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Evaluation of phenolic content and antioxidant activity of various solvent extracts of *Salvadora persica* stem

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Abstract
Plants are the storehouse of a large number of pharmacologically important compounds having antioxidant activities. *Salvadora persica*, commonly called miswak plant, is well documented for its antidiabetic, antihyperlipidemic and antimicrobial activities. In the present study antioxidant activity of five different extracts of *S. persica* stem along with total phenolic content were investigated by different antioxidant assays that include free radical (DPPH, superoxide, hydroxyl) scavenging activity, reducing power assay and metal chelating capacity. Total phenolic content in methanolic extract was 32.50 mg/g gallic acid equivalent. The results depicted that methanolic extract possessed the highest antioxidant activity which gets enhanced with increasing concentration. Antioxidant activities showed good correlation with total phenolic content suggesting the role of phenolics in quenching free radicals. These results indicate that methanolic extract of *S. persica* stem displayed significant antioxidant activities establishing its role as source of natural antioxidants.

Keywords: *Salvadora persica*, miswak, antioxidants, free radicals, phenolics

Introduction

Reactive oxygen species (ROS) generated in biological systems as a result of aerobic respiration creates oxidative stress resulting in numerous metabolic disorders such as cardiovascular disease, diabetes mellitus, hypertension, Alzheimer disease, atherosclerosis, and even cancer [1, 2, 3]. Many synthetic drugs have antioxidant properties but also have adverse side effects so the focus is shifted towards finding natural antioxidants from plant sources. Antioxidants by their free radical scavenging activity and metal ion chelation ability prevent the oxidation of other molecules thereby protecting the cells from harmful effects of reactive oxygen species. Plant based antioxidants are generating immense interest due to their low toxicity, economic viability, and strong pharmacological activities. Plant antioxidants are secondary metabolites reported from leaves, fruits, roots, stem, seeds and could serve as therapeutic agents in minimizing various chronic and degenerative diseases [4, 5].

Salvadora persica L. belonging to the family Salvadoraceae is a small oil yielding medicinal and multi-purpose tree distributed in saline, arid or semi-arid reasons of Africa and Asia. In dry regions because of its dense canopy it is used as shelter beds and windbreaks besides providing food for camels and goats. It is popularly known as miswak and has been used for centuries in Middle East countries for oral hygiene [6, 7]. Phytochemical analysis of *S. persica* revealed the presence of various phytochemical constituents *viz.* flavonoids, glycosides, sterols, terpenes, alkaloids, saponins, fatty acids (linoleic and stearic acid), and minerals such as fluoride, silica, potash etc. [8]. Different parts of the plant have been reported for their antibacterial, anti-inflammatory, hypoglycemic, hypolipidemic and analgesic properties [9, 10]. To explore the antioxidant potential of *S. persica* stem the present study was planned to assess the total phenolic content and free radicals scavenging activity using different *in vitro* assays.

Material and Methods

1. Preparation of extracts

For the present study stem samples of *S. persica* were collected from the campus of CCSHAU, Hisar, Haryana and herbarium specimen was authenticated at FRI, Dehradun with the accession number 10786. Different extracts (petroleum ether, benzene, chloroform, methanol, and water) of stem were prepared by cold percolation method. Different concentrations of extracts (from 0.1mg/ml to 0.5mg/ml) were used for the determination of total phenolic content and antioxidant activity.

2. Assessment of total phenolic content and antioxidant activity

Total phenolic content (TPC) was estimated by modified Folin-Ciocalteu's method [11] and represented in the form of mg gallic acid equivalent/g of extract. DPPH free radical scavenging activity of the extract was assayed by method of Lee *et al* [12]. Decrease in absorbance was measured at 517 nm and compared with standard ascorbic acid (10-50µg/ml). Potential of stem extracts to scavenge superoxide radical was estimated by reduction of NBT (Nitro blue tetrazolium) and compared with BHT (Butylated hydroxytoluene) [13]. Hydroxyl radical scavenging activity of extracts were measured with TBA (Thiobarbituric acid) reaction as described by Kunchandy and Rao [14] and compared with ascorbic acid. Metal chelating activity of extract was assessed by their ability to inhibit ferrozine-Fe^{2+}complex formation and measured at 562 nm [15]. Reducing power of each extract was measured according to Yen and Duh [16] and expressed in terms of OD 700 nm and high OD indicates high reductive potential. All the analysis were done in triplicate and percentage inhibition was calculated using that formula.

