Phytochemical and Antioxidant Studies of *Salvadora persica* L. Stem & Twig

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ABSTRACT

Salvadora persica L. commonly known as *Miswak* and have immense medicinal value as anti-microbial and in prevention of tooth decay. Present study deals with phytochemical and antioxidant evaluation of *S. persica* twig and stem. Chloroform and ethanolic extracts from *S. persica* twig and stem are screened for antioxidant activity using, DPPH free radical scavenging activity. Methanolic extract has been studied through HPTLC. In *S. persica* twig, IC_{50} of chloroform extract and ethanolic extract was found to be 181.33 ± 1.15 μg/ml and 197.00 ± 1.73 μg/ml respectively, however in stem, IC_{50} of chloroform extract and ethanolic extract was found to be 187.33 ± 0.57 μg/ml and 235.66 ± 1.52 μg/ml respectively. Ascorbic acid is used as standard which showed IC_{50} 2.03 ± 0.06 μg/ml. In HPTLC studies the ferulic acid content was found to be 0.026% and 0.082% in *S. persica* stem and twig respectively. All the extracts showed significant in vitro antioxidant activity, chloroform extract showed the most potent activity followed by ethanolic extract. Ferulic acid, a potential antioxidant present in this species, has been studied through HPTLC. The presence of ferulic acid has not yet been reported and quantified in this species which may be utilized for the proper standardization of the drug.

Key words: Antioxidant activity, DPPH, Ferulic acid, *Salvadora persica*.

INTRODUCTION

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.[1-3] *S. persica* is widely distributed in India, Africa, Saudi Arabia, Iran, Israel and Pakistan.[4]

It has been reported that extracts of miswak posses various biological properties, including significant antibacterial,[5,6] antifungal,[7] and anti-plasmodial effects.[8] Phytochemical investigation revealed that it contains alkaloids, glycosides, flavonoids, carbohydrates, tannins, saponins and steroids.[9] It also contains oleic, linoleic, stearic acids, esters of fatty acids and aromatic acids, and some terpenoids. The major components from the essential oil of S. persica stem have been identified as 1,8-cineole (eucalyptol) (46%), α-caryophellene (13.4%), β-pinene (6.3%), and 9-epi-(E)-caryophellene.[10] GC-MS analysis of the volatile oil extracted from *S. persica* leaves revealed benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and β-caryophyllene as important constituents.[11]

Sticks from *S. persica* have been analyzed for their soluble and total content of fluoride, calcium, phosphorus, and silica. There was a substantial amount of silica in the ashes of miswak.[12] Three lignin glycosides have been reported from the stem of *S. persica*.[13] The minor components such as volatile oils, flavonoids may, to some degree augment the pharmacological effects of the plant.[14] An indole alkaloid salvadoricine known to occur in leaves have also been isolated

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from undifferentiated tissues of *S. persica*.[15] It has been shown to contain trimethylamine, salvadorine, chloride, fluoride, silica, sulphur, mustard oil, vitamin C, resins and traces of tannins, saponins, flavonoids and sterol.[16] Roots also contain alpha amylase.[17] The antioxidant activity of *S. persica* stem has been performed earlier;[5] however present study was performed to compare the antioxidant property of stem and twig of *S. persica*. Considering the value of this drug in traditional practices, the present study was performed to develop comparative phytochemical parameters and evaluation of antioxidant potential on stem and twig of this species.

MATERIALS AND METHODS

Chemicals and Reagents

1,1Diphenyl -2-picrylhydrazyle (DPPH), was obtained from Sigma Aldrich Co. All other chemicals used were of analytical grade.

Plant material

The plant specimen i.e. dried stem and twig of *S. persica* were collected from Barhmer, Rajasthan, India in 2008. The plant was authenticated by Dr. AKS Rawat, NBRI. A voucher specimen (262542) has been submitted in institute's herbarium.

Preparation of Plant Extracts

The fresh plant material was collected, thoroughly washed with water to remove all debris and then shade dried. The dried material was powdered by using electric grinder at 100 mesh size. Extraction was performed by soxhlation process in two steps. Firstly the powdered material was defatted under soxhlet assembly using 250 mL of 98% ether for 6 hours. This is followed by 9 hours soxhlation of defatted powder by using 250 mL of chloroform as solvent. Same procedure was used to obtain ethanolic extract, after defatting of crude drug it was extracted with 250 mL of 99.9% ethanol for 6 hours.

