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STUDY OF ANTIMICROBIAL ACTIVITY OF *LANTANA INDICA* LINN. AGAINST SOME HUMAN PATHOGENS. AND ITS PHYTOCHEMICALS.

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ABSTRACT

Medicinal plants are the wealthy source of the antimicrobial agent and curative. In the present study we carried out the antimicrobial effect and analysis of phytochemical constituents of different parts of *Lantana indica* Linn (*Verbenaceae*). Pathogenically significant bacteria (*Salmonella typhi*-MTCC-733, *Staphylococcus aureus*- MTCC-7443, *Pseudomonas aeruginosa* MTCC-7296, *Mycobacterium tuberculosis*-MTCC-300) and fungus (*Microsporum canis*-MTCC-2820, *Epidermophyton floccossum*-MTCC-613, *Trichophyton rubrum* –MTCC-296, *Aspergillus candidus* –MTCC-1989) were selected for the study. The extract of root, stem, leaf and flower of plant were prepared in methanol and aqueous solutions and different doses (25 µl, 50 µl, 100 µl, 200 µl, 300 µl and 400 µl) were selected for study by agar disk diffusion method. We found significantly effective antibacterial property of *Lantana indica* at concentrations of 25 µl to 400 µl against *Pseudomonas aeruginosa* and at concentration of 400 µl against *Salmonella typhi* and *Staphylococcus aureus* but found completely ineffective against *Mycobacterium tuberculosis.* The significantly effective antifungal activity was found only at 400 µl concentration against *Epidermophyton floccossum* and *Trichophyton rubrum* but found completely ineffective against *Microsporum canis* and *Aspergillus candidus.*The secondary metabolites of extract effective against microorganisms was evaluated and recognized as saponins, tannins, cardiac glycosides, alkaloids, flavonoids, steroids and terpenoids. The HPLC profiling was also performed and was identified as Lantadens A, B, C, D which is effective agent against above microorganisms.

KEYWORDS: Antimicrobial activity, Medicinal plants, Aqueous extract, Methanol extract, Human pathogens, HPLC.

INTRODUCTION

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed.[1] Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and wellbeing and the bio prospecting of new plant-derived drugs.[2] Also an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies.[3] These medicinal plants play a vital role for the development of new drugs. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties that can be used to treat several chronic as well as infectious diseases. In Indian medicinal system, (the Ayurveda) different plants are employed as medicines to cure various diseases.[4] It is believed that plant extracts showing target areas other than those used by antibiotics will be active against drug resistant microbial pathogens. Plant extracts and phytochemicals with antimicrobial properties are of great significance in therapeutic treatments. In this context, in recent years the activity of phytochemical extracted from traditional medicinal plants with antimicrobial properties have been studied extensively by researchers from various countries. The findings obtained from such research may lead to the validation of traditionally used medicinally important plants.

Lantana indica belongs to family *Verbenaceae*, is a shrub, native to India.[5] It has been used as a sudorific, intestinal antiseptic, diaphoretic and in the treatment of tetanus, rheumatism and malaria in the Ayurvedic system of medicine.[6] Essential oil obtained from the leaves of *L. indica.* contains chemical compounds were trans-caryophyllene, α-selinene , globulol, trans-caryophyllene oxide, α-guaiene , valencene , humulene and β-eudesmene.[7] *L indica* root contains, oleanolic acid, 3-ketooleanolic acid, (+)-24-hydroxy-3-oxoolean-12-en-

28-oic acid, 3β,24-dihydroxyolean-12-en-28-oic acid and 3,24-dioxo-olean-12-en-28-oic acid.[8,9]

The test organism *Salmonella typhi* is the only one that is pathogenic exclusively for humans, in whom it causes typhoid or enteric fever. This disease is characterized by the sudden onset of a sustained and systemic fever, severe headache, nausea, and loss of appetite.[10] *Staphylococcus aureus* is often found as a commensal associated with skin, skin glands, and mucous membranes, particularly in the nose of healthy individuals.[11] It is one of the main causes of hospital- and community-acquired infections (nosocomial) which can result in serious consequences.[12] *Pseudomonas aeruginosa* is the most common pathogen isolated from patients who have been hospitalized longer than one week. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs)[13] and bacteremia. *Mycobacterium tuberculosis* is a causative agent of tuberculosis[14] typically attacks lungs.

