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FREE RADICALS SCAVENGING AND INHIBITION OF LIPID PEROXIDATION BY *SALVADORA PERSICA LINN.*

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ABSTRACT

*Salvadora persica (*Salvadoraceae) is widely used in Unani system of medicine. Its root and stem were extracted using solvents of different polarities and explored for *in-vitro* free radical scavenging activity and lipid per-oxidation assay in rat brain homogenate. Preliminary assays of six different extracts of *Salvadora persica*, scavenges 1,1-diphenyl-2-picrylhydrazyl stable free radicals in concentration dependent manner. All studied extracts possess electron donating ability and reduce ferric ion to ferrous in a cell free system at pH-7.4. Also, 250 and 500µg/ml alcoholic and hydro alcoholic extracts reduced the activated lipid per-oxidation in rat brain homogenate. Further, both extracts reduced the lipid per-oxidation to the extended time period (1 to 12 hour after initiation of lipid per-oxidation). Root extracts were found more effective when compared to stem in scavenging 1,1-diphenyl-2-picrylhydrazyl, reducing ferric ion and inhibiting the lipid per-oxidation. Hydro-alcoholic extracts were found more effective in scavenging the 1,1-diphenyl-2-picrylhydrazyl and reducing the ferric ions. The significant correlations exist between extract concentrations and percentage scavenging activity of radicals in all models. These results clearly indicate that *Salvadora persica* is effective free radical scavenger and chain breaking antioxidant.

KEY WORDS: Antioxidant, DPPH, Free Radical Scavenging, Miswak, Salvadora persica L., Toothbrush tree,

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INTRODUCTION

Free radicals may be designated as molecular sharks that damage molecules in cell membranes, mitochondria, DNA and are very unstable, tends to rob electrons from the molecules in the immediate surroundings in order to replace their own losses[1]. Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals but also some non-radical derivatives of oxygen; these include hydrogen peroxide, hypochlorous acid and ozone[2]. Numerous disorders like rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases and gastrointestinal ulcerogenesis are reported as ROS mediated[3,4]. The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of ageing[5,6]. The oxygen intermediates differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission, modification of polypeptides and lipid per-oxidation etc[7]. Screening of compounds which scavenge the free radicals, could lead to promising compounds. Most of the antioxidants used in therapy are derived from natural sources. About 28% drugs approved by the FDA between 1981 and 2002 are either natural products or chemicals derived from them[8,9]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects. Hence the attention has been given to naturally occurring antioxidants. Therefore, identification of effective antioxidants and free radical scavengers from plants origin is an ideal strategy for new drug development. Hence, present study was design to explore the antioxidant and free radical scavenging activity of *Salvadora persica* in a prospective way.

*Salvadora persica (*SP) also known as miswak, toothbrush tree and mustard tree, distributed mainly in tropical and sub tropical Asia. The plant is a large, evergreen profusely branched shrub or a small tree up to 4-6 m tall. SP has been used commonly as toothbrush to strengthen the gums[10]. The fresh root barks and leaves have been used in folk medicine for the treatment of a wide range of medical problems such as cough, asthma, scurvy, piles, rheumatism, leprosy, gonorrohoea, headache and hepatic disorders[11]. Various phytochemical studies on *Salvadora persica* reported the presence of alkaloids salvadorine, trimethylaine and salvadoricine[12]. Essential oil from the roots of SP contain benzyl isothiocyanate (70%) with other components such as α-pinene, camphene, benzaldehyde, β-pinene, myrcene, δ-3-carene, limonene, terpinolene, benzyl nitrile, umbellulone, β-elemene, γ-muurolene, myristicin, β-caryophyllene and longifolene[13]. Proteins,tannins, steroids, flavonoids and terpenoids in *Tagetes erecta* flower extracts which therefore encourages antioxidant studies[14]. Various pharmacological activities on SP including, *in-vivo* antimicrobial activity especially on lactobacilli and streptococcus mutans,[15] with moderate secretory activity significantly high acetyl cholinesterase inhibiting ability, antifertility activity in male rats[16]. The aqueous extract of SP leaves possesses analgesic activity and reduces carrageenan induced inflammation in rat paw[17]. Aqueous and alcoholic extract from leaves of SP reduce elevated urinary oxalate levels and deposition of stone-forming constituents in the kidneys of calculogenic rats[18]. In our previous study we showed root hydroalcoholic extract of SP reduces carrageenan induced inflammation in rats and scavenges ABTS [2,2-azino bis (3-ethyl benzo-thiazoline-6-sulphonic acid)] and superoxide radicals in a cell free system[19]. In present study we aimed to explore the antioxidant potential of different extracts (root and stem) of SP using solvent of different polarity (alcoholic, hydro alcoholic, aqueous).

