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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTI-MICROBIAL ACTIVITY OF *SALVADORA PERSICA* L.

Baba Fakruddin K[1]., Ranganayakulu G S[1] and Muralidhara Rao D[2]*

[1]Department of Botany, Rayalaseema University, Kurnool-518002, INDIA
*[2]Department of Biotechnology, Sri Krishnadevaraya University, Ananthapuramu-515002, INDIA

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ABSTRACT

In India and abroad various plant species including *Salvadora persica L.* is being used as the sources of herbal medicine. This work was mainly concerned with the identification of the therapeutic properties of *Salvadora persica L.* leaf and bark extracts. The SP-LF-ETH, SP-LF-ACET, SP-LF-WATE, SP-BRK-ETH, SP-BRK-ACET and SP-BRK- WATE extracts of *Salvadora persica L.*, showed solvent dependent anti-microbial activity, the extracts of ethanol and acetone extracts showed high antimicrobial activity than water extracts. These extracts were further studied and analyzed different phytochemicals in both leaf and bark samples. The *Salvadora persica L.* possess more alkaloids, carbohydrates, Steroids, Saponins, flavonoids, glycosides, phytosterols, proteins and tannins in ethanol and acetone extracts. The antimicrobial activity of leaf and stem bark of *Salvodara persica L.* with different extracts were analyzed and discussed.

INTRODUCTION

Traditional medicine has a long history of serving people all over the world from ancient period (Aibinu *et al*., 2004). The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs (De Pasqual., 1984). There is an evidence of herbs being used in the treatment of diseases and for revitalizing body systems in almost all ancient civilizations. The Vedas form the earliest literature in India called Rigveda, Yajurveda, Samaveda and Atharvanaveda revels importance of herbal medicine (Aibinu *et al*., 2004).

Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine (Shanmugapriya *et al*., 2012). Medicinal plants are resources of new drugs. It is estimated that there are more than 2, 50,000 flowering plant species.

The toothbrush tree, *Salvadora persica, L.,* locally called miswak, is a member of family Salvadoraceae. It has been used by many communities as toothbrushes and has been scientifically proven to be very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods (Almas *et al.,* 2014). *Salvadora persica* widely distributed in India, Africa, Saudi Arabia, Iran, Israel and Pakistan (Anonymous, 1972). In India and abroad various herbs including *Salvadora persica* is being used as the sources of herbal medicine (Howaida *et al*., 2003; Mohamed, 2013; Lobo *et al*., 2010). In the present study the extracts of leaf and stem bark of *Salvadora persica* revealed to posses the most potent bioactive components having important phytochemicals and antimicrobial effects, which might be used to formulate effective herbal medicine

Corresponding author:* **Muralidhara Rao D
Department of Botany, Rayalaseema University, Kurnool-518002, INDIA

MATERIALS AND METHODS

Collection of plant material

The plant samples (leaves and stem bark) of *Salvodara persica* L. were collected from the field areas of Nallamala forest, Kurnool district, Andhra Pradesh. The leaves and stem bark were washed thoroughly with tap water followed with sterilized distilled water for the removal of dust and sand particles, then shade dried at room temperature. Samples were homogenized to fine powder by a mechanical grinder and used for experimental analysis.

Preparation of plant extracts

5g of each sample powder were sequentially extracted with solvents namely ethanol, acetone and also with water by soxhelt apparatus for 48 hours. Then it was filtered through whatman No.1 filter paper. The crude extracts were obtained by dissolving a known amount of dry extract in different solvents such as ethanol, acetone and aqueous to obtain a stock

solution of 1000 µg/ml. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (50, 25 and 10 µg/ml). Crude extracts were analyzed for phytochemical and antimicrobial studies.

Preliminary phytochemical analysis

The individual extracts were subjected to qualitative chemical investigation for the identification of different phytochemical compounds and plant secondary metabolites by using standard protocols followed by mayer's and wagner's procedures.

Antibacterial and antifungal activity

The method called agar well plate method was adapted for screening of antimicrobial study. The hot sterile medium was poured into the sterile petri plates to form a 2-3mm thick. The plates were lawn cultured with bacterial broth and fungal spore suspension. Then make a hole with a diameter of 6-8 mm is punched aseptically with a sterile tip, and then poured a volume 150 µl of crude extract at desired concentrations (50, 25, 10 µg) were introduced into the each well. Then agar plates were incubated under ambient conditions in an incubator at 37°c for 24 hours depending upon the test organisms. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested, then evaluated the inhibition zones.

Preparation of Standard Solutions: Ciprofloxacin was the positive reference (control) standard with the dilutions of 50, 25, 10 µg/mL.

Test Organisms (Bacterial and Fungal stains)

The microorganisms used for the present study include bacteria like *Enterococcus faecalis*, *Staphylococcus aereus* are gram positive and *Klebsiallea pneumonia*, *Escherichia coli* are gram negative species. Fungal species includes *Candida albicans*, *Aspergillus fumigates*, *Dreschlera turcica* and *Fusarium verticillioides*. All bacterial and fungal species were procured from biotechnology lab, S.K.University, Anantapur. The bacterial and fungal stock cultures were maintained on different nutrient media which were stored at 4°c.

