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Screening of *Salvadora persica* leaves for antioxidant properties and total phenolic content

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Abstract

The objective of this research was to investigate the antioxidant properties of *Salvadora persica* leaf extracts using various in vitro assays and compare them with the total phenolic content. Petroleum ether, benzene, chloroform, methanol, and aqueous extracts were assessed for their ability to scavenge free radicals such as DPPH, superoxide, and hydroxyl radicals along with metal chelating capacity and reducing power. Estimation of phenolic content was done using the Folin-ciocalteu reagent and expressed as mg/g Gallic acid equivalent. The results demonstrated that all the solvents exhibited significant concentration-dependent free radical scavenging activity. Among the five extracts, the methanolic extract displayed the highest antioxidant activity and contained 52.64 mg/g Gallic acid equivalents (GAE) of phenolic content. The study identified significant correlation between the total phenolic content and the antioxidant assays suggesting role of phenolic compounds to the antioxidant activity of *S. persica* extracts. These findings suggest that the methanolic extract of *S. persica* is rich in phenolic compounds, which may account for its potent antioxidant activity. Consequently, these results emphasize the potential of *S. persica* as a valuable source of natural antioxidants and provide scientific support for its use in treating various health conditions.

Keywords: *Salvadora persica*; Plant extract; Antioxidant activity; Total phenolic content; DPPH radical

1. Introduction

Reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anion (O_2•-), peroxyl (ROO•), reactive hydroxyl (OH•), and hypochlorous acid (HOCl), are major contributors to degenerative disorders such as atherosclerosis, Alzheimer's, cancer, diabetes, and cardiovascular diseases. These are produced from both exogenous sources like stress, cigarette smoke, ionizing radiation, and dyes, as well as endogenous sources during normal oxidation processes in the body. Free radicals have the potential to initiate chain reactions and cause damage to biological molecules leading to mutations and cellular dysfunction. Antioxidants play a crucial role in preventing the oxidation of biomolecules by scavenging free radicals. The natural antioxidant system in the body maintains a balance between free radical generation and their neutralization under normal physiological conditions. However during disease state the accumulation of free radicals results in oxidative stress, causing damage to cellular components and contributing to various diseases. Synthetic drugs like Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) possess antioxidant properties but also have associated side effects. As a result, there is a growing interest in exploring natural antioxidants of plant origin [1, 2, 3].

Medicinal plants offer promising potential as safer, affordable, and easily accessible remedies for combating free radical-induced damage. Plant-based antioxidants, derived from leaves, fruits, roots, stems, and seeds, have gained significant attention due to their low toxicity, economic viability, and strong pharmacological activities. India, with its abundant floral diversity, serves as a treasure trove of many medicinally important plants, used in traditional folklore and herbal medicines. While numerous plant species have been studied for their pharmacological compounds, the full therapeutic

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potential of many plant species remains unknown. Therefore, there is a need for further exploration and investigation of the therapeutic applications of these plants to combat various chronic and degenerative diseases [4,5]

Salvadora persica, commonly known as the toothbrush tree or miswak, is widely distributed in India, Africa, Saudi Arabia, Iran, Israel, and Pakistan, primarily in dry and arid regions, saline lands, and coastal areas. In Middle East countries it is primarily used in dental care to treat toothaches, gum diseases, and for teeth cleaning. Its leaves possess several medicinal properties and have been used in the treatment of various conditions such as piles, scabies, leucoderma, teeth strengthening, asthma and cough, while a poultice made from them is applied to painful piles and tumours. Phytochemical investigations have revealed the presence of alkaloids, glycosides, flavonoids, carbohydrates, tannins, saponins, steroids, oleic acid, linoleic acid, stearic acid, esters of fatty acids and aromatic acids, and some terpenoids. *S. persica* extracts have been found to possess various biological properties, including significant antibacterial, antifungal, antidiabetic and anti-plasmodial effects [6,7,8]. To further explore the antioxidant potential of *S. persica* leaves, the present study aims to assess the total phenolic content and free radical scavenging activity using different in vitro assays.