MATERIALS AND METHODS

Chemicals Details

1, 1-diphenyl-2-picryl hydrazyl (DPPH), o-phenanthroline, ferric chloride, ascorbic acid, and EDTA were procured from Haryana Scientific & Engg. Corp., Rohtak. Root and stem of *Salvadora persica* were collected in March- April month from Kharainti, Meham Teh. Rohtak. District, Haryana (India), the plant material was authenticated by Dr. Ashok Kumar Sharma, M.D. (Dravyaguna Vigyan), Prof. & Head of Department Shri Baba Mastnath Ayurvedic Degree College, Asthal Bohar, Rohtak.

Extraction process

The shed dried plant material (either root or stem) were grinded and powdered material (100 g) was used for extraction. The six different extracts, three from each root and stem were prepared by hot continuous percolation method in a Soxhlet apparatus. Following six extractions were collected separately and dried in vacuo: [1] Root extracted with 99% ethanol (root-AA, yield: 09.15%); [2] Root extracted with 70% ethanol (root-HA, yield: 11.65%); [3] Root extracted water (root-W, yield: 12.25%); [4] Stem extracted with 99% ethanol (stem-AA, yield: 10.05%); [5] Stem extracted with 70% ethanol (stem-HA, yield: 11.45%) and [6] Stem extracted with water (stem-W, yield: 11.95%). These extracts were condensed by re-distillation and dried in vacuum desiccators to obtain a final extract residue.

DPPH radical scavenging activity[20]

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability by using stable free radical DPPH (100 µM) in ethanol. The equal volumes of different extracts were incubated with DPPH in graded concentrations of (10 to 500 µg/ml) for 20 min. at room temperature and by using a digital UV/VIS spectrophotometer (model 371E) absorbance was recorded at 517 nm. The experiment was performed in triplicate. Scavenging ability was measured by decreased DPPH absorbance in test sample when compared to standard DPPH solution. Results were expressed as percentage inhibition of DPPH by comparing with blank.

$$\%\text{age Scavenging} = \frac{\text{Absorbance of control sample- Absorbance of test sample}}{\text{Absorbance of control sample}} \times 100$$

Reduction of ferric-ions[21]

The electron donating capability (reducing ability) was studied by ferric chloride reduction in cell free system. The reaction mixture contained 1.0 ml of phosphate buffer (pH 7.4), 100 µM ferric chloride and 0.5 ml of test compounds of different concentrations (10-500 µg/ml). After 3 min. of incubation, EDTA (100 µM) and ortho-phenanthroline (300 µM) were added, reaction was allowed for 10 min at room temperature and by using a digital UV/VIS spectrophotometer (model 371E) absorbance was recorded at 510 nm. Ascorbic acid was used as standard as equivalent to 100% reduction of ferric ions, comparative reduction of Fe^{3+} by *Salvadora persica* extract was calculated.

