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EVALUATION OF ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *Lantana camera* FLOWERS AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus*

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ABSTRACT

Present study was aimed to determine the anti-MRSA effect of *Lantana camara* flower ethanol extract. These flowers are used to treat microbial infections in traditional medicinal practices of India. The ethanolic extract of *Lantana camara* flowers were subjected to preliminary antimicrobial activity by agar well diffusion method and found to be active against both Gram-negative *Escherichia coli* as well as Gram-positive methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* strains. Biochemical property of the extract was established by phytochemical analysis. The preliminary biochemical tests showed the presence of reducing sugars, flavonoids, glycosides, tannins, and phenolic, these groups of compounds have previously been reported to exhibit anti-microbial effects.

KEY WORDS: *Lantana camara*, flower, *Staphylococcus aureus*, MRSA.

INTRODUCTION

Lantana camara L. is a flowering ornamental plant belonging to the Verbenaceae family. In English, the plant is known as Lantana, Wild Sage, or Surinam Tea Plant. The plant is native to Central and Northern South America but is also common in India where it probably was introduced about two centuries ago. A number of languages are present and spoken in different regions of India. The plant is known as Raimuniya in Hindi, Chaturangi and Vanacehdi in Sanskrit, Arippu and Unnichedi in Tamil, Aripoov, Poochedi, Konginipoo and nattachedi in Malayalam, Thirei, Samballei and Nongballei in Manipuri, Tantani and Ghaneri in Marathi, Pulikampa in Telegu, and Kakke and Natahu in Kannada. [1]

Various ethnomedicinal uses have been reported for the plant. In Central Kenya, wet leaves are crushed and inhaled or leaves are crushed and directly applied during ear, nose and throat diseases. [2] The traditional healers in Kancheepuram district of Tamil Nadu, India use a handful of flower which is ground with coconut oil and applied to head to get relief from headache. [3]

Reported phytoconstituents from various parts of the plant include essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, saponins, steroids, triterpenes (22 beta-acetoxylantic acid, lantic acid, 22 beta-dimethylacryloyloxy lantanolic acid, 22 beta-angeloyloxylantanolic acid, lantanolic acid), sesquiterpenoids, and tannins. [1, 4-8]

The plant reportedly possess diverse pharmacological properties, which include antimicrobial, hepatoprotective, cytotoxic, antifertility, antiurolithiatic, anti-inflammatory, antimotility, antidiabetic, mosquito larvicidal, antioxidant, and wound healing activity. [1] One of its phytoconstituents, 22 beta-acetoxylantic acid, has been found to be active against *Staphylococcus aureus* and *Salmonella typhi*. [8] Crude leaf extract of the plant has been found to show promising inhibitory activity against *S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Vibrio cholerae*, and *Candida albicans*. [9] Leaf extract of the plant showed inhibitory activities against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Staphylococcus saprophyticus*. [5] Antimicrobial efficacy of flavonoids and crude alkaloids isolated from the plant has been shown against *E. coli*, *Proteus mirabilis*, *S. aureus*, *C. albicans* and *Trichophyton mentagrophytes*. [10] Ethanolic leaf extract has been shown to be active against *S. aureus*, *S. typhi*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli*. [11] Methanol and acetone extracts of aerial parts of the plant showed maximum inhibitory activity against *S. aureus*, *B. subtilis* and *B. cereus*. [12] Leaf extract showed antibacterial activity against *S. aureus*, *E. coli* and *K. pneumoniae*. [13]

Microorganisms are notorious for developing resistance to antibiotics. Multi-drug resistant microorganisms such as methicillin resistant *S. aureus* (MRSA), vancomycin resistant *Enterococci* (VRE) and the extended spectrum β-lactamase (ESBL) producing Gram-negative bacilli are creating major concerns and have become global problems. [14] To combat these multi-drug resistant microorganisms, new antibiotics need to be discovered, and the plant kingdom can form a useful source for such antibiotics. Already several plants have been shown to possess potential for combating particularly MRSA. Ethanolic extract of leaves of

Baeckea frutescens reportedly showed antibacterial activity against MRSA clinical isolates. [15] A fraction obtained from *Acalypha wilkesiana* has been shown to reverse the Ampicillin Resistance in MRSA via inhibition of Penicillin-Binding Protein 2a. [16] Several compounds have been isolated from mature carpels of *Manglietiastrum sinicum*, which demonstrated moderate inhibitory activity against MRSA. [17] Allicin, a compound present in *Allium sativum*, is also known to be capable of killing MRSA. [18] The objective of the present study was to evaluate the antibacterial potency of ethanol extract of *L. camara* flowers against MRSA.

METHODS

Collection of plant materials

Fresh flowers of *L. camara* were collected from their natural habitat in and around Bangalore, Karnataka, India. A voucher specimen of *L.camera* was submitted at Botanical survey of India (BSI), Central National Herbarium, Howrah.

Surface stérilisation of flowers

The petals were separated from the other parts of the flower and washed thoroughly with tap water to obtain petals free of dust and soil. The petals were next washed with 5% sodium chloride solution to remove the surface contaminants. Then the petals were rinsed with distilled water to remove the excess sodium chloride solution. The water was drained and the flowers dried in shade on a clean filter paper.

Ethanol extraction

Cleaned petals were dried using liquid nitrogen and powdered. 5g of the powder was extracted with 33.3ml ethanol for 24h on a rotary shaker at ambient temperature at 25 rpm and then allowed to stand for 5hrs. The extract was filtered and the residue re-extracted with ethanol as before. The two filtrates were combined and the ethanol evaporated to dryness. The extract was dissolved in 1% DMSO for further uses.

