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Preliminary Phytochemical Screening and Antimicrobial Activity of *Salvadora persica* Linn. Extracts against Oral Pathogens

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Abstract

Salvadora persica Linn. is used traditionally as hypotensive, emollient, laxative, diuretic, febrifuge, skin cleanser and in treatment of urinary infections, gall stones, bronchial asthma and diarrhoea. The present study was designed for evaluation of phytochemical and anti-microbial aspects of *S. persica* stem and bark extracts against oral pathogens. The antimicrobial screening of extracts was examined by agar well diffusion method at 200 mg/ml sample concentration and minimum inhibitory concentrations (MICs) by two fold serial dilution method. Ofloxacin was used as positive control to determine the sensitivity of the strains. The results showed that MeOH extract was more active than other extracts in its antimicrobial activity ranged 14.0 ± 0.50-22.3 ± 0.76 mm respectively. MIC values were recorded between 3.12 to 25 mg/ml for all selected pathogens. Phytoconstituents analysis of plant extract revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins. The results corroborate the traditional uses of *S. persica* in treatment of dental diseases.

Keywords: Antimicrobial activity; Oral pathogens; Phytochemical; *Salvadora persica*

Introduction

Our mouth contains a variety of microorganisms but few specifically engage in dental caries. Bacterial invasion causes demineralization and destruction of hard tissues of teeth. The acid production by bacteria causes accumulation of tooth surface, finally producing dental caries. Several bacteria are responsible for dental caries and periodontal infections i.e. *Streptococcus mutans*, *S. sobrinus*, *Lactobacillus acidophilus*, *Actinomyces* spp., *Nocardia* spp., Camphylobacter, Fusobacterium, Haemophilus, Prevotella, Porphylomonas, Veillonella [1-4]. Some of these organisms produce high level of lactic acid causing fermentation of dietary sugars and are resistant to the adverse effect of low pH [5].

Salvodara persica Linn. is a branched, evergreen shrub, belonging to family Salvadoraceae. It is commonly known as Jhak, Miswak, Kharjal in hindi, Brihatpilu in sanskrit and Tooth brush tree in english. Stem and bark is used as a dental diseases and stimulant in low fevers. Root decoction is used against gonorrhea and vesicle-catarrh. Root extract is used to relieve the pain due to spleen troubles. Leaves are used in treatment of asthma, cough and piles. Fruits possess carminative and diuretically properties and used in treatment of rheumatisms [6,7].

Various components of *S. persica* have been reported to have beneficial biological properties, including significant antibacterial and antifungal activity [8]. Furthermore, *S. persica* extracts are reported effective against some periodontal pathogens involved in dental plaque development [9,10]. In present study, phytochemical and antimicrobial aspects of *S. persica* are subjected to explore against to dental pathogens

Materials and Method

Plant material

The stem and bark of *S. persica* was collected from Bikaner, Rajasthan and authenticated at botanical survey of India (BSI), Northern Regional Centre, Dehradun. Plant materials were shade dried at room temperature. The dried parts were grounded to powder with a help of an electric grinder and stored in an air-tight container for future use.

Preparation of extract

Plant extracts were prepared by immersing separately 200 gm of dried powder in 600 ml of four different solvents i.e., petroleum ether (PET), acetone (ACE), methanol (MeOH) and aqueous (H_2O) by using soxhlet assembly and extracted for 72 h through successive methods [11]. Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30-60°C and stored in sterile bottles at 4°C until further use. The yield of PET extract was 7.2 gm, ACE extract 8.1 gm, MeOH extract 7.5 gm and H_2O extract 9.6 gm respectively. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/ml for antibacterial testing.

Microorganisms used

In present study, total 52 patient's samples were collected from OPD, Aggarwal Dental Clinic, Haridwar and other pathological centre, Haridwar. The isolated oral pathogens were identified according to published guidelines [12]. *Staphylococcus aureus* (MTCC 1144), *Streptococcus mutans* (MTCC 890), *S. sanguinis* (ATCC 10556), *S. sobrinus* (ATCC 33478), *S. salivarius* (MTCC 1938), *Lactobacillus acidophilus* (MTCC 10307) and *Candida albicans* (MTCC 227) were

procured from IMTECH, Chandigarh and national chemical lab (NCL), Pune. In isolates *S. aureus*, *S. mutans*, *S. sanguinis*, *S. sobrinus*, *S. salivarius* and *L. acidophilus* were most bacteria identified.

Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Slants were prepared by transferring a loop full culture from stock cultures to test tubes of mueller-hinton broth (MHB) for bacteria that were incubated without agitation for 24 h. While *C. albicans* was incubated for 48 h at 37°C.

Antimicrobial activity

The antimicrobial activity of different extracts was determined by agar well diffusion method [13] by using mueller hinton agar (MHA) medium no. 173 (Hi media Pvt Ltd., Mumbai, India). 0.1 ml of 12-16 h incubated cultures of bacterial and fungal species were mixed in molten medium and poured in pre-sterilized Petri plates. Plates were allowed to solidify for 5-10 minutes. A cork borer (6 mm diameter) was used to punch wells in medium and filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. Efficacy of extracts was compared with broad spectrum antibiotic ofloxacin (positive control). Plates were incubated at 37°C for 24-48 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 antimicrobial 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

Two-fold serial dilution method [14] was used to determine the minimum inhibitory concentration (MIC). 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 of crude MeOH extract were prepared separately and dissolved in 1 ml of DMSO. An aliquot of 1 ml of bacterial suspension (1.5 × 106) was inoculated into each tube (Figure 1).

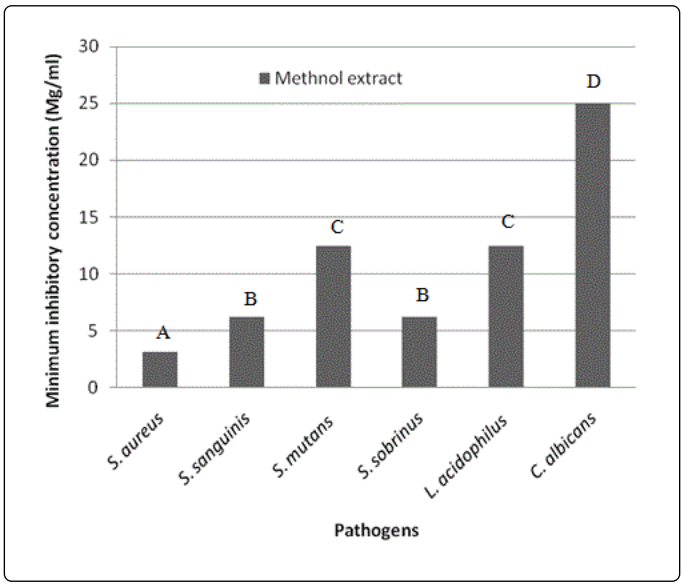

Figure 1: Minimum inhibitory concentrations (MICs) of methanol extract of *S. persica*. The inhibitions were noted at A) 3.12 mg/ml against *S. aureus* B) 6.12 mg/ml against *S. sanguinis* and *S. sobrinus*, C) 12.5 mg/ml against *S. mutans* and *L. acidophilus* and D) 25 mg/ml against *C. albicans*.

Control tubes were inoculated with same quantity of sterile distilled water. All tubes were incubated at 37°C for 24-48 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 24-48 h.

Phytochemical screening

The phytochemical analysis of plant extracts were carried out by standard qualitative methods [15,16].

Test for alkaloids

The test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloid.

Test for flavonoids

On addition of conc. HCl in methanolic extract of the material, a red colour appeared which indicated the presence of flavonoids.

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/terpenoids

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 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 were diluted with water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Results and Discussion

In results, plant extracts showed significant antimicrobial activity against all the selected pathogens at 200 mg/ml (Table 1). MeOH extract showed the maximum antimicrobial activity against the *L.*

acidophilus and *S. mutans* followed by H_2O, ACE and PET extract. MeOH extract showed best activity against *L. acidophilus* (22.3 ± 0.76 mm) and *S. mutans* (21.6 ± 0.76 mm) followed by *S. aureus* (19.3 ± 0.28 mm), *S. sobrinus* (19.3 ± 0.76 mm), *S. salivarius* (18.0 ± 0.50 mm), *S. sanguinis* (18.6 ± 0.76 mm) and *C. albicans* (14.0 ± 0.50 mm). According to Al-Bayati et al. [17] H_2O and MeOH extracts of *S. persica* were investigated for its antimicrobial activities against seven isolated oral pathogens including *S. aureus*, *S. mutans*, *S. faecalis*, *S. pyogens*, *L. acidophilus*, *P. aeruginosa* and *C. albicans*. The ethanol and MeOH extracts of *S. persica* extracts showed antibacterial activity against *S. aureus*, *E. faecalis* and *K. pneumoniae* [18].