The final extracts were passed through Whatman No. 1 filter paper. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use. The crude extracts were obtained by dissolving a known amount of dry extract in 98% methanol to obtain a stock solution of 1000 µg/ml. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (25, 50, 100, 125,150, 200, 250, 300 and 500 µg/ml).

Physicochemical and Phytochemical Studies

Physicochemical and Phytochemical studies like extractive values, total ash, acid insoluble ash, total sugar, starch, tannin, and phenols were calculated from the shade-dried and powdered (60 mesh) plant material.[18-21]

Antioxidant Activity (DPPH Free Radical Scavenging Activity)

Antioxidant activity of the plant extracts and standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical by the method previously described.[22] The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 50 µg/ml. 0.002% DPPH solution in methanol was prepared. Then 2 ml of this solution was mixed with 2 ml of sample solutions (ranging from 25 µg/ml to 500 µg/ml) and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using a Shimadzu spectrophotometer against methanol. The blank used was 2 ml of methanol with 2 ml of DPPH solution (0.002%). The optical density was recorded and percentage of inhibition was calculated using the equation: % of inhibition of DPPH activity = (A–B) /A × 100; where A is optical density of the blank and B is optical density of the sample.

HPTLC Studies

Air dried (45-55°C) powdered stem and twig of *S. persica* (2.0 g) in triplicate were extracted separately with 3 X 20 ml methanol. Extracts were concentrated under vacuum and redissolved in methanol, filtered and finally made up to 100 ml with methanol prior to HPTLC analysis. Reagents used were from Merk (Germany) and standard ferulic acid was procured from Sigma-Aldrich (Steinheim).

Chromatographic Conditions

Chromatography was performed on Merk HPTLC precoated silica gel 60GF_{254} (20 X 20 cm) plates. Methanolic solutions of samples and standard compound ferulic acid of known concentrations were applied to the layers as 6 mm-wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat V automated TLC applicator with nitrogen flow providing a delivery speed of 150 nl/s from application syringe. These conditions were kept constant throughout the analysis of samples.

Detection and Quantification of Ferulic Acid

Following sample application, layers were developed in a Camag twin trough glass chamber which was pre-saturated with mobile phase of toluene: ethyl acetate: formic acid (5:5:1) till proper separation of bands up to 8 cm height. After development, layers were dried with an air dryer and ferulic acid was simultaneously quantified

Table 1: Phytochemical Screening of *S. persica* (Twig & Stem)								
	Hexane		Chloroform		Alcohol		water	
	Twig	Stem	Twig	Stem	Twig	Stem	Twig	Stem
Alkaloids	-	-	+	+	+	-	-	+
Glycosides	-	-	-	-	+	+	+	+
Tannins	-	-	-	-	+	-	+	+
Saponins	-	-	-	-	+	+	+	+
Flavonoids	-	-	-	-	+	+	+	+
Protein	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	+	+	+	+