% Inhibition = A (control)-A (sample or standard)/A (control) × 100

Where
A(control) = absorbance of the control
A (sample or standard) = absorbance of sample extract or standard

3. Statistical analysis
One way ANOVA along with Duncan's multiple range test were done with level of significance at p <0.05. Correlation between TPC and different antioxidant tests was analysed by Pearson correlation coefficient.

Result & Discussion
Total phenolic content (TPC)
Phenolic compounds, ubiquitous in plants, are secondary metabolites arising from shikimate or polypropanoid pathways. The antioxidant property of phenolic compounds is due to their capability of losing protons or chelating metal ions [17]. Thus, to determine antioxidant activity of plant extract it is important to find out the quantity of phenolic compounds. Figure 1 shows the phenolic content in different extracts at different concentration. Of the five extracts methanolic extract exhibited highest TPC content (32.50mgGAE/g). Phenolic content in different extracts decreased in the order: methanol> aqueous> chloroform> petroleum ether> benzene extracts.

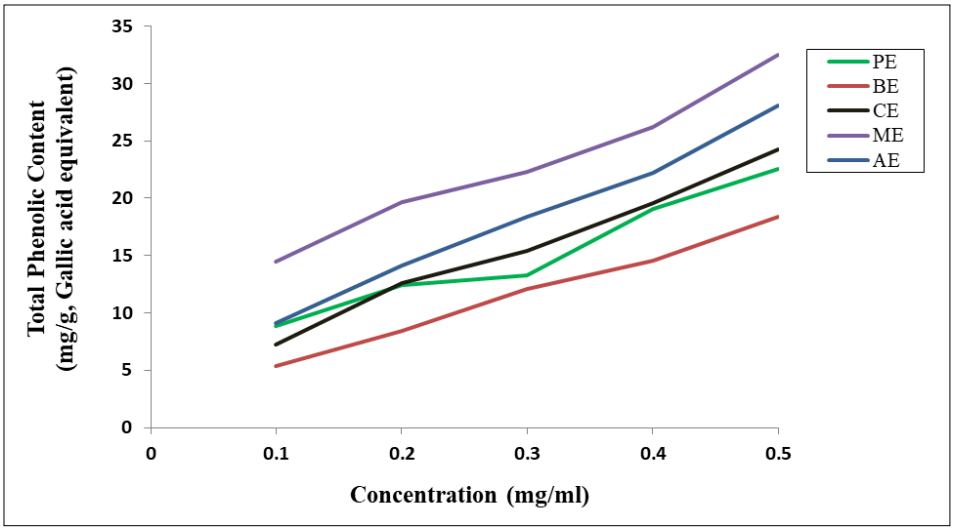

Fig 1: Total phenolic content (TPC) of stem extracts of *S. persica* (PE- petroleum ether extract, BE- benzene extract, CE-chloroform extract, ME- methanol extract, AE- aqueous extract).

DPPH radical scavenging activity
DPPH radical scavenging activity is the most widely used direct and inexpensive method that depends on the elimination of DPPH radical in presence of antioxidants. DPPH radical produces purple colour in alcoholic solution and on being reduced to DPPH-H it turns to pale yellow which can be measured quantitatively by decrease in absorbance at 517 nm [18,19]. *S. persica* stem extracts have significant DPPH scavenging capability which increases with increase in concentration from 0.1mg to 0.5mg/ml. At 0.5mg/g concentration free radical scavenging activity of methanolic, aqueous, chloroform, petroleum ether and benzene extract was 65.07%, 42.92%, 29.23%, 27.58% and 22.52% respectively, however it was lower than that of ascorbic acid (84.80% at 50 µg/ml) (Table 1).

Table 1: DPPH free radical scavenging activity (%) of stem extracts of *S. persica*

Stem extracts							
Concentration (mg/ml)	**PE**	**BE**	**CE**	**ME**	**AE**	**Conc. (µg/ml) of AS**	**AS**
0.1	6.21±0.73^{e}	7.27±0.40^{e}	5.23±0.17^{e}	25.47±0.35^{e}	10.54±0.31^{e}	10	20.84±0.62^{e}
0.2	10.38±0.22^{d}	10.42±0.87^{d}	10.19±0.54^{d}	32.78±0.25^{d}	17.11±0.48^{d}	20	38.63±0.40^{d}
0.3	16.31±0.51^{c}	15.05±0.71^{c}	18.95±0.17^{c}	57.82±0.78^{c}	25.82±0.14^{c}	30	75.17±0.60^{c}
0.4	22.46±0.43^{b}	19.26±0.83^{b}	23.34±0.61^{a}	61.36±0.62^{b}	39.63±0.45^{b}	40	80.28±0.12^{b}
0.5	27.58±0.36^{a}	22.52±0.46^{a}	29.23±0.86^{a}	65.07±0.93^{a}	42.92±0.87^{a}	50	84.80±0.66^{a}

Values are expressed as mean±S.D., (n=3).
Values with in the column not sharing common superscript letters (a-e) differ significantly at p<0.05 by Duncan's multiple range test.
PE- Petroleum ether, BE- Benzene extract, CE- Chloroform extract, ME- Methanol extract, AE- Aqueous extract, AS- Ascorbic acid.

