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Assessment of Antimicrobial Activity of Essential Oils from Lantana camara

Shikha Tiwari[1], Ashish Saraf[2]* and Jasmeet Kaur Sohal[3]
[1,2*,3]School of Sciences, MATS University, Raipur, C.G.
***Corresponding Author:** Ashish Saraf, School of Sciences, MATS University, Raipur, C.G.

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Abstract
Lantana camara, belonging to the Family Verbenaceae, has been a significant coniferous plant in Ayurvedic and indigenous medicinal practices. Clinical experiments and animal studies substantiate the application of *Lantana camara* for its anti-spasmodic, carminative, anti-tumor, anti-inflammatory, anti-malarial, anti-ulcerogenic properties, as well as its efficacy in treating mental stress and trauma, and its anti-microbial, insecticidal, fungicidal, and anti-asthmatic effects. This study was designed to analyze the essential oils and evaluate the antibacterial activity of Lantana camara essential oil. Phytochemical research revealed the presence of several secondary metabolites, including alkaloids, saponins, tannins, glycosides, and terpenoids. The antibacterial efficacy of Lantana camara against *Staphylococcus aureus* yielded favourable outcomes.
Keywords: *Lantana camara,* essential oils, antimicrobial, *Staphylococcus aureus*

Introduction

The utilization of medicinal plants for therapeutic reasons is a longstanding practice across several civilizations. About 80% of all recognized natural products derive from plants and play a crucial role in the development of novel value medications (Phillipson and Wright, 1991; Owolabi et al., 2007). *L. camara* is a low-lying, tough, hairy evergreen shrub (Verbenaceae). Known by various common names include Blacksage, cuasquito, angel lip, flowered sage, shrub verbena, white sage and wild sage all over the world, this is a major weed of which there are around 650 kinds in over 60 nations or island groupings. Mostly used as an herbal remedy, *L. camara* also finds use as mulch and in some places as firewood. Cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera (Abu-Shanab et al, 2006) are also treated using it. Some nations plant it as a barrier to contain or drive away animals. Apart from verbascoside, which has antibacterial, immunosuppressive and anticancer effects, extracts from the lantana leaves show antimicrobial, insecticidal and nematicidal action (Adiguzel et al, 2005). Separated and under research as possible nematocides are lantanoside, linaroside, and camarinic acid. Lantana oil is occasionally used for leprous and scabies, antibacterial for wounds, treatment of skin allergies.

Plant metabolites clearly have biological diversity since 47 commercially marketed medications have come from 39 tropical forest plants. Different terpenoids, steroids, and flavonoids have been isolated from phytochemical investigations conducted by different groups of workers on different areas of the plant. Three novel pentacyclic triterpenoids—camaryolic acid, methylcamaralate, and camangeloyl acid—were discovered in course of research on the contents of the aerial sections of *L. camara*. The plant has also been shown to have fungitoxic (Saxena and Sharma, 1999), autotoxic (Arora and Kohli, 1993) and antioxidant activity (Romero and Saavedra, 2005) but is also poisonous to grazing animals (Morton, 1994)

Lantana genus is also known worldwide for its bioactive secondary metabolites and essential oils. Essential oils are

fragrant, highly concentrated essences of plants which are considered to exemplify the soul or life-source of the plant. Essential oils are approximately 75-100 times more concentrated than dried herbs (Harborne and Baxter, 1998). The chemical composition and aroma of essential oils from Lantana species can provide valuable psychological and therapeutical benefits. The leaf oil of L. camara exhibits antimicrobial activities (Saxena and Sharma, 1999).

There have been reports that Lantana plant oil has insecticidal properties (Patil et al., 1997) as well as repellent properties directed against bees, mosquitoes, and cattle flies (Negi et al, 2019). In addition, researchers have investigated the chemical make-up of the essential oil that is derived from the flowers and leaves of the Indian Lantana plant (Khan et al., 2002). Apart from reported compounds this plant also offer β-sitosterol 3-O-β-bglucopyranoside, octadececanoic acid, docosanoic acid, palmitic acid, camaric acid, lantanolic acid etc. *Lantana camara* possess a unique triterpene 22 betaacetoxylantic acid and the recognized triterpenes lantic acid, 22 betadimethyl acryloyloxy lantanolic acids and lantanolic acid and 22 beta-angeloyloxy lantanolic acid. Against Salmonella typhi and Staphylococcus aureus, 22 beta-acetoxylantic acid exhibited antibacterial action. Furthermore displaying antimutagenic action was this molecule and 22 beta-dimethylacryloyloxylantonolic acid (Agarwal et al, 1986).