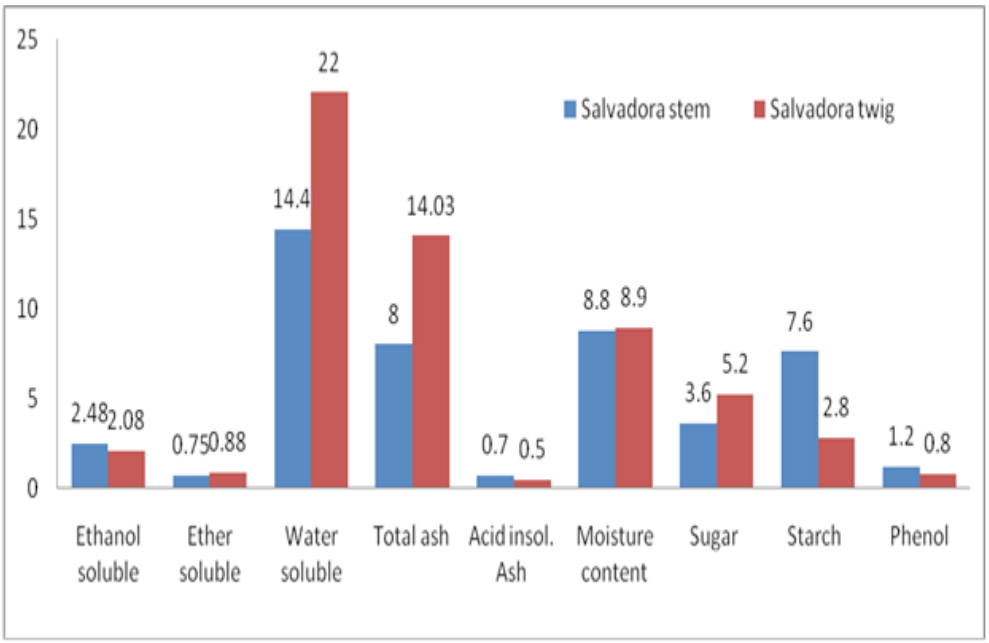

Figure 1: Percentage physicochemical values of *S. persica* stem and twig

using Camag TLC scanner model 3 equipped with Camag Wincats IV software. Following scan conditions were applied: slit width, 5 mm x 0.45 mm; wavelength, 320 nm; and absorption-reflection mode. In order to prepare calibration curves, stock solution of ferulic acid (1 mg/ml) was prepared and various volumes of the solution were analyzed through HPTLC, calibration curves of peak area vs. concentration were also prepared.

RESULTS

Phytochemical screening

Phytochemical screening for the hydro-alcoholic extract showed positive test for flavonoids, proteins, carbohydrates, glycosides, phenolic compounds and saponins, Table 1.

Physicochemical Studies

Parameters such as moisture content, extractive values (Water, alcohol and ether soluble), total ash and acid insoluble ash values, total sugar, total starch, and total phenolics were determined, Figure 1.

Antioxidant activity

Invitro antioxidant study of *S. persica* stem (SS) & twig (ST) was performed using two different extracts viz. ethanolic and chloroform extract. In this study ST chloroform extract showed the least IC_{50} value of 181.33 ± 1.15 µg/ml. Standard ascorbic acid showed IC50 value of 2.03 ± 0.06 µg/ml, Table 2.

HPTLC Studies

Calibration curve of ferulic acid showed r^2 of 0.949 and R_f of ferulic acid was found to be 0.69 ± 0.006. Quantification of ferulic acid in the samples of *S. persica* stem and twig has been performed and the ferulic acid was found to be 0.026% and 0.082% respectively. A Densitogram and Banding pattern obtained from extract shows ferulic acid, Figure 2 and 3.

Table 2: Antioxidant activity of *S. persica* (Twig & Stem)	
Sample	**DPPH assay IC-50(µg/mL)**
AA-std	2.03 ± 0.06
SS-chloroform	187.33 ± 0.57
SS-ethanolic	235.66 ± 1.52
ST-chloroform	181.33 ± 1.15
ST-etanolic	197.00 ± 1.73

AA- Ascorbic Acid; SS- Salvadora Stem; ST- Salvadora Twig

Figure 2: HPTLC profiles of Methanolic extract of *S. persica* stem (SS), twig (ST) & Standard (Ferulic acid)

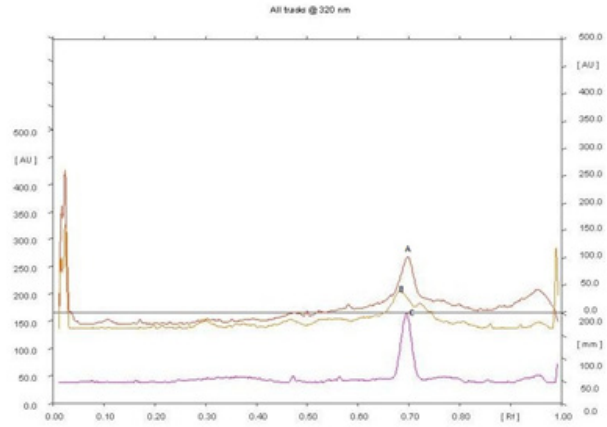

Figure 3: Densitometric chromatogram of *S. persica* stem, twig and ferulic acid
(A: Methanolic Extract of twig; B: Methanolic extract of stem; C: Standard Ferulic acid)

DISCUSSION

Ascorbic acid (standard compound) showed highest significant and potent antioxidant activity in DPPH free radical scavenging method. Chloroform extract showed the most potent activity followed by ethanolic extract.

Presence of phenolic compounds in *S. persica* suggests that the antioxidant activity may be due to the polyphenolic content. Identification of all chemical constituents in extract those are responsible for antioxidant activity requires further investigation, the crude extracts merits further experiments *in vivo*. Ferulic acid, a potential antioxidant present in this species, has been studied through HPTLC. The presence of ferulic acid has not yet been reported and quantified in this species which may be utilized for the proper standardization of the drug.

Present study showed new natural antioxidant that can replace the synthetic ones to be used in foods and cosmetics. Thus, the effective source of *S. persica* could be employed in all medicinal preparations to combat myriad diseases associated with oxidative stress and related disorders.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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