Microsporum canis is a Zoophilic dermatophyte. It is a common agent of ringworm in animals but is also frequently associated with human infection.[15] This species invades hair, skin and rarely nails. *Trichophyton rubrum* is an anthropophilic fungus, which infection is restricted to man only and more frequent in urban population due to modern way of life.[16] It causes chronic ringworm infections of skin, hair and nails, especially in toe webs, soles and palms. *Epidermophyton floccosum* is also an anthropophilic dermatophyte. It causes chronic ringworm infections of skin and nails. It is not known to invade hair. *Aspergillus candidus* has been claimed to be involved in a wide range of human infections including invasive aspergillosis[17] aspergilloma, otomycosis[18] brain granuloma and onychomycosis.[19, 20]

MATERIALS AND METHODS

Collection and identification

The plant of *Lantana indica* was collected from Rajnandgaon 50 km around the district head quarter in January 2012 to April 2012. The Latitude and Longitude of Rajnandgaon district is between 20º07′ and 22º29′ N and 80º23′ and 81º24′ E. Rajnandgaon district is situated in Chhattisgarh state of India. The identification and authentication of the plant was carried out at Department of Botany, Govt V.Y.T PG. Autonomous College, Durg (C.G.) India and further verified by Botanical Survey of India, Allahabad (U.P.).

Solvent Extraction

All parts (root, stem, leaves and flowers) of *Lantana indica* were collected then washed thoroughly for surface sterilization and shade dried for seven days and stored in powdered form. The powdered plant material was extracted with solvents methanol and distilled water in the ratio of 1:10 and then processed with Soxhlet Extraction Unit (MSW, India) for 48hrs. Freshly prepared extracts were categorized in to different concentrations (25, 50,100,200,300 and 400µl) for further investigations. The extracts thus obtained were used for the *in vitro* studies.[21]

Collection of Microorganisms

Four human pathogenic bacteria, *Salmonella typhi* (MTCC-733), *Staphylococcus aureus* (MTCC-7443), *Pseudomonas aeruginosa* (MTCC-7296), *Mycobacterium tuberculosis* (MTCC-300) and four human pathogenic fungi *Microsporum canis* (MTCC - 2820), *Epidermophyton floccossum* (MTCC - 613), *Trichophyton rubrum* (MTCC - 296) and *Aspergillus candidus* (MTCC - 1989) were selected for present study. All these strains were obtained from the (MTCC) Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology, Chandigarh, India and were selected for the study on the basis of their clinical importance.

Antimicrobial study

The antimicrobial activity was performed by the agar disk diffusion method described by.[22] The disk of 6.00mm of Whatman filter paper no. 1 was saturated with plant extracts with different concentrations (25µl, 50µl, 100µl, 200µl, 300µl and 400 µl) and allowed to dry. The impregnated disks were then placed on to the surface of a suitable solid agar medium to test antimicrobial activity of plant extract. The bacteria seeded plates containing *Salmonella typhi. Staphylococcus aureus, Pseudomonas aeruginosa,* were incubated for 24 hrs at 37^0 C and plates containing *Mycobacterium tuberculosis* was incubated for three weeks at 37^0 C. Fungal seeded plates were incubated for 72 hrs at 25^0C, except *Trichophyton rubrum* which is incubated at 30^0C for 72 hrs. The microbial growth was determined by measuring the diameter of zone of inhibition.

Phytochemical Evaluation

a). Preliminary Phytochemical Screening

Plant's parts with positive result were subjected for phytochemical evaluation.The presence of Saponins, Tannins, Alkaloids, Terpenoids, Steroids, Flavonoids and Cardiac glycosides were detected by using appropriate phytocamical screening methods.[23, 24, 25]

b). HPLC profiling

The secondary metabolite Lantadenes was analysed using C_{18} column of HPLC. HPLC separation was performed in a Dionex prep HPLC system coupled with Gynkotek GINA 50 auto sampler and Dionex UVD 3403 Photo Diode array Detector and ELSD-4-Luna (Evaporative Light Scattering Detector). C_{18} preparative HPLC column (10µ, 250mm × 21.2mm) was used. Sep Pack DSC-18 Supleco 10g cartridge was used for prep of HPLC fractionation. MBC spectra were optimized for a 10 ng range JH-C of 9 Hz and NOSEY experiment was carried out with a mixing time of 0.8 sec.