2. Material and methods

For this study, leaf samples of *Salvadora persica* were collected from CCSHAU, Hisar, Haryana and identified using the herbarium of FRI, Dehradun with the accession number 10786. Collected samples were dried in the shade, ground in a mechanical grinder, and subjected to cold percolation using petroleum ether, benzene, chloroform, methanol, and aqueous solvents to obtain extracts. Varying concentration of extracts ranging from 0.1 mg/ml to 0.5 mg/ml was used for estimation of total phenolic content and antioxidant activity.

The total phenolic content was estimated using a modified method based on Singleton and Rossi [9], and the concentration of phenolic content was calculated by creating a calibration curve using Gallic acid as a standard. The antioxidant activity of the extracts was assessed by their ability to decolorize stable DPPH from violet to yellow following the method described by Lee et al. [10]. Superoxide anions generated in the test solution by PMS-NADH system was estimated using the NBT reduction method outlined by Liu et al. [11]. The hydroxyl radical scavenging activity of the extract was measured by the TBA (Thiobarbituric acid) reaction, as recommended by Kunchandy and Rao [12] and compared with ascorbic acid. Metal ion chelation by the various extracts was determined by their capacity to inhibit ferrozine-Fe^{2+}complex formation according to Dinis et al. [13] and measured at 562 nm. The reducing capacity of the solvents was evaluated by analysing their ability to reduce the ferric cyanide complex to the ferrous form, following the method by Yen and Duh [14], where an increase in absorbance indicated an increase in reducing ability.

The statistical analysis was conducted by expressing the results as mean ± standard deviation. The percentage scavenging activity was calculated using the formula:

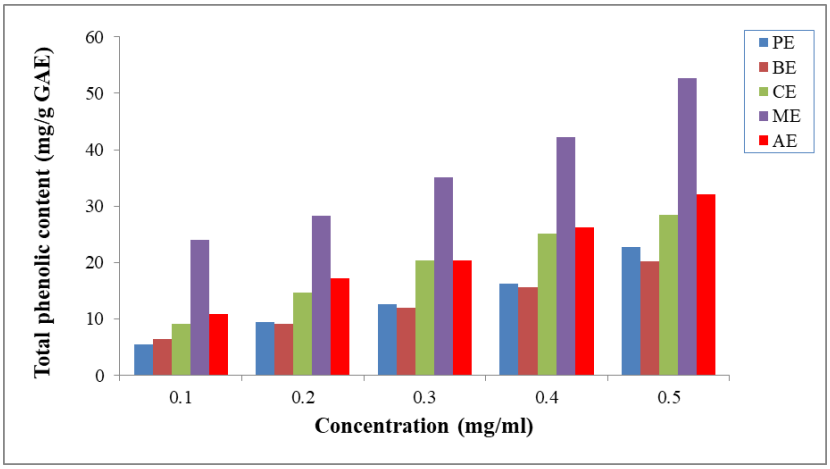
$$\text{Scavenging (\%)} = (A_{control} - A_{sample})/A_{control} \times 100.$$

The data was subjected to one-way ANOVA, and the significance of differences between concentrations was determined using the DMRT test ($P<0.05$). Correlation between TPC and antioxidant assays was done by Pearson's correlation coefficient analysis.

3. Results and discussion

3.1. Total phenolic content (TPC)

Total phenolic content represents the cumulative amount of phenolic compounds present in a given extract or sample. The antioxidant activity of phenolics is attributed to their ability to act as hydrogen donors or electron transfer agents, thereby neutralizing free radicals and reactive oxygen species (ROS). Chemical structure of phenolic compounds, such as the number and position of hydroxyl groups, affects their antioxidant capacity; more hydroxyl groups tend to exhibit higher antioxidant activity. Additionally, the presence of conjugated double bonds and specific functional groups in phenolics contributes to their radical scavenging ability. A higher total phenolic content (TPC) of a plant generally corresponds to greater antioxidant activity, indicating that the phenolic compounds present in the extract contribute to its overall antioxidant capacity [15]. Among five different extracts methanolic extract contained highest TPC (52.64 mg/g GAE) followed by aqueous, chloroform, petroleum ether and benzene extracts (Figure 1). Higher TPC in methanolic extracts exhibit its greater capacity compared to other extracts in scavenging free radicals and management of oxidative stress.



PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract

Figure 1 Determination of Total phenolic content (TPC) of *S. persica* leaves extracts

3.2. DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely used method to evaluate the antioxidant activity of plant extracts. This assay measures the ability of a compound to neutralize the stable free radical DPPH by electron donation, which results in the reduction of the purple DPPH solution to a yellow-colored solution. The degree of discoloration is proportional to the antioxidant activity of the tested compound that can be measured quantitatively by decrease in absorbance at 517 nm [16]. Ascorbic acid was used as a reference compound, and at concentrations ranging from 10 µg/ml to 50 µg/ml, it displayed inhibition rates of 20.84% and 84.80%, respectively. The percentage of antioxidant activity showed a dose-dependent increase for all the extracts tested. Methanolic extract exhibited the highest scavenging potential, followed by the chloroform, aqueous, benzene, and petroleum ether extracts (Table 1). The IC_{50} values of all the extracts were higher compared to ascorbic acid, indicating that they possessed weaker antioxidant activity than the reference compound, as higher concentrations of extracts were required to achieve a similar level of DPPH radical scavenging.

Table 1 DPPH free radical scavenging activity (%) of leaf extracts of *Salvadora persica*

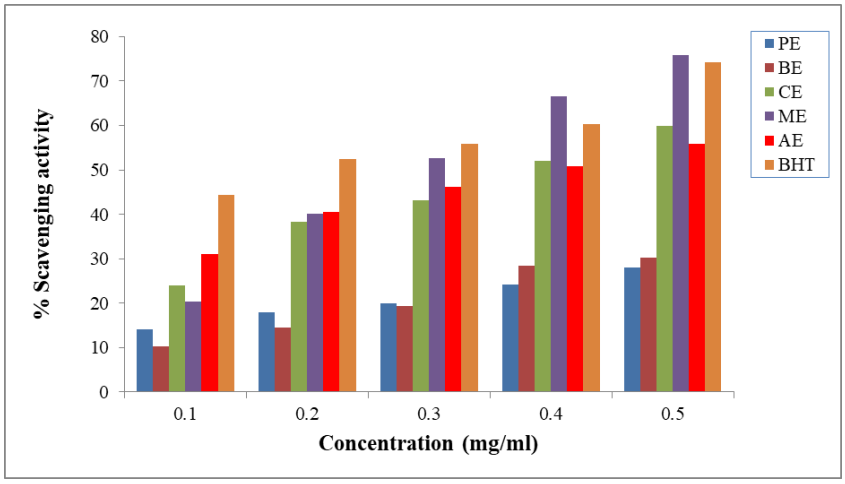
Leaf extract							
Concentration (mg/ml)	**PE**	**BE**	**CE**	**ME**	**AE**	**Conc. (µg/ml) of AS**	**AS**
0.1	14.66±0.49^{e}	12.31±0.45^{e}	34.85±0.32^{e}	30.18±0.92^{e}	10.48±0.29^{e}	10	20.84±0.62^{e}
0.2	17.51±0.43^{d}	21.73±0.22^{d}	38.61±0.28^{d}	45.21±0.43^{d}	21.36±0.47^{d}	20	38.63±0.40^{d}
0.3	20.47±0.16^{c}	30.49±0.35^{c}	42.37±0.12^{c}	66.73±0.18^{c}	26.59±0.25^{c}	30	75.17±0.60^{c}
0.4	24.65±0.73^{a}	34.56±0.92^{b}	45.78±0.62^{b}	76.62±0.11^{b}	32.39±0.78^{b}	40	80.28±0.12^{b}
0.5	25.12±0.91^{a}	38.79±1.04^{a}	56.14±0.75^{a}	80.25±0.42^{a}	40.28±1.13^{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