Over the course of several hundred years, lantana has been utilized as a medicinal plant, making it an abundant alternative for research. Investigating whether or not their traditional applications are supported by genuine pharmacological effects or whether or not they are solely based on folklore is a fascinating endeavour. As a result, the current work has been centered on isolating and partially purifying a bioactive molecule derived from *Lantana camara*, as well as investigating the antibacterial potential of these molecules *in vitro*.

Materials and methods

Plant sample

Lantana camara plant was collected from Nawa Raipur (C.G.) in month of September 2022. The plant material was identified by Dr. Ashish Saraf, Prof. & Head School of Sciences, MATS University, Raipur, Chhattisgarh and further identified by Prof. K.K. Shukla, Botanist, SoS in Biotechnology, Pt. R.S. University, Raipur, Chhattisgarh. A voucher specimen was deposited in the Herbarium of School of Sciences, MATS University, Raipur.

In this study essential oils were extracted by hydro distillation method (Kjeldahl condensation method). The authentic raw material of test plants of *Lantana camara* (Leaves and Fruit) were washed with distilled water several times and shade dried. Fresh plant material was powdered in a blender and subjected to hydro distillation using Clevenger type apparatus for essential oil extraction. The extraction was carried out by distilling 15g of plant powdered material with a 150ml of different solvents for preparation of extracts *viz*. acetone, ethyl-acetate, methanol (methanolic), ethanol (ethanolic) and distilled water (aqueous), for 4-5 hrs. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) (Mathur *et al.,* 2011). The oil were collected in air tight containers, dried over anhydrous sodium sulphate and stored at -4°C for further use. The crude extract was obtained by dissolving a known amount of the dry extract in 95% subsequent solvent to obtain a stock solution of desired concentration.

Test Organisms

Bacterial and Fungal Culture

Microorganisms used in the present study were listed in table 1. Microorganisms were obtained from Microbial Type Culture Collection (MTCC) Chandigarh, Punjab, Fungal Germplasm Collection Centre Department of Biological Sciences, R.D. University, Jabalpur and Microbial Solution Research Laboratory, New Rajendra Nagar, Raipur.

Table 1. Different microorganism used in the investigation

Bacterial species	Yeast species	Fungal
A. hydrophilla (MTCC 966)	*C. albicans* MTCC 822	*A. niger* FGCC# 492 (M)

A. faecalis (MTCC 2763)	*C. utilis* MTCC 847	*A. flavus* FGCC# 133 (P)
*A. eutrophus**	*C. tropicalis* FGCC# 026	*F. oxysporum* FGCC# 503
B. subtilis (MTCC 1789)	*K. fragilis* (NRRL 2415)	*F. moniliforme* FGCC# 193
B. cereus (MTCC 633)	*R. aurantocia* FGCC#SH- 7(0)	*A. alternata* FGCC# 418 (P)
*B. brevis**	*Rhodotorula rubra**	*M. gypsum* FGCC# 67
B. megaterium (MTCC 2343)	*S. cerevisiae* MTCC- 1732	*C. lunata* FGCC# 280
*E. aerogenes**	*Schizosaccharomyces sp.**	*T. harzinum* MTCC 792
*E. facalis**		*S. rolfsii* MTCC 406
*E. faecium**		*M. phaseolina* MTCC 794
E. coli (MTCC 1591)		
K. pneumoniae (MTCC 2405)		
P. aeruginosa (MTCC 779)		
P. fluorescens (MTCC 1748)		
S. typhi (MTCC 531)		
*S. paratyphi**		
S. typhimurium (MTCC 1008)		
S. aureus (MTCC 187)		
*S. epidermidis**		
V. cholerae (MTCC 1168)		
V. heamoparalyticus *		

Determination of Antimicrobial Activity of Plants Extract

The desiccated plant extracts were solubilised in the identical to achieve a final concentration of 30 mg/ml and subsequently sterilised using filtration through 0.45µm Millipore filters.