Statistical analysis

For statistical validation of data to test the significance level of zone of inhibition, student "t" test was applied.

RESULT

The methanolic extract of leaves of *Lantana indica* showed significant antibacterial activity against *Salmonella typhi* and *Staphylococcus aureus* at 400 µl concentration only. But it was found significant against *Pseudomonas aeruginosa* at different concentrations (25 µl, 50 µl, 100 µl, 200 µl, 300 µl and 400 µl) . At 25 µl concentration the reported zone of inhibition was 07.93 ± 00.24 mm (P= 03.81 >2.78 at 5% P), at 50 µl concentration it was 08.33 ± 00.44 mm (P=02.98 >2.78 at 5% P), at 100 µl concentration it was 10.93 ± 00.24 mm (P = 03.18 >2.78 at 5% P), at 200 µl concentration**,** it was 11.66 ± 00.18 mm (P= 03.63 >2.78 at 5% P), at 300 µl concentration**,** it was 15.39 ± 00.12 mm (P = 03.20 >2.78 at 5% P) and at 400 µl concentration it was18.39 ± 00.12 mm (P = 03.20>2.78 at 5% P).The same extract showed significant antibacterial property (P=03.53>2.78 at 5% P) with zone of inhibition 15.46 ± 00.22 mm against *Salmonella typhi* at 400 µl concentration only and against *Staphylococcus aureus* both the methanolic extract of leaf and aqueous extract of stem showed zone of inhibition of 16.46 ± 00.32 mm and 07.13 ±00.08 mm respectively but the significant zone of inhibition was reported only in leaf extract (P = 03.47>2.78 at 5% P) at 400 µl concentration.

Leaf, stem and root of *Lantana indica* were found as the rich antifungal sources and significantly effective against *Trichophyton rubrum* and *Epidermophyton floccossum.* Against *Trichophyton rubrum* only the methanolic extract of leaf showed significant antifungal property (P=3.40 > 2.78 at 5% P) with zone of inhibition 13.46 ± 0.42mm. Against *Epidermophyton floccossum*, the methanolic extract of leaf showed significant antifungal property (P=3.90 > 2.78 at 5% P) with zone of inhibition 15.26 ± 0.39mm and the methanolic extract of root showed significant result (P=3.55 > 2.78 at 5% P) with zone of inhibition 22.06 ± 0.54mm and aqueous extract of stem showed significant antifungal property (P=3.59 > 2.78 at 5% P) with zone of inhibition 14.99 ± 0.27mm.