Test organisms

Wild type strains of *S. aureus* [methicillin-resistant (MRSA) and methicillin-sensitive (MSSA)] were collected from pus samples of patients at the Department of Microbiology & Serology, Lab Medicine, NH Health City, Bangalore, India. MSSA ATCC 29213 and MRSA ATCC BAA-1026 were used as control.

Agar well diffusion method

Gram positive *Staphylococcus aureus* overnight cultures adjusted with 0.5 Mac Farland's standard were swabbed on the Muller-Hinton media plates using a sterile swab and allowed to stand for 15 minutes. A sterile 6mm well borer was used to make wells in the media and extract was added into the well with sterile micro-pipettes and incubated overnight at 37°C and checked for the development of zone of inhibition around the well. The experiment was done in triplicate. The zone of inhibition was calculated as described before. [19] Essentially, the diameter of the inhibition zone was measured around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all three replicates and the average values were determined.

Preliminary phytochemical screening of ethanolic extracts

Analysis of various groups of phytochemicals was conducted according to procedures as previously described. [20]

1. Test for Carbohydrates (Benedicts Test)

Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appearing green was taken as an indication of the presence of reducing sugars.

2. Test for Proteins (Biuret Test)

Small quantity of extract was dissolved in a few ml of water. To this test solution 4% NaOH solution and a few drops of 1% $CuSO_4$ solution was added. Appearance of violet color indicated presence of proteins.

3. Test for Steroids (Salkowski Test)

To 2ml of extract, 2ml of chloroform and 2ml of concentrated H_2SO_4 was added. The solution was shaken well. Chloroform layer turning red and acidic layer showing greenish yellow fluorescence indicated the presence of steroids.

4. Test for Alkaloids (Hager's Test)

To 2-3ml of extract Mayer's reagent was added. Formation of yellow precipitate indicated the presence of alkaloids.

5. Test for Flavonoids (Lead acetate Test)

To a small quantity of extract, lead acetate solution was added. Formation of yellow precipitate indicated the presence of flavonoids.

6. Test for Tannins and Phenolic compounds ($FeCl_3$ Test)

On addition of 5% $FeCl_3$ solution to the extract, appearance of a bluish black color indicated the presence of tannins and phenolic compounds.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of reducing sugars, flavonoids, glycosides, tannins, and phenolics in the ethanol extract.

Preliminary screening for antibacterial activity

A preliminary *in-vitro* antibacterial screening was done with 20 mg/ml of the ethanol extract against Gram positive *S. aureus* (various strains) using the agar well diffusion method. The various zones of inhibition obtained are shown in Table 1 and represent the mean value of three replicate experiments. The results show that the ethanolic flower extract is active against all the *S. aureus* strains.

Table 1. Inhibition zone of *L. camara* ethanolic flower extract against MRSA

Organism and Zone of inhibition (mm)			
MRSA from pus (Wild Strain)	***S.aureus* (MSSA) isolated from pus (Wild Strain)**	***S. aureus* ATCC 29213**	***S. aureus* ATCC BAA-1026**
25	16	18	26

Concentration-dependent zone of inhibition against different *S. aureus* strains

The zone of inhibition of various concentrations of extract (10, 12, 14, 16, 18, 20, 30, 40 and 50 mg/ml) was checked against MSSA ATCC 29213, MRSA (wild type) and MRSA ATCC BAA-1026). The results are shown in Table 2 and represent the mean values of 3 replicate experiments. The results suggest that the ethanolic extract was active against all MRSA strains, including wild type MRSA strain.

Table 2. Concentration-dependent zone of inhibition of *L. camara* ethanolic flower extract against different *S. aureus* strains

Concentration of the extract (mg/ml)	Zone of inhibition (mm)		
	MSSA ATCC 29213	MRSA (Wild strain)	MRSA ATCC BAA-1026
50	16	26	26
40	12	18	20
30	10	15	16
20	8	11	15
18	6	0	0
16	0	0	0
14	0	0	0
12	0	0	0
10	0	0	0

DISCUSSION

The number of reports [5, 8-13] on the *in-vitro* antimicrobial activities of various parts of *L. camara* suggest that the plant may have the potential for discovery of new antimicrobial components, and in fact one such potential antimicrobial component, namely, 22 beta-acetoxylantic acid, has been already reported from the plant. [8] This report, however, present for the first time, to our knowledge, the antibacterial efficacy of ethanol extract of flowers of the plant against various strains of *S. aureus* including MRSA. Although the active principle(s) were not identified in this preliminary study, phytochemical screening showed the presence of reducing sugars, flavonoids, glycosides, tannins, and phenolics in the ethanol extract.

A number of scientific studies have shown the inhibitory efficacy of the above group of compounds against microorganisms. For instance, chloroform and methanol fractions of *Euphorbia milii* have been shown to be active against *S. epidermis* and *K. pneumoniae*; glycosides and tannins were present in the fractions. [21] Hot aqueous extract of *Acacia nilotica* leaves have been found to inhibit *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *B. cereus*, *S. aureus*, and *Streptococcus uberis* and fungal pathogens *Aspergillus niger* and *Aspergillus fumigatus*. Phytochemical screening of the aqueous extract showed the presence of glycosides and phenolic compounds among the constituents. [22] Flavonoids from *Baeckea frutescens* and *Commiphora pedunculata* have been found to be active against MRSA. [15, 23] Novel quercetin glycosides have been described as potent anti-MRSA agents. [24] Six phlorotannins, isolated from *Eisenia bicyclis*, showed inhibitory activity against MRSA. [25] Thus components from the various groups of phytochemicals present in ethanol extract of *L.*

camara flowers may be responsible for the observed MRSA effect, the isolation and identification of which may lead to discovery of lead compounds and novel anti-MRSA drugs.

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