RESULTS AND DISCUSSION

Preliminary Qualitative and quantitative Phytochemical Studies

We analyzed qualitative and quantitative analysis of different phytochemical compounds in leaf and stem bark of *S. persica*. Leaf and stem bark was carried out by using the soxhlet apparatus with different solvents (Acetone, Ethanol and Water). It was observed that, ethanol extracts of leaf and bark crud extracts contain more phytochemicals such as alkaloids, carbohydrates, flavonoids, phenols and tannins respectively. Whereas, rest of the extracts of the plant materials contain few of the phytochemicals such as leaf acetone extract contains carbohydrates, phenols and tannins results were depicted in Table no 1a. In bark ethanol extract contains alkaloids, phenols and tannins and water extracts contains only carbohydrates respectively results were depicted in Table no 1b. Several reports stated that ethanol and acetone extracts shows more phytochemical than water extracts (Ravichandra *et al*., 2014; Souad Akroum *et al.,* 2012; Satish nayak *et al*., 2003).

Table 1a Preliminary Phytochemical Screening of Salvadora persica Leaf Extracts

Sl. No.	Test	Acetone Extract	Ethanol Extract	Water Extract
i	Alkaloids	-	+	-
ii	Carbohydrates	+	+	+
iii	Steroids	-	-	-
iv	Glycosides	-	-	+
v	Saponins	-	-	-
vi	Flavonoids	-	+	-
vii	Phenols	+	+	-
viii	Tannins	+	+	-

Table 1b Preliminary Phytochemical Screening of *Salvadora persica* Bark Extracts

Sl. No.	Test	Acetone Extract	Ethanol Extract	Water Extract
i	Alkaloids	-	+	-
ii	Carbohydrates	-	-	+
iii	Steroids	-	-	-
iv	Glycosides	-	-	+
v	Saponins	-	-	-
vi	Flavonoids	-	-	-
vii	Phenols	+	+	-
viii	Tannins	-	+	-

Anti microbial activity

We estimated antimicrobial activity of *Salvadora persica* leaf and bark in different extracts on common bacterial and fungal species. The disc diffusion method was used to determine the zone inhibition range. Efficiency of crude drug on different micro organisms was calculated based on the inhibition zone in diameter (mm), results were represented in table no.2a. The SP-LF-ETH and SP-BRK-ETH showed significant activity against *Staphylococcus aureus, Enterococcus faecalis* in gram positive and *Escherichia coli, Klebsiella pneumonia* in gram negative at the concentration of 50 µg. SP-LF-ACET, SP-LF-WATE, SP-BRK-ACET, SP-BRK- WATE extracts showed less significant activity. Similarly Gabin Thierry *et al.,* 2015 the ethanol extracts of *Tectona grandis* shows maximum bacterial inhibition.

Table 2a Anti microbial activity of *Salvadora persica leaf and bark extracts of different extracts*

Microorganisms	Staphylococcus aureus			Enterococcus faecalis			Klebsiella pneumonia			Escherichia coli		
Concentration in µg	50	25	10	50	25	10	50	25	10	50	25	10
SP-LF-ETH	16	14.5	12.5	15	12.5	10	17	14	12	18	16	13
SP-LF-ACET	10	-	-	9	-	-	9.5	-	-	11	10	9
SP-LF-WATE	14	12	11	13	11	9	12	10.5	-	15	12	9.5
SP-BRK-ETH	14	12	9.5	15	12	9	10	-	-	13	12	-
SP-BRK-ACET	11	10	-	10	9	-	11	9	-	12	10	9
SP-BRK- WATE	14	12	10	14	10	9	12	10	9	14	12	10
Standard (Ciprofloxacin)	18	15	14	18	16	14	17.5	15	13	17.5	15	12

*Data showing zone of inhibition in mm

In Anti fungal activity the SP-LF-ETH and SP-BRK-ETH showed significant activity against *Candida albicans, Asperigillus fumigates, Dreschlera turcica* and *Fusarium verticillioides* at the concentration of 50 µg. SP-LF-ACET, SP-LF- WATE and SP-BRK-ACET extracts showed less significant activity when extracts were compared with fluconazole which is used as a positive control. Similarly Somchit *et al.,* (2003) reported antifungal activity in *Cassia alata*. Results were placed in table no.2b

Table 2b Anti fungal activity of *Salvadora persica leaf and bark extracts of different extracts*

Microorganisms	Candida albicans			Aperigillus fumigatus			Dreschlera turcica			Fusarium verticillioides		
Concentration in µg	50	25	10	50	25	10	50	25	10	50	25	10
SP-LF-ETH	14	12	10	14	13.5	11	14	11.5	10	15	14	10
SP-LF-ACET	11	9	-	11	9	-	13	10	9	12	10	-
SP-LF-WATE	10	-	-	13	11	9	12	9	-	14	12	10.5
SP-BRK-ETH	13	11.5	9	10.5	-	-	15	12	9	14	13	10
SP-BRK-ACET	11	9.5	-	9	-	-	11.5	9	-	10	9	-
SP-BRK- WATE	12.5	11	9	10	-	-	11	10	9.5	9	9	-
Standard (Flucanazole)	17	15.5	12.5	17	16	13	17	15.5	12.5	17	16	13

***Data showing zone of inhibition in mm**

CONCLUSIONS

Present study has been concluded that *Salvadora persica* extracts using ethanol and acetone solvent were most effective against *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli and Enterococcus faecalis*. Out of all the extracts from *Salvadora persica* maximum significant antimicrobial activity showed by leaf extract ethanol solvent against bacterial as well as fungal species, *Klebsiella pneumonia, Escherichia coli, Fusarium verticillioides, Aperigillus fumigates and Candida albicans*. The results of present work have justified the traditional indirect use of plant in curing diseases. Some of these observations have helped in developing drugs for therapeutic use in different diseases.

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