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Screening of Antimicrobial Properties of *Jasminum sambac* Linn. Leaf Extracts against Dental Pathogens

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ABSTRACT

In present study, antimicrobial efficacy of *Jasminum sambac* leaf extracts was evaluated against six bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *S. pyogenes*, *S. sobrinus*, *S. sanguinis* and *Lactobacillus acidophilus*) and one fungi (*Candida albicans*) causing dental infections. Results showed that methanol extract was more efficient in comparison to other extracts. The zone of inhibition ranged between 12.3±0.57-17.3±0.57 mm examined at 200 mg mL^{-1}, respectively. Minimum inhibitory concentration were recorded for methanol extract at 3.12-25 mg mL^{-1}. Phytochemical analysis of extracts showed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins. The results conclude the traditional uses of *J. sambac* in treatment of dental diseases.

Key words: Antimicrobial activity, dental pathogens, *Jasminum sambac*, minimum inhibitory concentration

INTRODUCTION

Oral microflora is a complex system comprises variety of bacterial and fungal organisms involved specifically/non-specifically in mutualistic relationship with host by preventing pathogenic species. About 700 microbial species are identified from oral microbiome (Palmer *et al*., 2008). Bacterial involvement can cause dental caries or cavities and periodontal diseases, simply pathological inflammatory condition of gum and periodontal tissues. Several bacteria are responsible for dental caries and periodontal infections including *Lactobacillus acidophilus*, *Streptococcus mutans*, *S. sobrinus*, *Actinomyces* spp., *Nocardia* spp., *Camphylobacter*, *Fusobacterium*, *Haemophilus*, *Prevotella*, *Porphylomonas* and *Veillonella* (Kononen *et al*., 1994; Marsh, 1992; Schupbach *et al*., 1995). If we focus on the microbial role, they ferment many sugars and resulting products are utilized by dental plaque bacteria (Prasad *et al*., 2008). Due to production of high level of lactic acid causing fermentation of dietary sugars and resistant to the adverse effect of low pH (Walsh, 2006).

Jasminum sambac Linn. (Oleaceae), commonly known as Chameli, is a shrub, about 1.5-2.0 m long, bearing small white flower. It is commonly distributed in all over tropical region of India. Its various parts are used in preparation of medicine, perfumes and aromatizing products (Abdoul-Latif *et al*., 2010). Other medicinal applications of *J. sambac* have been reported in curing insanity, skin diseases, ulcers, sight weakness, leprosy and suppression of puerperal lactation (Mittal *et al*., 2011).

Currently, herbal medicines have received greater attention because of their multiplicity of curing diseases, safety and being well tolerated remedies when compared with the conventional drugs. Herbs had been priced for their medicinal, flavouring and aromatic abilities for long time (Srivastava *et al*., 2013). Present days, phytoconstituents have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). Therefore, phytochemicals with adequate antimicrobial efficiency can be used for the treatment of numerous infectious diseases (Balandrin *et al*., 1985). The present study was aimed to investigate antimicrobial properties of *J. sambac* against selected pathogens causing dental infections.

MATERIALS AND METHODS

Plant material: The leaves of *J. sambac* was collected from Haridwar, Uttarakhand and authenticated at Botanical Survey of India (BSI), Deharadun. Leaves were washed in fresh running water, dried under shade at room temperature, crushed by using pestle and mortar and powdered in an electric grinder.

Preparation of extract: Plant extracts were prepared by immersing 200 g of powdered seeds in 600 mL of four different solvents including Petroleum Ether (PET), Acetone (ACE), methanol (MeOH) and aqueous (H_2O) loaded in Soxhlet assembly and extracted for 72 h through successive method (Ahmad *et al*., 1998). Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30°C. Residues were stored at 4°C until further use. Extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 200 mg mL^{-1} for agar well diffusion method. The yield of PET extract was 5.1 g, ACE extract 6.5 g, MeOH extract 8.7 g and H_2O extract 9.4 g, respectively.

Test microorganisms: The pathogenic organisms were selected for the study were *Staphylococcus aureus* (MTCC 1144), *Streptococcus mutans* (MTCC 890), *S. sanguinis* (ATCC 10556), *S. sobrinus* (ATCC 33478), *S. pyogenes* (MTCC 442), *Lactobacillus acidophilus* (MTCC 10307) and *Candida albicans* (MTCC 227) procured from IMTECH, Chandigarh and NCL, Pune.