Percentage reduction of Ferric ion by test compound:

$$\%\text{age Reduction} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of ascorbic acid}} \times 100$$

Lipid per-oxidation[22]

The brain isolated from healthy albino rat (180-200 gm) was used as lipid source. Brain homogenate (10% w/v) was prepared in 150 mM KCl and centrifuged at 800 g for 10 minutes. The supernatant was collected and used immediately to study *in-vitro* lipid per-oxidation. Briefly, the reaction mixture contained 0.3 ml of brain homogenate, KCl (100 µM), ascorbic acid (100 µM), ferric-chloride (100 µM), 0.5 ml of graded concentrations of extracts and final volume was made with buffer. After incubating at room temperature for 20 minutes, 1.0 ml of thiobarbituric acid-trichloroacetic acid (TBA-TCA) reagent was added. The resulted mixtures were heated at 80^0C for 20 minutes, cool and centrifuged for 10 minutes at 1000 rpm and by using a digital UV/VIS spectrophotometer (model 371E) recorded the absorbance at 532 nm. Control and standard (curcumin 10 µM) were carried out at similar manner. Percentage inhibition of thiobarbituric acid reactive substance (TBARS) formation by extract/standard drug (curcumin) was calculated by comparing with control. All experiments were carried out in triplicate and results are the means of one such individual experiment. Percentage inhibition of lipid per-oxidation by test compound:

$$\%\text{age inhibition} = \frac{\text{Absorbance of control sample} - \text{Absorbance of test sample}}{\text{Absorbance of control sample}} \times 100$$

RESULTS

DPPH radical scavenging activity

Root and stem extracts of SP scavenged the DPPH stable free radicals in a concentration dependent manner (10-500 µg/ml). All the extracts showed maximum scavenging activity at 400µg/ml, beyond this biphasic effect was observed with reduced scavenging activity. The root-HA showed maximum activity (88.15%), root-AA (73.0%), while root-W showed a maximum activity (71.85%) at concentration 400 µg/ml of root extracts and stem extracts also be in same manner. The stem-HA showed maximum activity (87.72%), stem-AA (64.0%), while stem-W showed a maximum activity (53.0%) at concentration 400 µg/ml. In all the cases biphasic effect was observed above concentration 400 µg/ml. The interaction of *Salvadora persica* root and stem extracts with DPPH radicals presented in Tab. and Fig.1a.

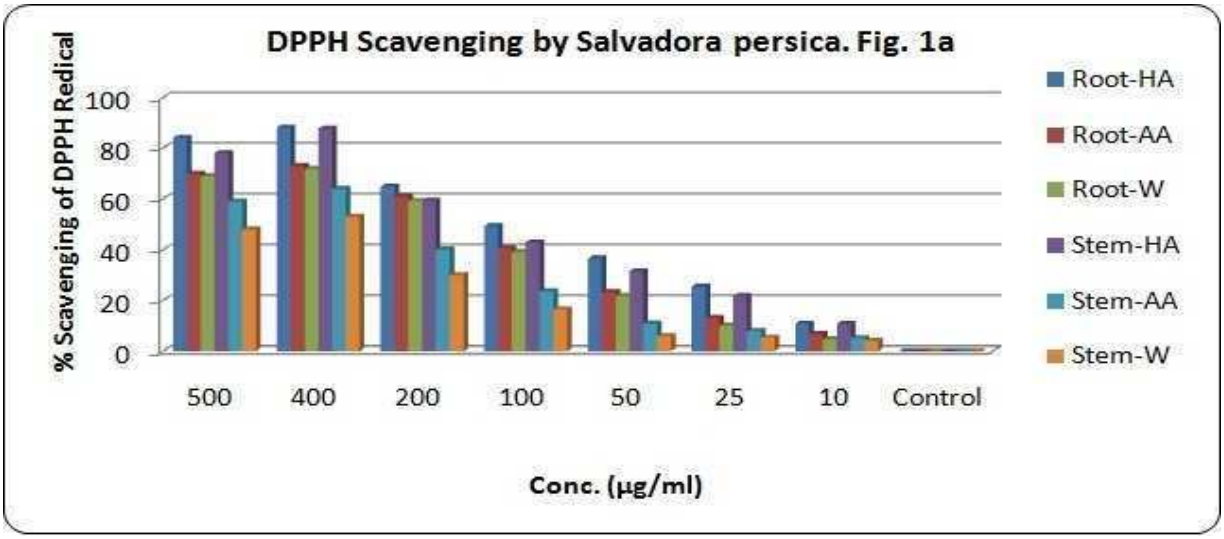
Table 1a

Interaction of Salvadora persica[a] root extracts with DPPH Radical.