3.3. Superoxide radical scavenging activity

The superoxide radical scavenging activity assay assesses the ability of plant extracts to neutralize the superoxide anion, which is generated during aerobic respiration and can lead to oxidative damage. The superoxide radical, although relatively weak, acts as an originator to more potent reactive oxygen species, such as H_2O_2, OH radical, peroxynitrite, and singlet oxygen, which can damage biomolecules and initiate lipid peroxidation. Antioxidants present in extract by neutralising superoxide radicals, prevent damage to biomolecules and tissues [17]. In the present study all the extracts and the standard compound BHT exhibited dose-dependent superoxide radical scavenging activity (Figure 2). Compared to the other solvents, the methanolic extract at a concentration of 0.5 mg/ml exhibited highest inhibition of

superoxide radicals (75.76 %). The IC_{50} values of the methanolic extract and the standard compound BHT were 0.27 mg/ml and 0.18 mg/ml, respectively.

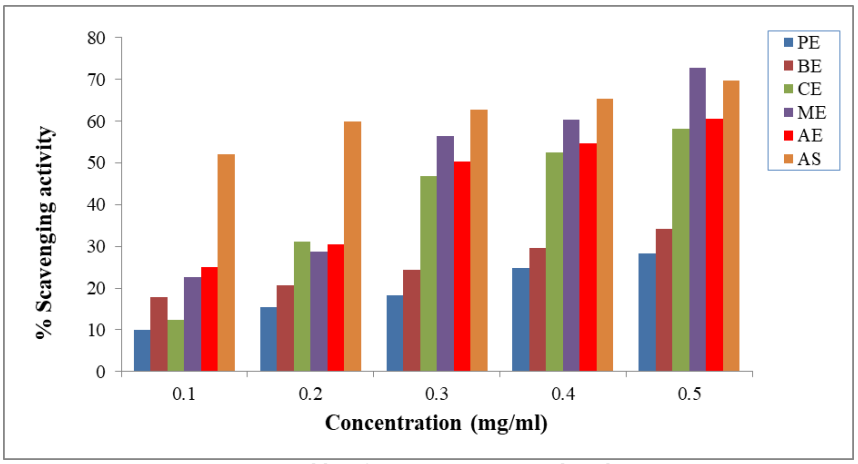


PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; BHT- Butylated hydroxytoluene

Figure 2 Determination of Superoxide radical scavenging activity (%) of *S. persica* leaves extracts

3.4. Hydroxyl radical scavenging activity

Hydroxyl radicals (OH•) are highly reactive and damaging free radicals that can cause oxidative stress and contribute to various diseases. Hydroxyl radicals are generated through various chemical reactions in the presence of metal ions and hydrogen peroxide. Plant extracts having antioxidant compounds can donate hydrogen atoms or electrons to the hydroxyl radicals, thereby neutralizing their reactivity and reducing their damaging affects [18]. Hydroxyl radical scavenging activity of plant extracts is typically expressed as the percentage inhibition of hydroxyl radicals at different concentrations. Higher percentages of inhibition indicate stronger scavenging activity and greater potential for reducing oxidative stress. Different extracts of *Salvadora persica* and the reference compound exhibited hydroxyl radical scavenging activity in dose dependent manner (Figure 3). Methanolic extract possesses highest activity followed by aqueous, chloroform, benzene and petroleum ether extracts. The IC_{50} value of methanolic extract and ascorbic acid was found to be 0.29 mg/ml and 0.18 mg/ml respectively.



