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***In Vitro* Assay of Herbaceous Extracts of *Salvadora persica* L. Against Some Pathogenic Microbes**

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ABSTRACT

The knowledge of plants as therapeutic agents goes back to the beginning of time. Ancient texts provide valuable clues of plants being used as potentially antiparasitic, antimalarial, anti-tumorous and antibacterial compounds. The plant was selected due to its application as traditional medicinal plant. The potent antimicrobial activities of alcoholic extracts of *Salvadora persica* L. (fresh and dry plant) were screened for *in vitro* activity against *B. subtilis*, *E. coli*, *Lactobacillus brevis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* using standard Agar Disc-Diffusion assay. Chloramphenicol and Miconazole were used as reference standards. The antimicrobial activity of alcoholic extracts of herbaceous parts of *S. persica* L. revealed a higher inhibition zone to *Proteus vulgaris* followed by other test organisms which exhibited less zone of inhibition such as *B. subtilis*, *Lactobacillus brevis*, *Staphylococcus aureus*, *E. coli*, *Candida albicans* and *Aspergillus niger*. This confirms that *S. persica* L. contains substances with antimicrobial properties. The phytochemical screening of the extracts revealed that the herbaceous parts of *Salvadora persica* L. contained carbohydrates, glycosides, sterols, terpenes, flavonoids, tannins and alkaloids but, are deprived of saponins, coumarins and anthraquinones. The spectra of activities displayed by the extracts can be attributed to the presence of these phytochemicals which signifies the potential of *Salvadora persica* L. plant as a source of therapeutic agents and may provide leads in the ongoing search for commercial extraction of essential components employed for varied purposes.

Key words: *Salvadora persica* L., antimicrobial activity, Phytochemical screening.

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INTRODUCTION

Salvodara persica L. is a much branched, ever green shrub or small tree, belonging to the family Salvadoraceae; Fresh bark is used as a vesicant. Root decoction is used against gonorrhoea and vesical-catarh. Root extract is used to relieve the pain due to spleen troubles. Bark decoction used as a tonic and stimulant in low fevers. Leaves are used in treatment of asthma, cough and piles. Fruits possess carminative and diuretically properties and used in treatment of rheumatism [1, 2]. Keeping this fact in the view we made an attempt to screen the antimicrobial nature of *S. persica* extracts against some pathogenic bacteria and fungi.

MATERIALS AND METHODS

PLANT MATERIAL

Salvodara persica L. fresh and dry plants were collected from Gorantla, Guntur district. The collected material was authenticated by Dr. T. Pullaiah, Department of Botany, Sri Krishna Devaraya University, Ananthapur. A voucher specimen has been deposited at K L University (KLU, DOB/BH/SP-01).

PREPARATION OF EXTRACT

S. persica fresh and dry plants were collected and stored in air tight bottles. The material was extracted with 150 ml of ethanolic solvent for 24 hours by using hot extraction. The extract was dried in a flash evaporator for 30 minutes and the left over powder was considered 100% different concentrations such as 100, 250, 500, 750 and 1000 µg/ml were prepared by re-dissolving the extracted powder in the same solvent which was used in the extraction. They were tested for antimicrobial activity using antibiotic sensitivity test [3]. The zones of growth inhibition around discs were measured and the area of inhibition zone was calculated. Simultaneously the activity of standard antibiotic Chloramphenicol was also tested against the bacteria under study (Table-1) in similar conditions, so as to compare the degree of inhibition by the fresh and dry plant extracts. Discs fed with corresponding solvents served as control.

Table No: 1. List of the selected test organisms (bacteria and fungi)

S.No	Name of the organism	Characteristic features	Diseases caused by the organisms
1.	<i>Bacillus subtilis</i> (ATCC 6633)	Gram + ve, rod-shaped, endospore forming, obligate aerobe.	Food poisoning, Opportunistic Pathogen.
2.	<i>Escherichia coli</i> (ATCC 25922)	Gram -ve, rod-shaped, facultative anaerobic endothermic.	Gastro-enteritis, urinary tract disease.
3.	<i>Lactobacillus brevis</i> (ATCC 8727)	Gram-positive, rod-shaped, non spore forming bacteria	Inflammatory effects in patients with chronic periodontitis, halitosis (Bad breath).

