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STUDY OF ANTIMICROBIAL ACTIVITY OF SOLVENT EXTRACTS OF LEAVES OF *LANTANA CAMARA*

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ABSTRACT

Medicinal plants are gaining global attention owing to the fact that the herbal drugs are cost effective, easily available and with negligible side effects. The beneficial effects of the medicinal plants in health care can be well judged from the WHO estimate that around 80% of the world population uses them in some form or the other. It is important to note that homeopathy and modern medicine have their roots in medicinal plants. In the present investigation, different polar (aqueous and methanolic) and non polar (hexane) solvent extracts of leaves of *Lantana camara* at 250µg/ml were investigated for antimicrobial potential against different microbial strains viz. *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus licheniformis*. The results showed that methanolic and aqueous extracts have significant antimicrobial activity in comparison to that of hexane extracts. Amongst all these extracts, methanolic extract had significant antibacterial activity against *Bacillus subtilis* (45 mm) and *Bacillus licheniformis* (50 mm) while aqueous extract was found to have antibacterial activity against *Staphylococcus aureus* (43 mm) while hexane extract was found to have no antibacterial activity against all the microbes studied. The Phytochemical screening results of the aqueous and methanolic extracts of the leaves of the plant showed the presence of tannins, steroids, saponin, glycosides and reducing sugars while alkaloids and flavanoids were found to be absent.

KEYWORDS: *Lantana camara*, leaves, antimicrobial activity, polar and non polar solvent extracts.

INTRODUCTION

Antimicrobial plant extracts have been recognized as a future source of new antimicrobials in the event of the current downturn in the pace at which these are being derived from micro-organisms.[1] The public is also becoming more aware of problems with over prescription and misuse of traditional antibiotics.[2] Resistance to anti-microbial agents is recognized at present as a major global public health problem. Infective diseases account for approximately one-half of all deaths in countries in tropical regions. In industrialized nations, despite the progress made in the understanding of microorganisms and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns.[3] Lantana is a perennial, summer-growing, erect or scrambling shrub, growing up to four metres high and often forming dense thickets. Flesh of the plant produces a strong, aromatic odour when crushed. The plant is a member of the *Verbenaceae* (verbena) family. *Lantana camara* has several therapeutic uses, mainly as herbal medicine.[4-6] There has been much work conducted in India on the chemical constituents of *Lantana camara*; extracts from the leaves exhibit antimicrobial, fungicidal, insecticidal, nematicidal, biocidal activity.[7-10] Lantana oil is used externally for leprosy and scabies. Plant extracts are used as medicine 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.[11]

Lantana camara though being a noxious weed has several minor uses, mainly in herbal medicine. There are

series of research studies conducted on the exploitation of chemical constituents present in different parts of the plant species. The studies demonstrate that extracts from the leaves can be employed to combat antimicrobial, fungicidal, insecticidal and nematicidal problems. Its potential to serve as biocide has also been illustrated in several researches.[12-14] In the present investigation, the study was performed in order to investigate the antimicrobial potential of different solvent extracts of leaves of *Lantana camara*.

MATERIALS AND METHODS

The chemicals and reagents used in the present study were of analytical grade procured from Ranchem, Mumbai, India and media was procured from Hi-Media, Mumbai.

Collection of plant materials

The leaves of *Lantana camara* plant were taxonomically identified and specimen was stored for future reference in NCFT, New Delhi, India.

Preparation of plant extracts

The leaves of the plant were washed with distilled water, dried under shade and pulverized. The method[15] was adopted for preparation of plant extracts with little modifications. Briefly 20g portions of powdered plant material (leaves) were soaked separately in different solvents i.e. hexane, methanol and distilled water on the basis of increasing polarity for 72h. Each mixture was stirred every 24h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30^0C.

Culture Media

For antibacterial activity, Nutrient agar medium (NAM) of Hi Media Pvt. Bombay, India was used.

Inoculum of the microbes

The bacterial cultures used for the study viz. *Staphylococcus aureus, Bacillus subtilis and Bacillus licheniformis* were inoculated into separately. Nutrient broth and incubated at 30^0C for 24h. The suspension was checked to provide approximately, 10^8 CFU/ml.