Antibacterial (comprising both Gram-positive and Gram-negative) and anti-yeast assays were subsequently conducted using the disc diffusion method, employing 100 µl of a suspension containing 10^8 cfu/ml of bacteria and 10^6 cfu/ml of yeast, which were inoculated onto nutritional agar and Sabouraud dextrose agar, respectively. The discs (6mm) were saturated with 10µl of the extracts (100µg/ml per disc) and positioned on the inoculated agar. Negative controls were generated utilising the identical solvents used to dissolve the plant extract. Gentamicin sulphate (10 mg/ml disc) served as a positive reference standard to assess the sensitivity of one strain from each bacterial species. The inoculation plates were incubated at 35±10°C for 24 hours for bacteria and 48 hours for yeast.

The antifungal activity was assessed using the poisoned food technique to examine the impact of essential plant extracts on the growth of human and plant pathogenic fungi. Twenty millilitres of sterilised and chilled (40°C) growth medium (PDA) containing 10 milligrammes of streptomycin were dispensed into pre-sterilized Petri dishes. Required quantities of plant extract at varying concentrations of 100, 300, 500, and 1000 µg were introduced into the plates. The assay plates were meticulously rotated to guarantee uniform distribution of the oil throughout the medium. In the control plates, the medium was augmented with sterilised distilled water to maintain the volume in lieu of plant extract. Following the solidification of the agar media, inocula of the test organism (discs with a diameter of 6 micrometres, excised from the periphery of a 7-day-old culture using a sterile cork borer) were aseptically positioned at the centre of each Petri dish in both the treated and control groups. The assay plates were subsequently incubated at 28 ± 10°C for six days. Upon

completion of the incubation period, the diameters of the fungal colonies in both the treated and control groups were measured. The experiment was conducted in triplicates, in multiples of three.

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

Percentage of mycelial inhibition = dc – dt

dc – average diameter of fungal colony in control sets

dt - average diameter of fungal colony in treated sets

Result

Filter paper disc diffusion bioassay obtained from various fractions of *L. camera and C. flexuosus* (Table 4.4) revels that the effect was varied significantly against various organism. Ethanolic fraction of *L. camera* was most effective against the tested organisms than methanolic fraction. Maximum inhibition was recorded against *E. coli* which was followed by *B. cereus*, *B. megaterium*, *B. brevis* and *A. faecalis* when ethanolic fraction was applied. Minimum antibacterial activity of both ethanolic and methanolic fraction was observed against *S. paratyphi*. *Staphylococcus aureus* (MTCC 187) reference strain and *Staphylococcus aureus* (MRSA) also showed inhibitory activity against ethanolic extract of *L. camera*. Whereas, other extracts *viz. e*thyl acetate, acetone and distilled water failed to do so, these fraction was most effective against *B. brevis, B. megaterium* and least effective against *S. typhi*. Aqueous extract of *L. camera* exhibited its maximum inhibition activity against *E. coli* followed by *B. megaterium* and *A. eutrophus* while minimum activity was recorded against *E. facalis*. Methanolic fraction also showed maximum activity against *R. rubra* followed by *R. aurantioca*, *S. cerevisiae* and *Schizosaccharomyces* sp. Ethyl acetate fraction showed good activity against *R. aurantioca* and *R. rubra*. Ethanolic and acetone fraction exhibited some moderate activity against these yeast cells. Aqueous extract was least effective against *R. rubra* and *S. cerevisiae*.