Drug Conc .ug/ml	Root- HA		Root-AA		Root-W		Stem-HA		Stem-AA		Stem-W	
	Ab.	%Sca.	Ab.	%Sca.	Ab.	%Sca.	Ab.	%Sca.	Ab.	%Sca.	Ab.	%Sca.
500	.107±.003	84.14	.204±.005	69.91	.210±.003	69.00	.148±.002	78.10	.277±.003	59.00	.352±.003	48.00
400	.080±.005	88.15	.183±.004	73.00	.190±.001	71.85	.083±.001	87.72	.246±.003	64.00	.322±.003	53.00
200	.237±.007	64.90	.263±.003	61.21	.275±.003	59.25	.275±.004	59.31	.406±.001	40.11	.475±.003	29.94
100	.342±.003	49.35	.403±.004	40.56	.411±.008	39.10	.386±.003	42.90	.518±.006	23.60	.566±.001	16.52
50	.428±.005	36.60	.520±.008	23.30	.528±.006	21.80	.463±.006	31.50	.604±.003	10.91	.638±.008	5.90
25	.503±.008	25.50	.599±.002	13.13	.607±.004	10.10	.529±.009	21.75	.624±.003	7.96	.642±.005	5.30
10	.602±.001	10.80	.632±.004	6.78	.643±.005	4.75	.603±.006	10.79	.643±.004	5.16	.650±.003	4.13
Control	.675±.004	00.00	.678±.002	00.00	.675±.006	00.00	.676±.004	00.00	.678±.002	00.00	.678±.002	00.00

[a] All values are mean (n = 3). Data are expressed as Mean ± S.D.

Figure1a
Interaction of Salvadora persica root extracts with DPPH Radical.



Reduction of ferric ion

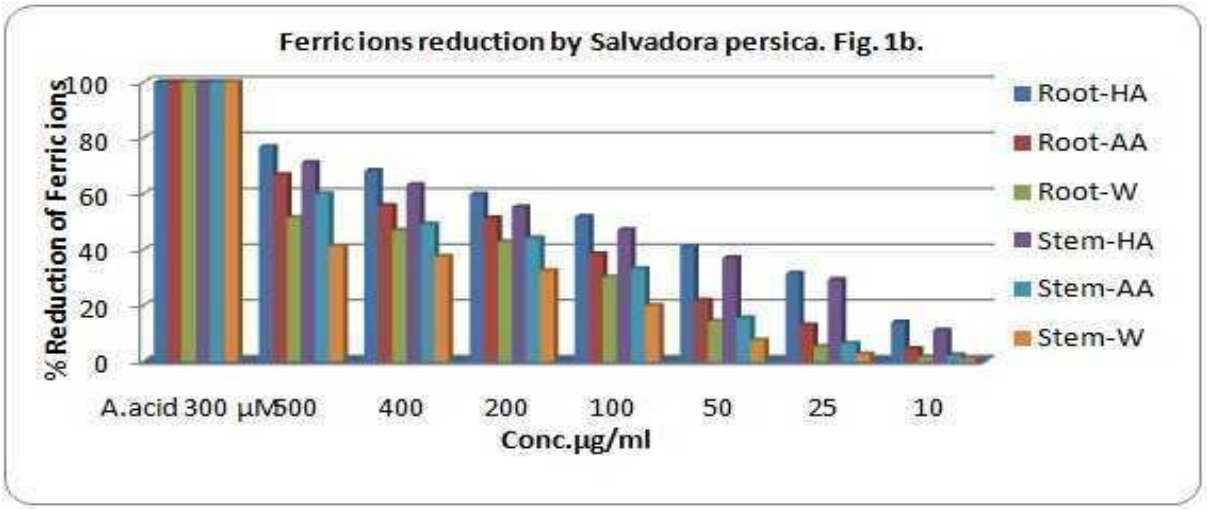
Reduction of ferric ion i.e. Fe^{2+}- Fe^{3+} couple is known to be involved in various free radical reactions. All of these extracts of root-HA, root-AA and stem-HA reduce Fe^{3+} in to Fe^{2+}at pH 7.4. In this study, reduction of Fe^{3+} into Fe^{2+} by ascorbate was taken as 100%. The root-HA and stem-HA (500 µg/ml) caused a significant reduction of Fe^{3+} to (76.83%) and (71.08%) respectively, while the root-AA (500 µg/ml) produced (66.75%). The stem-AA and root-W (500 µg/ml) caused a significant reduction of (60%) and (51.36%) respectively, while stem-W (500 µg/ml) produced a reduction of 41.29% as shown in Tab. and Fig. 1b.