Determination of diameter of zone of inhibition by well diffusion method

The agar well diffusion method[16] was modified. The culture medium was inoculated with the bacterial cultures separately suspended in Nutrient broth. A total of 8 mm diameter wells were punched into the agar and filled with leaves extracts (250µg/ml) and DMSO in which the extracts were dissolved. DMSO was used as negative controls. Erythromycin (25mg/ml) was used as standard for determination of antibacterial activity. The plates were kept at 30^0C for 24 h for determination of antibacterial activity of any of the extracts and solvent blanks. The procedure for assaying antibacterial activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

Determination of Minimum Inhibitory Concentration (MIC)

MIC value of potent leaves extracts was determined by the modified method adopted.[17-18] Leaves extract (s) were prepared in highest concentration (250 µg/ml) in sterile distilled water and is serially diluted with N-saline (0.85 % NaCl) and similar quantity of bacterial suspension was added to different test tubes and incubated for 24-48 h. The inhibition of turbidity appeared in the minimum dose at which total growth of bacteria gets killed is known as minimum lethal concentration (MLC) or minimum fungicidal concentration (MFC) while little turbidity appeared in the minimum amount of dose of plant extract which inhibits the growth of bacteria is known as Minimum Inhibitory Concentration (MIC).

Phytochemical screening of the extract

The portion of the dry extract of the leaves was subjected to the Phytochemical screening using the modified method adopted.[19-20] Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars and cardiac glycosides.[21]

Test for alkaloids

The 0.5 g of the leaves extract was dissolved in 5 ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Dragendroff's reagent Turbidity or precipitation was taken as indicator for the presence of alkaloids.

Test for tannins

About 0.5 g of the sample was dissolved in 10 ml of boiling water and was filtered. Few ml of 6% FeCl3 was added to the filtrate. Deep green colour appeared confirmed the presence of Tannins.[22]

Test for flavanoids

About 0.2 gm of the leaves extract was dissolved in methanol and heated for some time. A chip of Mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was indicator of the flavanoids.

Test for saponin

About 0.5 g of the leaves extract was stirred with water in the test tube. Frothing persists on warming was taken as a evidence for the presence of saponin.

Test for steroids

Salkowaski method was adopted for the detection of steroids. About 0.5 g of leaves extract was dissolved in 3ml of chloroform and filtered. To the filtrate, conc. H_2SO_4 was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring.[23]

Test for cardiac glycoside

About 0.5 g of the leaves extract was dissolved in 2ml of glacial acetic acid containing 1 drop of 1% $FeCl_3$. This was under laid with conc. H_2SO_4. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

Test for reducing sugars

1ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

RESULTS

In the present study, polar extracts (aqueous and methanolic) extracts of leaves of *Lantana camara* were screened for antibacterial activity against pathogenic and drug resistant *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus licheniformis*. The results showed that, methanolic and aqueous extracts had significant antibacterial potential in comparison to hexane extract. The methanolic extract had significant antibacterial activity against *Bacillus subtilis* (45 mm) and *Bacillus licheniformis* (50 mm) while aqueous extract was found to have antibacterial activity against *Staphylococcus aureus* (43 mm) while hexane extract was found to have no antibacterial activity against all the microbes studied. The results are shown in **Table 1**; **Figure 1** and **Figure 2**. The potent extracts showed MIC values from 30-150µg/ml while MBC/MLC values from 75 to 175µg/ml against the pathogens studied. The results are shown in **Table 2**. It was found that, tannins, steroids, saponin, glycosides and reducing sugars were present in the polar extracts of the leaves of the plant while alkaloids and flavanoids were absent. The results are shown in **Table 3**.