Superoxide radical scavenging activity
Superoxide anion is generated during aerobic respiration by reduction of molecular oxygen through an electron transfer. Although superoxide itself is a weak oxidant but inside cell it may lead to the generation of hydroxyl radical (OH·) and peroxynitrite, both of which are very powerful oxidants targeting proteins, lipids, carbohydrates and DNA in cells causing tissue damage and associated oxidative stress [20,21]. During present study on antioxidant potential of *S. persica* stem, methanolic extract was found to be most

active followed by aqueous> chloroform> petroleum ether> benzene extracts (Figure 2). The percentage inhibition of the superoxide radical by methanolic extract varied from 29.62% (at 0.1mg/ml) to 60.17% (at 0.5mg/ml). The IC_{50} value of methanolic extract was 0.34 mg/ml which was double to the standard drug BHT (0.17 mg/ml).

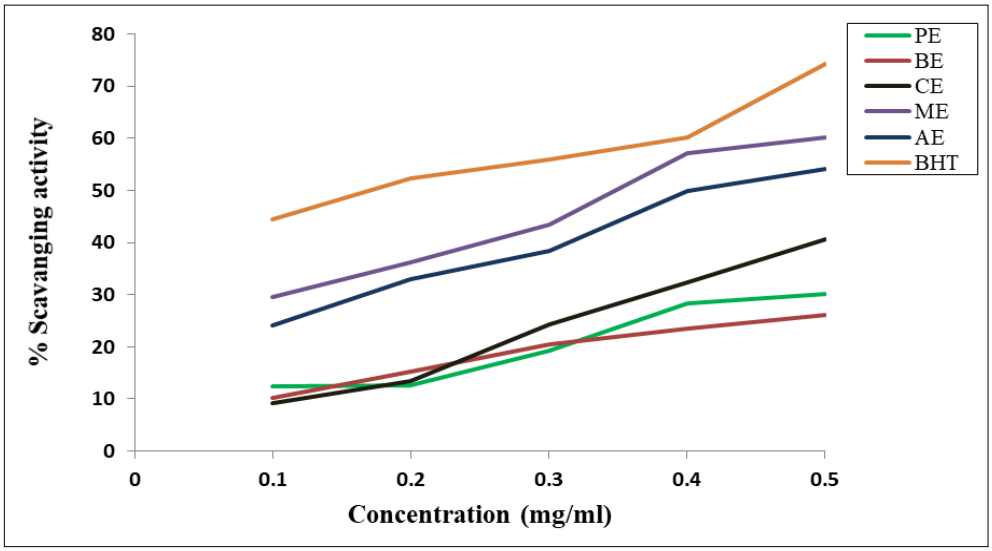

Fig 2: Superoxide radical scavenging activity (%) of stem extracts of *S. persica* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, BHT- Butylated hydroxytoluene).

Hydroxyl radical scavenging activity

Hydroxyl radicals being highly reactive interact directly with DNA causing DNA breakage and resulting in mutagenesis and cancer [22,23]. In the reaction mixture hydroxyl radicals were produced by Fenton reaction between H_2O_2 and Fe^{2+} and addition of plant extracts resulted in decline rate of absorption at 536 reflecting hydroxyl radical scavenging activity of the samples. Comparison of antioxidant activity of different extract and standard ascorbic acid is shown in Figure 3. All the extracts showed significant dose dependent inhibition of hydroxyl radical in the following order: methanolic > petroleum> aqueous> chloroform> benzene indicating a potent hydroxyl scavenging effect. The IC_{50} value of methanolic extract and ascorbic acid was found to be 0.38 mg/ml and 0.092 mg/ml respectively.