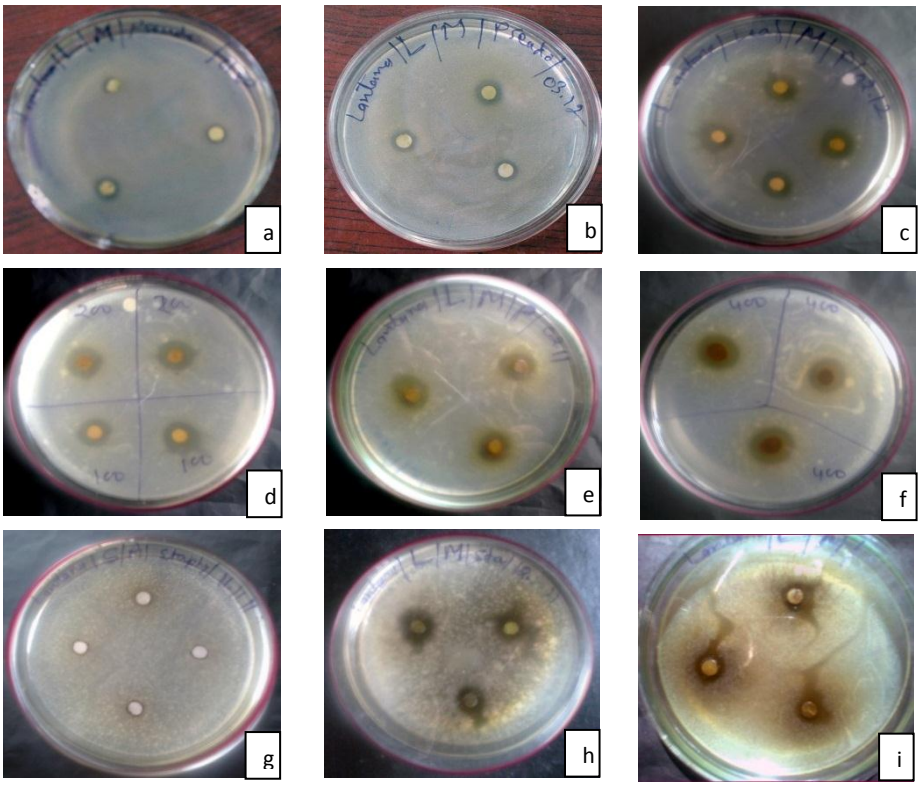


Figure 01: Antibactirial effect of *Lantana indica.*

Plate a – Showing antibacterial effect of methanolic extract of leaf against *Pseudomonas aeruginosa* (ZOI – 07.93 mm) at 25 µl concentration.

Plate b – Showing antibacterial effect of methanolic extract of leaf against *Pseudomonas aeruginosa* (ZOI – 08.33 mm) at 50 µl concentration.

Plate c – Showing antibacterial effect of methanolic extract of leaf against *Pseudomonas aeruginosa* (ZOI – 10.93 mm) at 100 µl concentration.

Plate d – Showing antibacterial effect of methanolic extract of leaf against *Pseudomonas aeruginosa* (ZOI – 11.66 mm) at 200 µl concentration.

Plate e – Showing antibacterial effect of methanolic extract of leaf against *Pseudomonas aeruginosa* (ZOI – 15.39 mm) at 300 µl concentration.
Plate f – Showing antibacterial effect of methanolic extract of leaf against *Pseudomonas aeruginosa* (ZOI – 18.39 mm) at 400 µl concentration.
Plate g – Showing antibacterial effect of aqueous extract of stem against *Staphylococcus aureus* (ZOI –07.13 mm) at 400 µl concentration.
Plate h- Showing antibacterial effect of methanolic extract of leaf against *Staphylococcus aureus* (ZOI – 16.46 mm) at 400 µl concentration.
Plate i – Showing antibacterial effect of methanolic extract of leaf against *Salmonella typhi* (ZOI –15.46 mm) at 400 µl concentration.

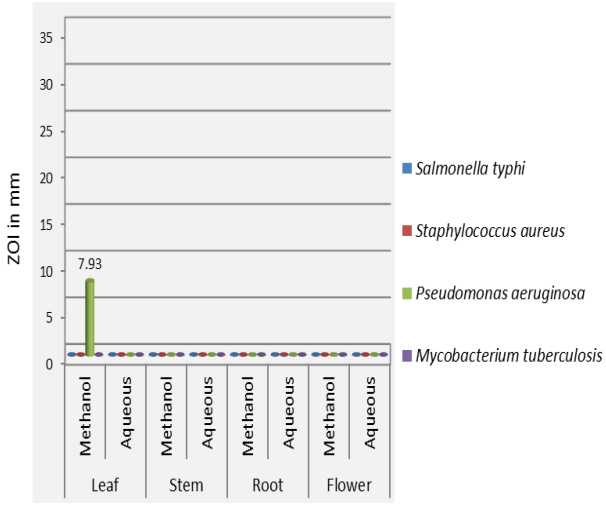


Fig. 2- Showing antibacterial effect of *Lantana indica* at 25 µl concentration.

The methanolic extract of leaf is showing zone of inhibition (07.93 mm) against *Pseudomonas aeruginosa* only.