S. No.	Microorganisms	*Diameter of the inhibition zone (mm)				Positive Control (Ofloxicin)
		PET	ACE	MeOH	H2O	
1	*S. aureus*	9.0 ± 0.50	12.6 ± 0.57	18.6 ± 0.28	17.6 ± 0.76	33.0 ± 0.50
2	*S. aureus* MTCC 1144	11.3 ± 0.57	13.6 ± 0.28	19.3 ± 0.28	17.6 ± 0.76	33.3 ± 0.28
3	*S. mutans*	12.0 ± 0.50	16.6 ± 0.28	20.6 ± 0.76	18.6 ± 0.57	33.6 ± 0.57
4	*S. mutans* MTCC 890	11.6 ± 0.28	16.0 ± 0.50	21.6 ± 0.76	18.3 ± 0.57	35.3 ± 0.28
5	*S. salivarius*	9.6 ± 0.28	18.3 ± 0.28	19.6 ± 0.76	19.6 ± 0.28	34.3 ± 0.57
6	*S.salivarius* MTCC 1938	10.6 ± 0.28	16.3 ± 0.28	18.0 ± 0.50	18.6 ± 0.28	35.0 ± 0.50
7	*S. sanguinis*	14.0 ± 0.50	19.0 ± 0.50	19.0 ± 0.50	19.6 ± 0.76	35.3 ± 0.57
8	*S. sanguinis* ATCC 10556	11.6 ± 0.28	18.3 ± 0.76	18.6 ± 0.76	18.0 ± 0.50	33.6 ± 0.28
9	*S. sobrinus*	13.3 ± 0.57	17.3 ± 0.76	19.3 ± 0.76	18.3 ± 0.57	32.6 ± 0.57
10	*S. sobrinus* ATCC 33478	12.3 ± 0.28	16.6 ± 0.57	19.3 ± 0.76	17.0 ± 0.50	32.6 ± 0.28
11	*L. acidophilus*	10.0 ± 0.50	16.0 ± 0.50	21.8 ± 0.76	20.0 ± 0.28	33.6 ± 0.28
12	*L. acidophilus* MTCC 10307	10.3 ± 0.28	16.6 ± 0.57	22.3 ± 0.76	20.3 ± 0.76	34.3 ± 0.57
13	*C. albicans* MTCC 227	7.3 ± 0.28	11.6 ± 0.57	14.0 ± 0.50	11.3 ± 0.76	27.0 ± 0.50

Table 1: Antimicrobial activity of *Salvadora persica* extracts against oral pathogens*: Zone of inhibition in millimetre (mm) in triplicate expressed as means and standard error of means.

The results for MICs of *S. persica* MeOH extract were recorded 3.12-25 mg/ml (Figure 1). The MICs were noted similar against *S. sanguinis* and *S. sobrinus* (6.12 mg/ml). Moreover, MeOH extract of this plant manifested a better MIC against *S. aureus* (3.25 mg/ml) and least MIC recorded against *C. albicans* (25 mg/ml). Firas et al. [19] reported MIC of H_2O extract of *S. persica* stem and bark against *S. mutans* at 0.0781 mg/ml. Thus, the activity observed for *S. persica* provides a rationale use in treatment of dental infections.

The phytochemical analysis of plant extracts disclosed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins which might be accountable for its antimicrobial potential (Table 2). Abdillahi et al. [20] reported the presence of flavonoids, alkaloids, steroids, terpenoids, saponins and carbohydrates in *S. persica* extracts. The H_2O extracts of *S. persica* contains important phytoconstituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides [21,22].

Solvents	Alkaloids	Glycosides	Flavonoids	Saponins	Steroids	Tannins	Terpenoids
PET	+	-	-	-	-	+	-
ACE	+	+	+	+	+	+	+
MeOH	+	+	+	+	+	+	+
H_2O	+	+	-	+	+	-	+

Table 2: The phytochemical screening of *Salvadora persica* crude extracts, +: Present, -: Absent.

Conclusion

The present study had shown that *S. persica* possess antimicrobial properties that support its medicinal values in herbal medicine for the treatment of dental ailments. The results significantly showed that *S. persica* stems and bark extracts possess a broad spectrum activity against a panel of bacteria and fungi responsible for the most dental diseases. This study can boast a new possibility for finding novel clinically effective antimicrobial compounds.

Conflict of Interests

The authors declare that they have no conflict of interests.

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