Antimicrobial activity: Antibacterial activity of different extracts was determined by agar well-diffusion method (Ahmad *et al*., 1998). *In vitro* antibacterial activity was screened by using Mueller-Hinton Agar (MHA) medium No. 173 (Hi media Pvt. Ltd., Mumbai, India). The 0.1 mL of 12-16 h incubated cultures of bacterial species were mixed in molten medium and poured in pre-sterilized petri plates. Plates were allowed to solidify for 5-10 min. A cork borer (6 mm diameter) used to punch wells in medium and filled with extracts of 45 µL of 200 mg mL^{-1} final concentration of extracts. The DMSO was used as negative control. Efficacies of extracts against pathogens were compared with broad spectrum antibiotic Ofloxacin (positive control). Ofloxacin was dissolved in double distilled water. Plates were incubated at 37°C for 24 h in BOD incubator. At the end of incubation, inhibition zones formed around the well were measured with transparent ruler in millimetre. Each sample was assayed in triplicate and mean values were observed. The antibacterial activity was interpreted from size of diameter of zone of inhibition measured to the nearest millimetre (mm) as observed from clear zones surrounding the wells.

Determination of Minimum Inhibitory Concentrations (MICs): Two-fold serial dilution method (Aboaba *et al*., 2006) was used to determine the Minimum Inhibitory Concentrations

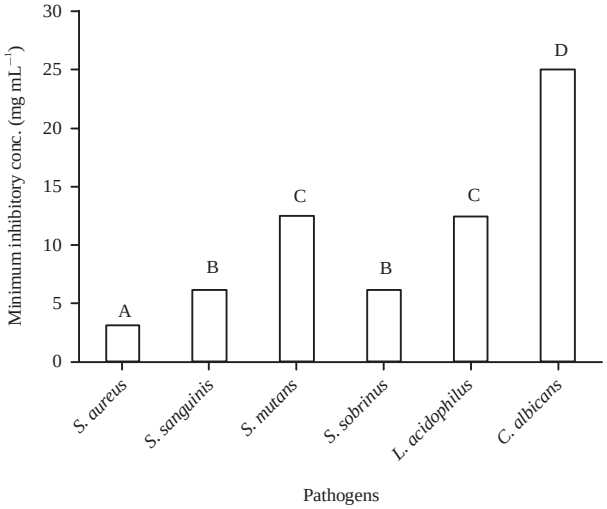

Fig. 1: Minimum inhibitory concentrations of methanol extract of *Jasminum sambac*. The inhibition is noted at A: 3.12 mg mL^{-1} against *S. aureus* B: 6.12 mg mL^{-1} against *S. sanguinis* and *S. sobrinus*, C: 12.5 mg mL^{-1} against *Streptococcus mutans* and *Lactobacillus acidophilus* and D: 25 mg mL^{-1} against *C. albicans*

(MICs). The MeOH extract was diluted double fold (2:2) with nutrient broth in a series of six test tubes. Concentration of 50, 25, 12.5, 6.25, 3.12 and 1.56 mg mL^{-1} of crude MeOH extract were prepared separately and dissolved in 1 mL of DMSO. An aliquot of 1 mL of microorganism suspension (1.5×10^6) was inoculated into each tube (Fig. 1). Control tubes were inoculated with same quantity of sterile distilled water. All tubes were incubated at 37°C for 24 h. The lowest concentration that did not permit any visible growth when compared with control was considered as the minimum inhibitory concentration. The contents of all tubes that showed no visible growth were cultured on MHA medium incubated at 37°C for 24 h.

Phytochemical screening: The phytochemical analysis of plant extracts were carried out by using standard qualitative methods for identification of various classes of active phytochemicals (Evans, 1996; Scalbert, 1991).

Test for alkaloids:

- Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids
- The test solution gave brown precipitate with the Dragendorff's reagent. The presence of brown precipitate showed positive test while absence of precipitate was negative

Test for flavonoids:

- On addition of conc. HCl in MeOH extract of material, a red colour appeared which indicated the presence of flavonoids
- Ethanolic solution of test material was added with small piece of magnesium ribbon, followed by drop wise addition of conc. HCl and change in colour noted. The colour was changed orange to red showed positive flavonoids test

Test for glycosides: The extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with Ba $(OH)_2$. The remaining extract contained the glycosides. The hydrolysis of the solution was done with conc. H_2SO_4 and after the hydrolysis the presence of sugar was determined with the help of Fehling's solution.

Test for steroids: The extract was mixed with 3 mL $CHCl_3$ and 2 mL conc. H_2SO_4 was poured from the side of the test tube and the colour of the ring at the junction of two layers was noted. A red colour showed the presence of steroids.

Test for tannins: Extract was added in 1% ferric chloride and the colour was observed. Bluish black colour appeared, which disappeared on addition of dilute H_2SO_4 follow a yellow brown precipitate showed the presence of tannins.

Test for saponins: Extracts (0.5 mg) were boiled with water (10 mL) for 2 min in a test tube and cooled. The mixture was shaken vigorously and left for 2-3 min. Formation of 1 cm layer of foam indicates the presence of saponins.