Table 1b
Reduction of ferric ions by Salvadora persica[a] root extracts.

Drug conc. ug/ml	Root- HA		Root-AA		Root-W		Stem-HA		Stem-AA		Stem-W	
	Ab.	%Red.	Ab.	%Red.	Ab.	%Red.	Ab.	%Red.	Ab.	%Red.	Ab.	%Red.
Asc.A.Std.300 µM	.410±.002	100	.406±.003	100	.405±.003	100	.408±.001	100	.410±.003	100	.402±.003	100
500	.315±.001	76.83	.271±.002	66.75	.208±.005	51.36	.290±.002	71.08	.246±.005	60.00	.166±.004	41.29
400	.280±.005	68.29	.226±.005	55.67	.190±.004	46.91	.258±.001	63.24	.201±.007	49.02	.151±.004	37.56
200	.245±.004	59.76	.208±.002	51.23	.173±.005	42.72	.225±.002	55.15	.181±.004	44.15	.130±.004	32.34
100	.212±.003	51.71	.156±.002	38.42	.122±.004	30.12	.192±.002	47.06	.136±.004	33.17	.080±.005	19.90
50	.168±.002	40.98	.088±.004	21.67	.058±.003	14.32	.151±.001	73.00	.063±.003	15.37	.030±.003	07.46
25	.129±.001	31.46	.052±.002	12.81	.021±.003	5.19	.119±.003	29.17	.026±.003	6.34	.010±.001	2.49
10	.057±.001	13.90	.018±.001	4.43	.006±.001	1.48	.045±.002	11.03	.009±.002	2.20	.003±.001	.75

[a] All values are mean (n = 3). Data are expressed as Mean ± S.D.

Figure1b
Reduction of ferric ions by Salvadora persica root extracts.



Lipid peroxidation in rat brain homogenate

The amount of thiobarbituric acid reactive substance (TBARS) was calculated and percentage inhibition of TBARS formed was compared with control and standard drug (curcumin). The root-AA and root-HA (500µg/ml) inhibited 64.35% and 45.07% formation of TBARS when compared to control respectively. The stem-AA and stem-HA (500µg/ml) inhibited 64.04% and 27.88% formation of TBARS respectively. Water extracts of root or stem doesn't inhibit the TBARS formation. Alcoholic and hydro-alcoholic extracts (root-AA, root-HA, stem-AA, and stem-HA) significantly inhibited the lipid per-oxidation at concentrations 250 and 500 µg/ml. Further, alcoholic and hydro-alcoholic extracts (root-AA, root-HA, stem-AA and stem-HA) inhibited the formation of lipid per-oxidation for a prolong time after initiation of lipid per-oxidation. Root-AA reduced the formation of TBARS from 64.25 to 59.79 % at 1hour and 12 hours post-initiation to lipid per-oxidation as shown in Tab. and Fig. 1c & 1d.

Table 1c
Lipid peroxidation inhibition by Salvadora persica[a] root extracts.