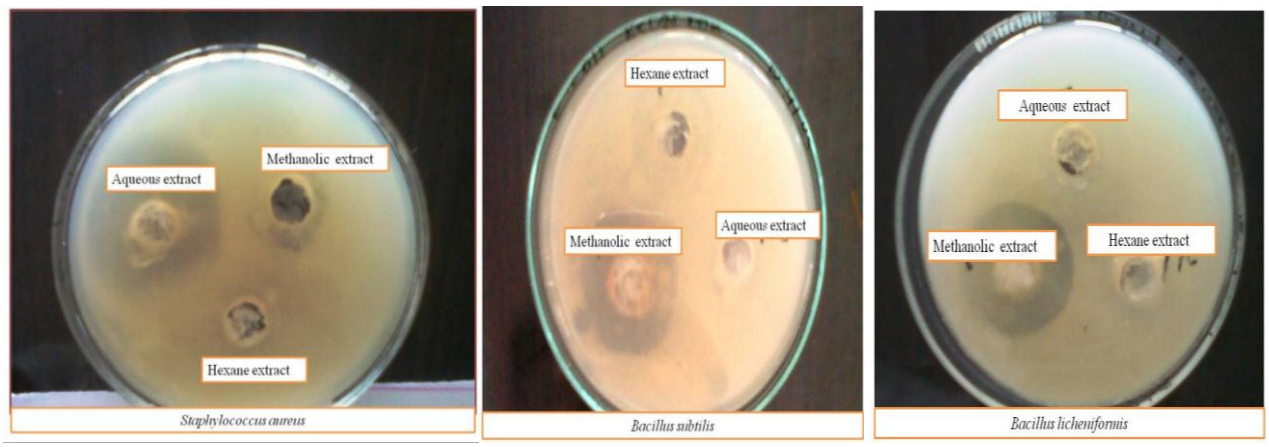

Figure 1: Antibacterial activity of solvent extracts of leaves of *Lantana camara*

Table 1: Antimicrobial activity of solvent extracts of leaves of *Lantana camara*

Plants/parts	Solvent extracts	Diameter of zone of inhibition (mm)		
	@ 250 µg/ml	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus licheniformis*
Lantana camara (Leaves)	Distilled water (Aqueous)	43.0	NA	NA
	Methanol	15.0	45.0	50.0
	Hexane	NA	NA	NA

***NA, No activity**

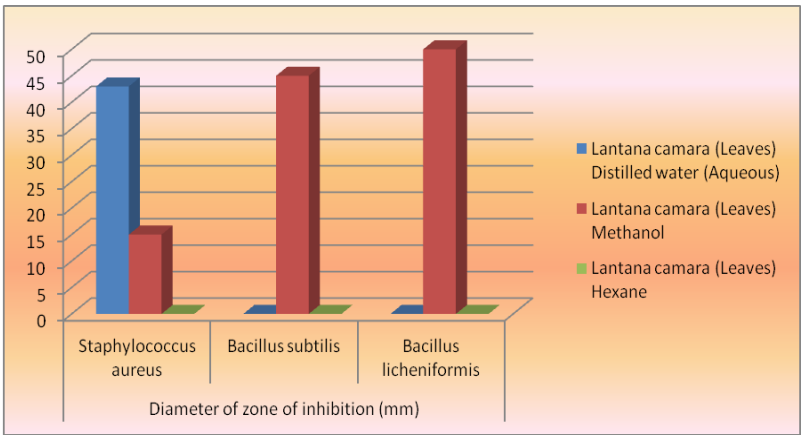

Figure 2: Graphical representation of antimicrobial activity of solvent extracts of leaves of *Lantana camara*

Table 2: MIC and MBC/MLC of the potent polar plants extracts

Plants/parts	Solvent extracts	MIC & MBC (µg/ml)					
		Staphylococcus aureus		*Bacillus subtilis*		*Bacillus licheniformis*	
		MIC	MBC	MIC	MBC	MIC	MBC
***Lantana camara* (Leaves)**	Distilled water (Aqueous)	50	100	NA	NA	NA	NA
	Methanol	150	175	50	100	30	75

***NA, No activity**

Table 3: Phytochemical screening of the potent extracts of leaves of *Lantana camara*

Plant	Phytochemical constituents						
	Alkaloids	Flavanoids	Tannins	Steroids	Saponin	Glycosides	Reducing sugars
Lantana camara (Leaves)	-	-	+	+	+	+	+

*+, present; -, absent

DISCUSSION

The present study findings are in the correlation with the previous studies performed. *Lantana camara* though being a noxious weed has several minor uses, mainly in herbal medicine. There are series of research studies conducted on the exploitation of chemical constituents present in different parts of the plant species. The studies demonstrate that extracts from the leaves can be employed to combat antimicrobial, fungicidal, insecticidal and nematicidal problems. Its potential to serve as biocide has also been illustrated in several researches.[24-26] Previous studies were reported on different medicinal plants of Garhwal region of North west Himalaya for different pharmacological properties.[27-30] The results of the current study thus confirm the presence of polar compounds in the leaves of the plant responsible for antimicrobial activity. The study thus leads to the basis of isolation and identification of active molecule (s) from leaves extract of the plant responsible for antimicrobial activity.

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