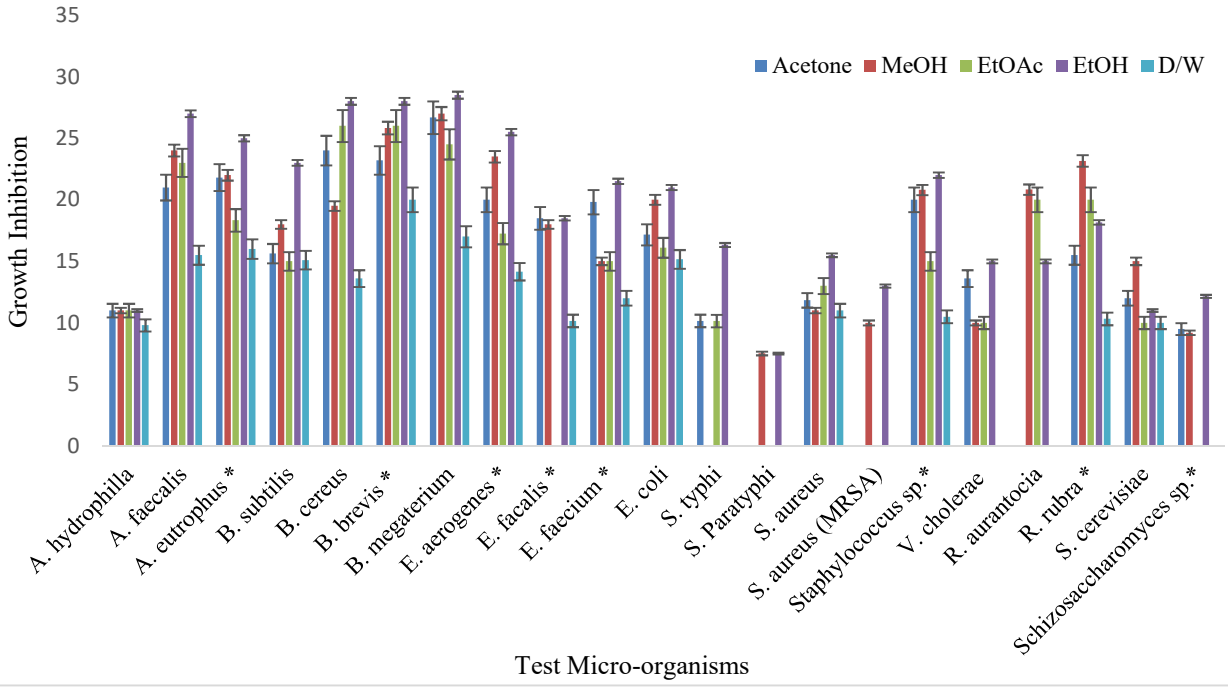
Table 2: Antimicrobial activity of extract of *Lantana camera* on some bacteria and yeast through DDM

Test micro organism	Acetone	MeOH	EtOAc	EtOH	D/W	Ref.
Aeromonas hydrophilla (MTCC 966)	11.00±0.05	12.00±0.00	11.00±0.02	15.0±0.21	9.80±0.02	38 ± 0.04
Alcaligenes faecalis (MTCC 2763)	21.00±0.15	24.00±0.09	23.00±0.20	27.16±0.02	15.50±0.09	35 ± 0.06
Alacalignes eutrophus *	21.80±0.23	22.00±0.07	18.33±0.09	25.00±0.09	16.00±0.16	32.1 ± 0.00
Bacillus subtilis (MTCC 1789)	15.63±0.26	18.00±0.10	15.00±0.01	23.00±0.00	15.10±0.20	33.0 ± 0.01
B. cereus (MTCC 633)	24.00±0.02	19.50±0.83	26.00±0.30	28.00±0.00	13.60±0.00	40 ± 0.07
B. brevis *	23.20±0.10	25.83±0.02	26.00±0.30	28.00±0.00	20.0±0.05	32 ± 0.04
B. megaterium (MTCC 2343)	26.67±0.23	27.00±0.09	24.80±0.50	28.50±0.16	17.00±	31.5 ± 0.05
Enterobacter aerogenes *	20.00±0.00	23.50±0.00	17.00±0.08	25.50±0.30	14.16±0.04	34.8 ± 0.07
E. facalis *	18.50±0.15	18.20±0.08	ND	18.50±0.30	10.16±0.07	35 ± 0.07