Drug conc. ug/ml	Root- AA		Root-HA		Root-W		Stem-AA		Stem-HA		Stem-W	
	Ab.	% Inh.	Ab.	% Inh.	Ab.	%Inh.	Ab.	% Inh.	Ab.	%Inh.	Ab.	% Inh.
500	.344±.004	64.35	.530±.003	45.07	.941±.006	2.48	.347±.003	64.04	.696±.005	27.88	.945±.005	2.07
Control	.965±.006	00.00	.965±.006	00.00	.965±.006	00.00	.965±.006	00.00	.965±.006	00.00	.965±.006	00.00
250	.399±.007	54.92	.507±.002	42.71	.868±.006	1.92	.403±.007	54.46	.679±.006	23.28	.870±.005	1.69
Control	.885±.005	00.00	.885±.005	00.00	.885±.005	00.00	.885±.005	00.00	.885±.005	00.00	.885±.005	00.00

[a] ***All values are mean (n = 3). Data are expressed as Mean ± S.D.***

Figure1c

Lipid peroxidation inhibition by Salvadora persica root extracts.

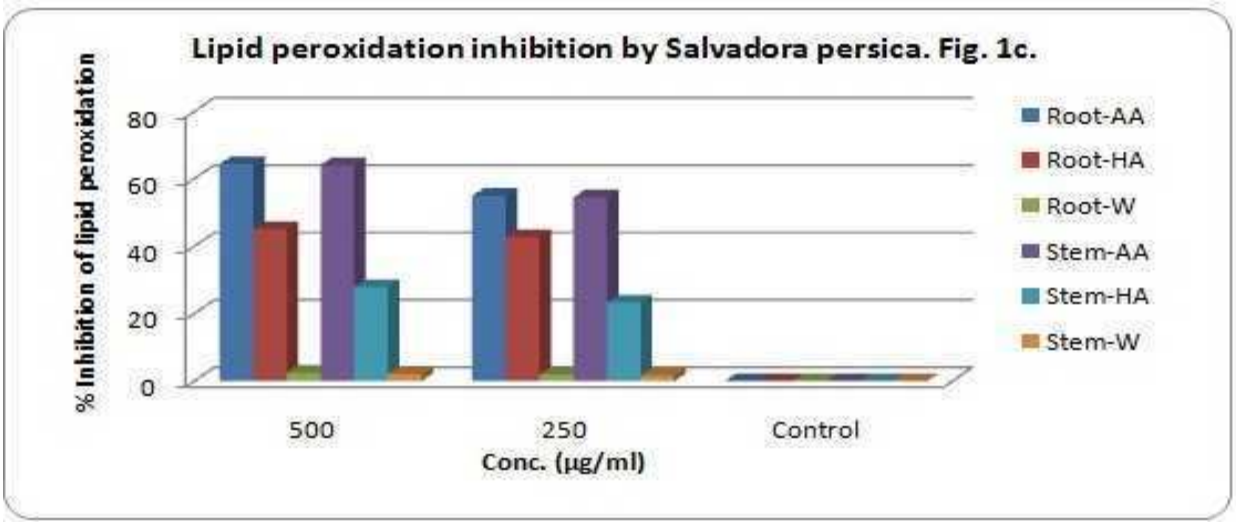


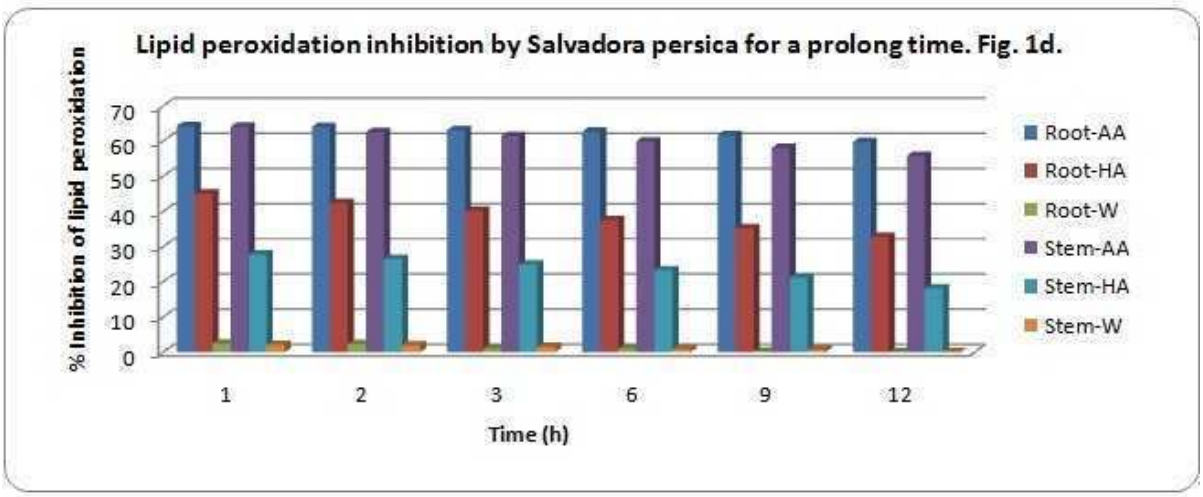
Table 1d

Lipid peroxidation inhibition by Salvadora persica[a] root extracts for a prolong time.

Time (h)	Root- AA		Root-HA		Root-W		Stem-AA		Stem-HA		Stem-W	
	Ab.	%Inh.	Ab.	%Inh.	Ab.	%Inh.	Ab.	%Inh.	Ab.	%Inh.	Ab.	%Inh.
1	.345	64.25	.530	45.07	.941	2.48	.346	64.15	.697	27.77	.945	2.07
2	.357	64.05	.572	42.40	.970	2.32	.372	62.54	.730	26.49	.973	2.01
3	.365	63.13	.592	40.20	.980	1.01	.382	61.41	.743	24.95	.975	1.51
6	.372	62.76	.624	37.54	.989	1.01	.400	59.96	.768	23.33	.990	00.90
9	.382	61.69	.646	35.21	.995	00.20	.418	58.07	.785	21.26	.990	00.70
12	.386	59.79	.645	32.81	.996	00.00	.424	55.83	.786	18.13	.990	00.00

Figure1d

Lipid peroxidation inhibition by Salvadora persica root extracts for a prolong time.



DISCUSSION

Free radicals have been a subject of significant interest among scientists in the past decade and their possible role in human diseases has gained importance now in days[23,24] . Antioxidants neutralize toxin and volatile free radicals that are defined as atoms or groups of atoms having an unpaired electron[6]. These also include related reactive oxygen species (ROS) that leads to free radical generation, causes the cascading chain reaction in biological system. In a normal, healthy organism or