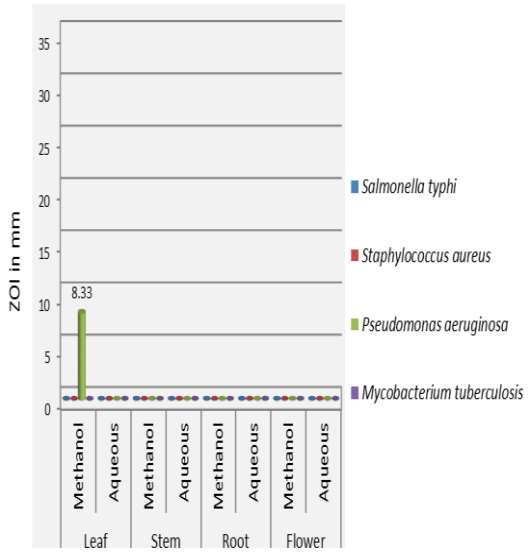


Fig .3 - Showing antibacterial effect of *Lantana indica* at 50 µl concentration

The methanolic extract of leaf is showing zone of inhibition (08.33 mm) against *Pseudomonas aeruginosa* only.

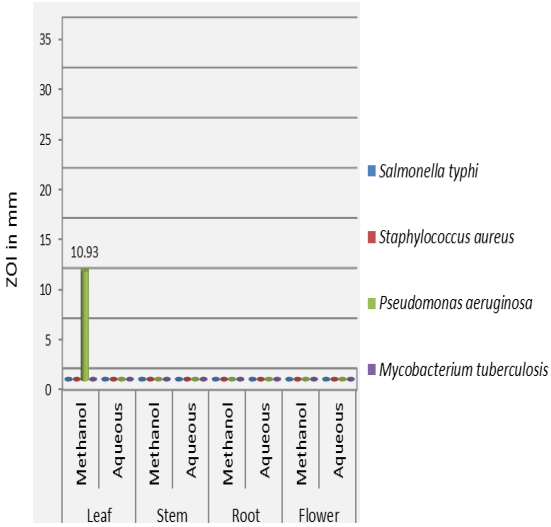


Fig. 4- Showing antibacterial effect of *Lantana indica* at 100 µl con.

The methanolic extract of leaf is showing zone of inhibition (10.93 mm) against *Pseudomonas aeruginosa* only.

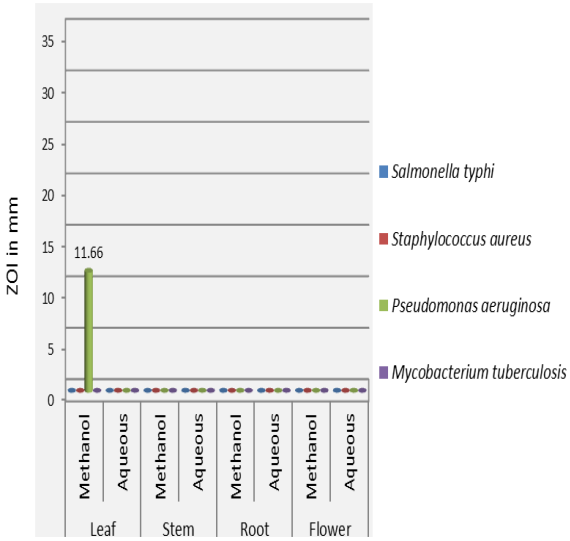


Fig. 5- Showing antibacterial effect of *Lantana indica* at 200 µl concentration

The methanolic extract of leaf is showing zone of inhibition (11.66 mm) against *Pseudomonas aeruginosa* only.

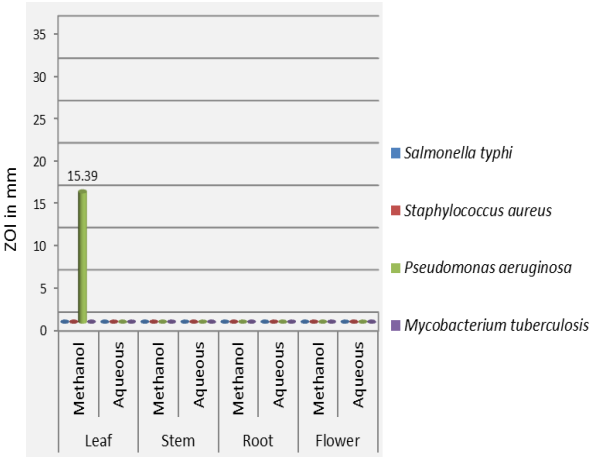


Fig . 6- Showing antibacterial effect of *Lantana indica* at 300 µl concentration

The methanolic extract of leaf is showing zone of inhibition (15.39 mm) against *Pseudomonas aeruginosa* only.