4.	<i>Proteus vulgaris</i> (ATCC 6380)	Gram –ve, rod shaped and extremely motile organism	Urinary tract infections.
5.	<i>Staphylococcus aureus</i> (ATCC 25293)	Gram +ve, coccus, facultative anaerobic, occur singly, in pairs, and irregular clusters, non-motile.	Chronic osteomyelitis, Meningitis, endocarditic.
6.	<i>Aspergillus niger</i> (NCIM 596)	Dichotomously branched, filamentous fungi.	Allergy, Asthma.
7.	<i>Candida albicans</i> (NCIM 670)	Dimorphic fungus.	Oral thrush, Gastritis, Cutaneous infection.

RESULTS AND DISCUSSION

The alcoholic extracts of both fresh and dry herbs of *S. persica* showed activity against all the tested microorganisms. The MIC varies from one organism to another organism i.e. 250 µg for *B. subtilis* and *Lactobacillus brevis*, 100 µg for *P. vulgaris*, 500 µg for *S. aureus*, 750 µg for *E. coli*, *C. albicans* and *A. niger*. Comparatively fresh plant extracts exhibited more activity than dry plant extracts (Table-2). Some values clearly indicate higher potency than standard antibiotics. Among the different concentrations of the extracts tested against the bacteria and fungi, fresh plant extracts were found very effective against *Proteus vulgaris* followed by other test organisms which exhibited less zone of inhibition such as *B. subtilis*, *Lactobacillus brevis*, *Staphylococcus aureus*, *E. coli*, *Candida albicans* and *Aspergillus niger*. To know the active principles of the extracts, they were subjected to preliminary phyto chemical screening [4-7], which revealed the presence carbohydrates, glycosides, sterols, terpenes, flavonoids, tannins and alkaloids but, are deprived of saponins, coumarins and anthraquinones (Table-3).

Table No. 2. Inhibitory activity of Fresh and dry herbaceous alcoholic extracts of *Salvadora persica* L.

Plant extract	Concentration µg/ml	Area of inhibition zone (mm ²)						
		A	B	C	D	E	F	G
Fresh Plant extract	100	-			31.43	-	-	-
	250	56.57		31.43	56.57	-	-	-
	500	97.48		56.57	88.00	31.43	-	-
	750	106.0	71.50	97.48	169.7	56.57	-	31.43
	1000	146.9	88.00	125.7	194.0	106.0	21.21.	43.21
Dry Plant extract	100	-	-	-	31.43	-	-	-
	250	21.21	-	-	43.21	-	-	-
	500	31.43	-	31.43	56.57	31.43	-	-
	750	88.0	56.57	56.57	125.7	56.57	21.21	21.21
	1000	146.9	71.50	125.7	169.7	88.00	31.43	31.43
Chloramphenicol (10µg/mL)		314	314	55.0	107	272	ND	ND
Miconazole (10µg/mL)		ND	ND	ND	ND	ND	154	167

(A) *Bacillus subtilis* (B) *E. coli* (C) *Lactobacillus brevis* (D) *Proteus vulgaris* (E) *Staphylococcus aureus*
 (F) *Aspergillus niger* (G) *Candida albicans*.

Table No: 3. Preliminary phytochemical studies of *Salvadora persica* L.

Phytochemical Constituents	Fresh Plant Extract	Dry Plant Extract
Alkaloids	++	+
Carboxylic acids	-	-
Coumarins	-	-
Fixed oils	-	-
Flavonoids	++	+
Phenols	-	-
Quinones	++	+
Resins	-	-
Saponins	-	-
Steroids	+	+
Tannins	+	+
Xanthoproteins	-	-
Glycosides	++	+

++ indicates high presence, + indicates present; - indicates absent

CONCLUSION

Phyto medicines are effective in treating most of the infectious diseases. Most of the secondary metabolites, serve as plant defence mechanisms against micro-organisms, insects and herbivores [8]. The herbaceous extracts of *S. persica* were found to contain carbohydrates, glycosides, sterols, terpenes, flavonoids, tannins and alkaloids. The antimicrobial activity of tested medicinal plant can be attributed to any of these constituents. Earlier reports have suggested the antibacterial activity of alkaloids. However, there was a marked difference in the level of activity shown by the various fractions. A detailed phyto chemical investigation and antimicrobial screening of secondary metabolites from these plant extracts may yield promising antimicrobial agents.

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