RESULTS

Dental infections are one of the most common disease globally. The results showed that *J. sambac* possess better antimicrobial properties against selected microorganisms (Table 1). The MeOH extract showed the maximum antimicrobial activity against tested strains in comparison to other extracts followed by PET, ACE and H_2O extract. The best activity of MeOH extract was noted against *S. pyogenes* (17.3±0.28 mm), *S. sanguinis* (17.3±0.57 mm) and *L. acidophilus* (17.3±0.57 mm) and moderately active against *S. sobrinus* (16.6±0.28 mm), *S. mutans* (15.6±0.57 mm) and *C. albicans* (12.3±0.57 mm), respectively. The activity of reference drug (ofloxacin) were higher in comparison to tested crude extracts at similar concentration.

The MICs values for MeOH extract were ranged at 3.12- 25 mg mL^{-1} (Fig. 1). *Jasminum sambac* presented similar MICs against *S. sanguinis* and *S. sobrinus* at 6.12 mg mL^{-1}, respectively. Moreover, MeOH extract of this plant manifested a better MIC against *S. aureus* at 3.25 mg mL^{-1} and least MIC against *C. albicans* at 25 mg mL^{-1}.

The phytochemical analysis of plant extract showed positive tests for all performed phytochemical tests. It disclosed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins which might be accountable for its antimicrobial potential (Table 2).

Table 1: Antimicrobial values of *Jasminum sambac* leaf extracts against dental pathogens

	*Diameter of the inhibition zone (mm)				
Microorganisms	PET	ACE	MeOH	H_2O	Reference drug (Ofloxacin)
L. acidophilus	10.3±0.28	13.3±0.28	16.3±0.76	14.3±0.57	34.3±0.57
S. aureus	10.3±0.57	11.3±0.28	15.0±0.50	14.6±0.57	33.3±0.28
S. mutans	10.0±0.50	12.3±0.57	14.3±0.57	14.6±0.57	35.3±0.28
S. pyogenes	10.6±0.28	12.3±0.28	16.3±0.76	15.6±0.28	35.0±0.50
S. sanguinis	11.6±0.28	13.3±0.57	16.3±0.57	15.3±0.28	33.6±0.28
S. sobrinus	10.3±0.28	12.6±0.28	16.6±0.28	16.0±0.50	32.6±0.28
Candida albicans	8.6±0.28	11.0±0.50	12.3±0.57	12.0±0.50	27.0±0.50

*Values are mean of three replicates expressed as means and standard error of means, cork borer diameter: 6 mm, PET: Petroleum ether, ACE: Acetone, MeOH: Methanol

Table 2: Phytochemical screening of *Jasminum sambac* leaf crude extracts

	Plant extracts			
Phytoconstituents	PET	ACE	MeOH	H_2O
Alkaloids	-	+	+	+
Flavonoids	-	+	+	+
Glycosides	-	+	-	+
Steroids	+	+	+	+
Saponins	-	-	+	+
Tannins	+	+	+	+

+: Present, -: Absent, PET: Petroleum ether, ACE: Acetone, MeOH: Methanol

DISCUSSION

The results obtained by this study figured out the antimicrobial properties of *J. sambac* leaf extracts tested against selected dental microorganisms by using agar well diffusion method. The efficacy of crude extracts was higher against bacterial strains in comparison to fungal organism. The antimicrobial activity of PET extract observed lower compared to other extracts. According to Al-Hussaini and Mahasneh (2011), ACE extract of *J. sambac* leaf extract was reported most active against six bacteria i.e. *S. aureus*, *Bacillus subtilis, B. cereus, E. coli, P. aeruginosa, Chromobacterium violaceum* and one fungi i.e. *C. albicans*. The leaf extracts were also reported active against *Xanthomonas campestris* (Gracelin *et al*., 2012), *C. albicans* (18.0±0.50 mm) and *Aspergillus niger* (10.0±0.30 mm) (Nandhini *et al*., 2015). Abdoul-Latif *et al.* (2010) reported the antimicrobial activity of essential oil and MeOH extract of *J. sambac* against *S. pyogenes*, *S. enterica*, *E. coli*, *S. dysenteriae*, *L. innocua* and *E. facealis*. The MICs values tested for MeOH extract were recorded least against *S. aureus* and moderate against *S. sanguinis* and *S. sobrinus*. These data represent the sensitivity of tested strains.

The phytochemical screening of *J. sambac* extract had shown that plant contains major phytoconstituents including alkaloids, flavonoids, steroids, reducing sugars, saponins and tannins. Sabharwal *et al*. (2012) also reported the presence of flavonoids, steroids, glycoside, tannins and fatty acids in flowers of *J. sambac*. Phytochemicals are responsible for various properties i.e., antioxidant activity, hormonal action, enzymatic activity, interference with DNA replication, antimicrobial activity etc. (Doughari, 2012). In conclusion, *J. sambac* leaf extracts possess a broad spectrum of activity against a panel of microorganisms responsible for the dental diseases. This study can boast a new possibility for finding novel clinically effective antimicrobial compounds from *J. sambac*.

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