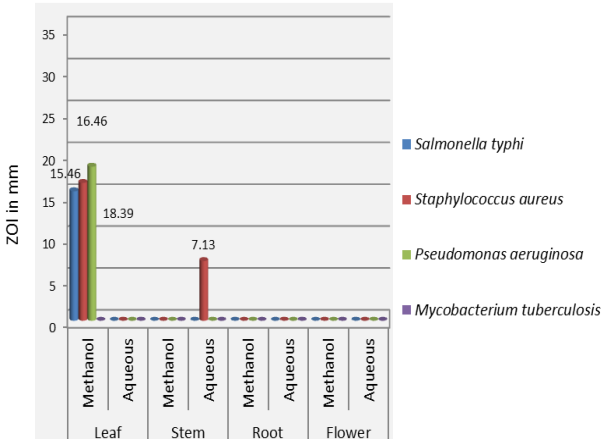


Fig .7- Showing antibacterial effect of *Lantana indica* at 400 µl concentration The methanolic extract of leaf is showing zone of inhibition (18.39 mm) against *Pseudomonas aeruginosa* and the aqueous extract of stem is showing lowest zone of inhibition (07.13 mm) against *Staphylococcus aureus*.

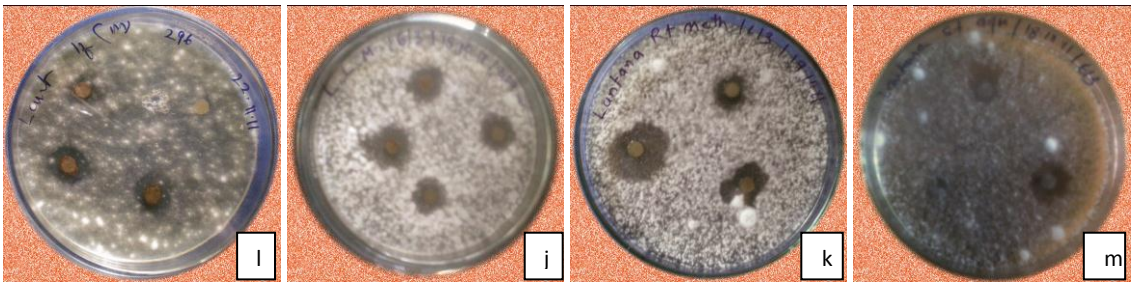


Figure 8:- Showing antifungal effect of *Lantana indica*

Plate j – Showing antifungal effect of *Lantana indica* leaf methanol extract 400µl against *Trichophyton rubrum* (ZOI 13.46mm ± 0.42)

Plate k – Showing antifungal effect of *Lantana indica* leaf methanol extract 400µl against *Epidermophyton floccossum* (ZOI 15.26mm ± 0.39)

Plate l – Showing antifungal effect of *Lantana indica* root methanol extract 400µl against *Epidermophyton floccossum* (ZOI 22.06mm ± 0.54)

Plate m – Showing antifungal effect of *Lantana indica* stem aqueous extract 400µl against *Epidermophyton floccossum* (ZOI 14.99mm ± 0.27)