E. faecium *	19.80±0.04	15.00±0.02	15.00±0.25	21.50±0.04	12.00±0.07	29.2 ± 0.8
Escherichia coli (MTCC 1591)	17.16±0.09	20.00±0.07	16.10±0.04	21.00±0.09	15.16±0.33	36 ± 0.02
Klabsiella pneumoniae (MTCC 2405)	-	-	-	16.00±0.02	ND	30.01±0.02
Pseudomonas aeruginosa (MTCC 779)	-	ND	-	12.20±0.09	-	27.2±0.12
P. fluorescens (MTCC 1748)	-	-	-	8.16±0.21	-	22.21±0.23
Salmonella typhi (MTCC 531)	10.16±0.03	-	10.50±0.15	16.33±0.02	ND	28.15±0.00
P. Paratyphi	-	7.50±0.25	-	7.50±0.15	-	22.58±0.01
S. typhimurium (MTCC 1008)	-	-	-	ND	-	25.66±0.58
Staphylococcus aureus (MTCC 187)	11.83±0.05	11.0v0.02	12.67±0.00	15.50±0.20	11.00±0.04	26.25±0.02
Staphylococcus aureus (MRSA)	ND	10.83±0.08	-	13.00±0.15	-	10.00±0.01
Staphylococcus sp.*	20.16±0.11	20.60±0.12	15.00±0.10	22.00±0.04	10.50±	27.00±0.38
Vibrio cholerae (MTCC 1168)	13.67±10.00	10.0±0.40	10.00v0.09	14.50±0.00	ND	26.18±0.00
V. heamoparalyticus	-	ND	-	ND	-	25.00±0.01
Candida albicans MTCC 1022	-	-	-	-	-	NT
Candida utilis MTCC 847	-	-	-	-	-	NT
Candida tropicalis FGCC# 026(Ct)	-	-	-	-	-	NT
Kluyveromyces fragilis NRRL 2415	-	-	-	-	-	NT
Rhodotorula aurantocia FGCC# SH-7(P)	ND	20.83±0.21	20.00±0.06	15.00±0.05	-	NT
Rhodotorula rubra *	15.50±0.05	23.16±0.23	20.00±0.30	18.16±0.05	10.33±0.10	NT
Saccharomyces cerevisiae MTCC1732	12.00±0.08	15.00±0.22	10.00±0.21	11.00±0.26	10.00±0.07	NT
Schizosaccharomyces sp.*	9.50±0.12	9.20±0.31	ND0.05	12.16±0.40	-	NT

- **Data are multiple of three observations, o values ± SEM, o NT- Not Tested, o –No Significant Inhibition**

Fig.1. Graphical Presentation of Antimicrobial activity of extract of *Lantana camera* through DDM



- Microorganisms against which no inhibitory activity was detected/recorded are not shown in the graph.

Discussion and Conclusion

Comparable outcomes with various plant extracts against Gram-positive and Gram-negative bacteria have been documented by other researchers (Gover and Moore, 1962). Twenty microliters of acetone extract from various plants, including Glycyrrhiza glabra, Cinnamomum cassia, and Juniperus oxycedrus, had the highest antibacterial efficacy against *B. brevis, B. cereus, B. megaterium, B. subtilis, P. aeruginosa, and S. aureus* (Leite et al, 2006). The antibacterial action may result from the presence of triterpene secondary metabolites in the extract. Barre *et al.* (1997) similarly identified a bioactive triterpene, 22 beta aceto xylantic acid, along with other triterpenes that exhibited antibacterial action against Staphylococcus aureus and Salmonella typhii. The antibacterial efficacy of several plant extracts against the phytopathogenic Xanthomonas campestris pathovars has been investigated and documented by other researchers (Mazumdar et al, 2004). The Soxhlet solvent extraction approach yielded remarkable antifungal efficacy from the methanolic extract of Senna alata, a Thai medicinal plant, against certain dermatophytes (Moshi and Mbwambo, 2005). Other researchers have also discovered the antifungal activities of root extracts from Terminalia sericea against Candida albicans and Aspergillus niger (Satish et al, 1999). The methanol extract from different sections of Lagerstroemia parviflora Roxb shown antifungal efficacy against Aspergillus and Penicillium species, producing significant zones of inhibition. Consequently, the extract demonstrated fungistatic properties (Sharma et al, 1998). The antifungal efficacy of the methanolic leaf extract of Indigofera suffruticosa was assessed against Aspergillus sp., Penicillium sp., and Fusarium sp. (Wuthi-udomlert, 1998).

This study emphasizes the utilization of solvent-extracted stem and leaf extracts of L. camara, which include a highly promising phytochemical that might be identified and perhaps incorporated into the repertoire of profitable antibacterial agents.

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Conflicting of Interests: The authors declare that they have no competing interest.

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