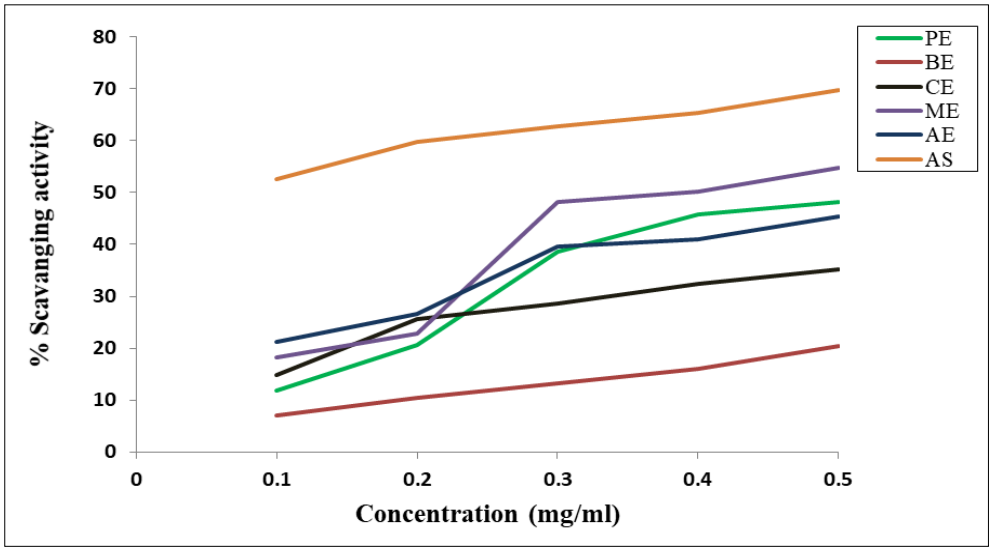

Fig 3: Hydroxyl radical scavenging activity (%) of stem extracts of *S. persica* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, AS- Ascorbic acid).

Metal chelating activity

Ferrous ions have the capacity to remove electrons from molecules leading to the generation of reactive oxygen species. Ferrous ion by Fenton and Haber-Weiss reactions catalyse lipid peroxidation leading to the production of hydroxyl radicals. Metal ion chelators can inhibit ROS production and free radical formation. Natural metal chelating compounds are preferred over synthetic chelating agents due to their associated health problems in long term use. Metal chelators present in plant extract remove ferrous ions before Ferrozine-Fe^{2+} complex formation decreasing the absorbance at 562nm in dose dependent manner [24,25]. Present results showed that methanolic extract was most effective in chelating metal ions followed by aqueous> chloroform> petroleum ether > benzene extracts (Figure 4). Mean IC_{50} value of methanolic extract was found to be 0.34 mg/ml while that of standard EDTA (Ethylenediamine tetraacetic acid) was 0.22 mg/ml.

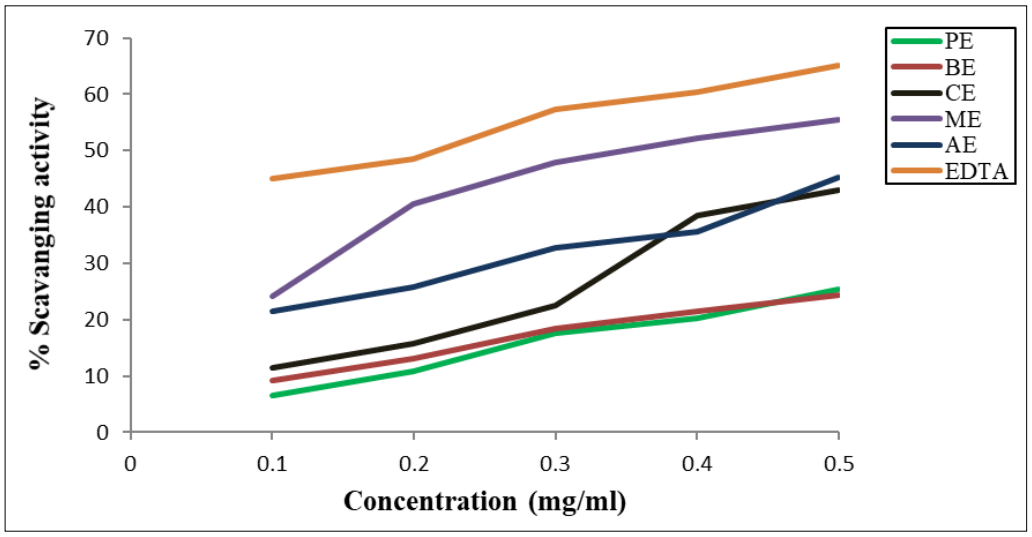

Fig 4: Metal chelating activity (%) of stem extracts of *S. persica* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, EDTA- Ethylenediamine tetra acetic acid).