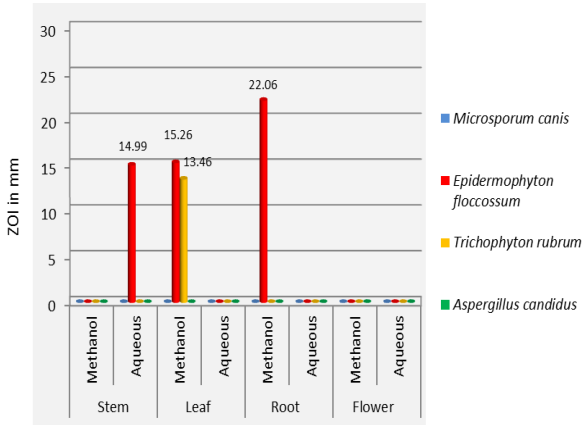
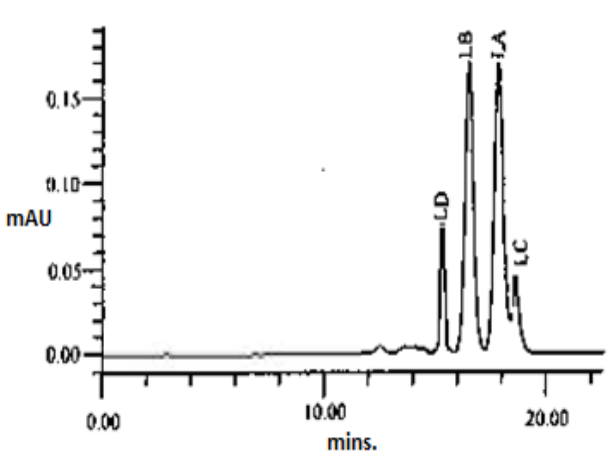
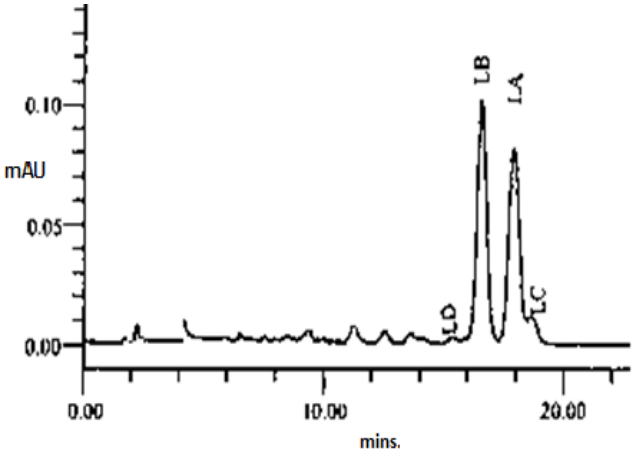


Fig. 9 - Showing Antifungal effect of *Lantana indica* at 400µl concentration. The methanolic extract of root is showing highest zone of inhibition 22.06mm against *Epidermophyton floccossum* and the methanolic extract of leaf is showing lowest zone of inhibition 13.46mm against *Trichophyton rubrum*.



standard Lantadene [LA – Lantadene A (RT -17.82); LB – Lantadene B (RT- 16.57); LC – Lantadene C (RT-18.84); LD- Lantadene D (RT- 15.88)]



Lantadene from *Lantana indica*. LA : RT - 17.68 (10.3ppm); LB : RT-16.46 (12.2ppm); LC : RT-13.73 (8.4ppm)

mAU- milli Absorbance Units

Figure 10:- HPLC-ELSD chromatogram of standard Lantadenes (LA, LB, LC and LD) (concentration=20 ppm each) at 210 nm, mobile phase consisting of methanol: water: acetic acid (95: 5.0: 0.04) on Sunfire (4.6 × 150 mm, 5 µm) column at a flow rate of 0.6. mL/min.

Table no. 1 showing phytochemical analysis of *Lantana indica* Roxb.

S N	Extract	C. Glycosides	Steroids	Terpenoids	Flavonoids	Saponins	Tannins	Alkaloids
1	Leaf M	+	+	+	+	_	+	_
2	Stem A	+	+	+	_	_	_	+
3	Root M	_	_	+	+	+	+	+

