *L. camara* containing a highly potential phytochemical which could be characterized and thus, find its way into the arsenal of lucrative antimicrobial drugs.

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