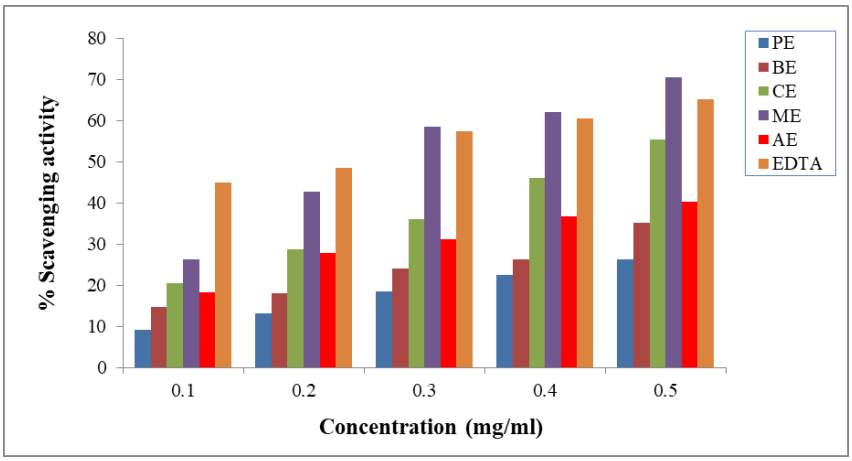
PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; AS-Ascorbic acid

Figure 3 Determination of Hydroxyl radical scavenging activity (%) of *S. persica* leaves extracts

3.5. Metal Chelating activity

Iron metal has the capability to generate free radicals through electron transfer processes. In biological systems, iron metal by reaction with superoxide anions (Haber-Weiss process) or hydrogen peroxide (Fenton reaction) leads to the

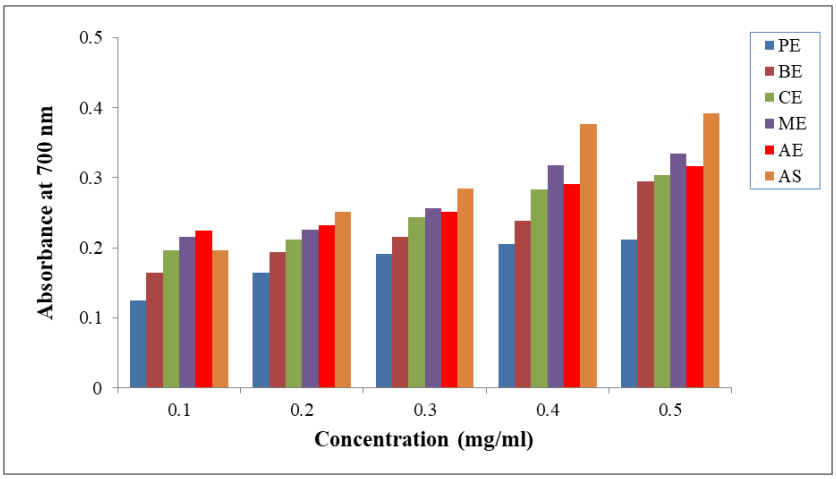
production of highly reactive hydroxyl radicals. Metal chelating activity refers to the ability of plant extracts to bind or chelate metal ions, preventing them from participating in oxidative reactions and reducing their potential to generate harmful free radicals [19]. In the current method, presence of metal chelators in plant extracts by removing ferrous ions disrupted the formation of the Ferrozine-Fe^{2+} complex, causing a dose-dependent decrease in absorbance at 562 nm. The methanolic extract exhibited remarkable metal chelating ability, followed by the chloroform, aqueous, benzene and petroleum ether extracts (Figure 4). The mean IC_{50} value of the methanolic extract was determined to be 0.24 mg/ml, which was comparable to that of the standard EDTA (0.22 mg/ml).



PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; EDTA- Ethylenediamine tetra acetic acid

Figure 4 Determination of Metal chelating activity (%) of *S. persica* leaves extracts

3.6. Reducing power assay



PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; As- Ascorbic acid

Figure 5 Determination of Reducing power assay of *S. persica* leaves extracts

This assay measures the ability of plant extracts to reduce or donate electrons to reactive species, indicating their potential as antioxidants. In the reducing power assay, the plant extract is mixed with a solution containing ferric (Fe^{3+}) salt. The ferric ions can be reduced to ferrous (Fe^{2+}) ions by the antioxidants present in the extract. The reduction is accompanied by a color change, typically from yellow to various shades of blue or green, measured spectrophotometrically at 700. The intensity of the color change is directly proportional to the reducing power or electron-donating capacity of the plant extract [20]. Different plant species and parts may exhibit varying levels of reducing power activity based on their specific phytochemical profiles. Strong reducing power indicates a higher antioxidant activity of the extract. Plant extracts and reference compound exhibited increased reducing power with increasing concentration as depicted in figure 5. Reducing power ability of different extract was in the order methanolic>aqueous>chloroform>benzene>petroleum ether extracts. Reducing power of methanolic extract was nearly comparable to standard ascorbic acid.

3.7. Correlation between antioxidant tests and total phenolic content

Phenolic compounds are known to possess strong antioxidant activity due to their redox properties, which allow them to scavenge and neutralize free radicals by donating electrons. It suggests that the presence of phenolic compounds, such as flavonoids, tannins, and phenolic acids, in plant extracts contributes to their antioxidant properties. These compounds can act as free radical scavengers, metal chelators, and reducing agents, effectively protecting cells and biomolecules from oxidative damage. Additionally, a strong correlation between TPC and antioxidant activity reinforces the potential therapeutic and health-promoting benefits of plant extracts rich in phenolic compounds [21,22]. In the present study the methanolic extract of *S. persica* exhibits a significant correlation between various antioxidant assays and the total phenolic content. The value of correlation coefficients for DPPH, superoxide, hydroxyl, and metal chelating assays were 0.928165, 0.961066, 0.953538 and 0.932116 respectively (Table 2). These values suggest a strong relationship between the antioxidant activity and phenolic compounds present in the extract.

The presence of various phenolic compounds reported in previous studies also suggests that phenolics play a role in the antioxidant activity of *S. persica*. The leaves contain flavonoids and flavonoid glycosides such as Kaempferol, Quercetin, Kaempferol 3-α-L-rhamnosyl-7-β-xylopyranoside, isorhamnetin-3-O-robinobioside, kaempferol-3-O-robinobioside, kaempferol-3-O-rutinoside, isorhamnetin-3-O-β-galactoside, astragalin, narcissin, isorhamnetin-3-O-β-D-glucoside, isorhamnetin-3-(2,6-di-hamnopyranosyl-galactopyranoside), Mauritanian, isorhamnetin-3-O-(2-Glc-rhamnosylrutinoside), and kaempferol 3-O-(2-Glc-rhamnosylrutinoside) [23,24]. From its stem three lignin glycosides and flavonoids rutin and quercetin were reported while Salvadoricine was detected from leaves of *S. persica*. Its roots were found to contain kaempferol, quercetin, rutin, and quercetin glucoside [25,26]. The reports suggest that phenolic acids and flavonoids may play a significant role in the antioxidant activity observed in the methanolic extract of the plant.

Table 2 Determination of correlation between total phenolics and antioxidant activity

Antioxidant activity	**Total phenolic content**	
	r	**R^2**
DPPH assay	0.928165	0.861
Superoxide radical scavenging assay	0.961066	0.923
Hydroxyl radical scavenging assay	0.953538	0.909
Metal chelating assay	0.932116	0.868

*significance at 95% confidence level

4. Conclusion

The results demonstrate remarkable free radical scavenging activity of the methanolic extract derived from leaves of *Salvadora persica*. The antioxidant activity exhibited by the plant can be attributed to the presence of phenolic compounds, which have the ability to donate electrons and neutralize free radicals. These findings provide validation for the therapeutic role of *S. persica* in combating oxidative stress. However, further studies are necessary to isolate and identify the specific bioactive compounds responsible for the antioxidant activity in *Salvadora persica*.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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