M – Methanol A – Aqueous +Present - Absent

DISCUSSION

Many earlier workers evaluated the antimicrobial properties for different parts of *Lantana* species against different pathogenic microorganism. Antibacterial activity of hexane extract of aerial parts of *Lantana hispida* against *Mycobacterium tuberculosis* have been reported.[26] Antibacterial activity of chloroform and methanol extracts of leaves of *Lantana camara* against *Mycobacterium tuberculosis* have been also carried out[27] but in our study the *Lantana indica* was found completely ineffective against *Mycobacterium tuberculosis.* Antimicrobial properties of ethyl acetate, methanol and aqueous extracts from the leaves of *Lantana indica* against *Staphylococcus aureus, Bacillus subtilis, Steptococcus pyrogens, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi* by agar well diffusion method have been investigated.[5] Antibacterial activity of methanol extract of leaves of *Lantana camara* against *Bacillus subtilus, Escherichia coli* and *Salmonella typhi* was done.[28] There are also report about antibacterial activity of crude and column extracts of flower and leaf of *Lantana camara* against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia faecalis.*[29] Antimicrobial activity of ether, acetone, chloroform, ethanol, methanol and aqueous extracts of leaves of *Lantana camara* against *Escherichia coli* and *Bacillus subtilis* has also been reported under *in vivo* condition.[30] In the present study *Lantana indica* was found effective against *Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa* as previously reported.[5,28,29]

Several authors have tried to investigate the antifungal effect of *Lantana indica* and found positive result against various pathogenic fungal strains along with dermatophytes. Antifungal nature of *Lantana indica* against some dermatophytes i.e. *Trichophyton tonsurans, Trichophyton rubrum, Trichophyton ajelloi, Trichophyton soudence* and *Microsporum gypseum* have been reported.[31] *Lantana indica* exhibited strong inhibitory action against *Candida albicans* and *Aspergillus niger.*[5,32] Antifungal activity of *Lantana indica* against *Aspergillus flavus* have been investigated.[33] Antimicrobial activity in ethyl acetate and methanol extract of leaves of *Lantana indica* have been reported.[34] Apart from *Lantana indica*, several other species of *Lantana* have been widely evaluated by various workers with respect to antifungal activity. The *Lantana achyranthifolia* exhibited antifungal activity

against *Aspergillus niger*, *Fusarium nomiliforme*, *Fusarium sporotrithum*, *Ttichophyton mentagrophytes*, and *Rhyzoctonia solani*.[35] *Lantana camara* containing highly potential phytochemicals which were effective against *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus A. niger*, *penicillium sp.*, *Sclerotium rolfsii* and *curvularia lunata*.[36] Notable antimicrobial activity in flower have been also reported.[37] Antifungal property in leaves of *L. camara* against *Aspergillus flavus*, *Aspergillus niger*, *Rhyzoctonia solani* and *Alternaria alternate* was done.[38]

The methanol and aqueous leaf extracts of *Lantana indica* contains flavonoidal glycosides, carbohydrates, proteins, triterpenoids and tannins showed the antimicrobial activities.[5] In our phytochemical study we found Tannin, Cardiac glycosides, Flavonoids, Steroids and Terpenoids from methanolic extract of leaf, Cardiac glycosides, Steroids and Terpenoids were found in aqueous extract of stem and Terpenoids, Flavonoids, Saponin, Tannin and Alkaloids were found in methanolic extract of root of *Lantana indica*. In HPLC profiling we found most active ingredients as Lantadenes from extract of the plant. Our prediction is Lantadenes is responsible compound for antimicrobial effect against *Salmonella typhi*, *Staphylococcus aureus* ,*Pseudomonas aeruginosa* and fungus *Trichophyton rubrum* and *Epidermophyton floccossum.* Several concepts support the idea that Lantadenes is directly responsible for lethality of tested microorganism.[39, 40] The anti-microbial activities of tannins of *Lantana indica* were well documented. The growth of many yeasts, fungi, viruses and bacteria were inhibited by tannins.[5, 41]

CONCLUSION

From the present study we have concluded that methanolic extract of leaves of *Lantana indica* was found effective against *Salmonella typhi* and *Staphylococcus aureus* at maximum concentration (400µl) but it has prominent antibacterial activity against *Pseudomonas aeruginosa* even at minimum concentration (25µl). In phytochemical analysis some authors have reported various active principles from plants *viz.*, flavonoids, glycosides, terpenoids and tannins but presence of cardiac glycosides from both methanolic and aqueous extract of leaf was first time noticed in the present study. The inhibitory effect of *Lantana indica* against *Epidermophyton floccossum* was not previously reported in other studies and is a major novelty of our study. Thus our study is significant from view point of the active ingredient based pathogenic antimicrobial properties of *Lantana indica* and it may be recommended for further pharmacological study.

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