Reducing power assay

Reducing power is generally associated with the presence of reductants that reduces ferricyanide complex to ferrous form. In the assay yellow colour of test solution changes to Perrusian blue indicating reducing power of plant extracts. Previous reports suggest that reducing potential is due to donation of electrons to break the chain reactions initiated by free radicals and observed a direct correlation between reducing power and antioxidant activities of plant extracts [25]. Figure 5 showed the reductive ability of plant extract in at varying concentration. Reducing power of extracts increased with increase in concentration. Methanolic extract has highest reducing power followed by benzene> chloroform> aqueous> petroleum ether extracts but lower than ascorbic acid.

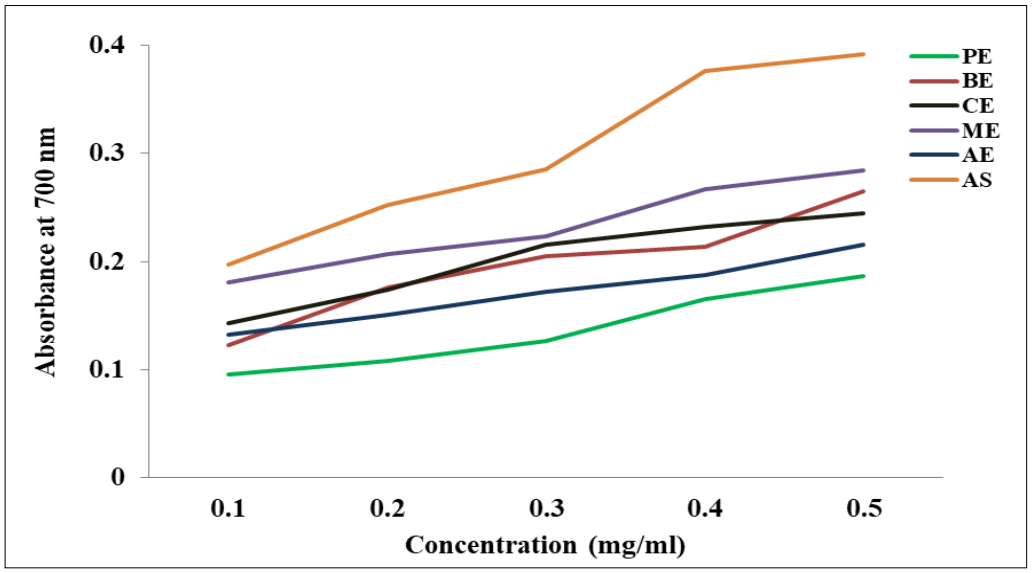

Fig 5: Reducing power assay of stem extracts of *S. persica* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, AS- Ascorbic acid).

Correlation between total phenolic content and antioxidant activity

Phenolic compounds having hydroxyl groups are important antioxidant compounds. They by donating electrons deactivate the free radicals and prevent the onset of degenerative diseases. Several previous reports suggest that phenolic compounds may be responsible for antioxidant potential of plant extracts [26,27]. For *S. persica* methanolic extract significant correlation was observed between various antioxidant assays and total phenolic content. The values of correlation coefficient for DPPH, superoxide, hydroxyl and metal chelating assay were 0.896908, 0.962759, 0.889708, and 0.924891 respectively (Table 2) indicating the role of phenolic compounds towards antioxidant activity. Various previous studies reported the presence of phenolic compounds in *S. persica* [28,29]. In the stem of *S. persica* flavonoids rutin and quercetin were found by Akhtar *et al* [30]. Similarly Ahmed *et al* [31] detected the presence of kaempferol, quercetin, rutin and quercetin glycosides from roots of *S. persica*. From different extracts of *S. persica* and *S. oleoides* thirteen phenolic acids and five flavonoids were detected with the help of RP-HPLC [32]. Maroid *et al* [33] in *S. persica* seed oil detected the existence of γ-tocopherol and α-tocopherol, both having antioxidant activity similar to vitamin E. These reports supports the view that phenolics may be the contributor of antioxidant activity if *S. persica.*

Table 2: Correlation analysis between different antioxidant assays with their respective total phenolic content at 0.5 mg/ml concentration in *S. persica* stem methanolic extract

Assays	Total phenolics in stem	
	r	R^2
DPPH radical scavenging	0.896908*	0.804*
Superoxide radical scavenging	0.962759*	0.926*
Hydroxyl radical scavenging	0.889708*	0.791*
Metal chelating assay	0.924891*	0.855*

r- correlation coefficient, R^2- coefficient of determination, *significance at $p<0.05$

Conclusion

This study reported significant correlation between total phenolic content and antioxidant activity of *Salvadora persica* thus reinforcing the belief that phenolic compounds are responsible for antioxidant activity. Further studies are required to isolate the bioactive compounds contributing to antioxidant activity.

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