human body, the generation of pro-oxidants in the form of ROS is effectively kept in check by various levels of antioxidant defense. Antioxidants present in various dietary supplements offered their beneficial effects by neutralizing these ROS during various disease conditions. Lipids, proteins and DNA are all susceptible to attack by free radicals and cellular damage induced by oxidative stress has been implicated in the etiology of numerous diseases.

DPPH radical is widely used as a model to investigate the scavenging potential of several natural compounds such as phenolic and anthocyanins or crude extract of plants[25]. The ability of stem and root extracts of SP to reduce DPPH radicals, supports its free radical scavenging activity. Our study indicates the proton donating property may be responsible for free radical scavenging activity of SP. Antioxidant compounds for example, sesamol, gallic acid poly-phenols reduce the Fe^{3+} to Fe^{2+} and are considered as chain breaking antioxidant for their proton donating activity[26]. SP reduces ferric ion at pH 7.4 which indicates its proton donating ability and supports its free radical scavenging activity. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. It was therefore to be expected that SP has potent antioxidant and radical scavenging ability. This activity is believed to be mainly due to their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Hence present study revealed antioxidant property of SP root and stem extracts.

Lipid per-oxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products[27]. TBARS, one of the products of lipid per-oxidation, has been studied widely as an index of lipid per-oxidation and as a marker of oxidative stress[28]. We observed incubation of SP extracts with brain homogenate reduced the lipid per-oxidation at great extent which indicates the protecting effect of SP against lipid per-oxidation and TBARS formation. It has been proved that free radicals initiated lipid per oxidation play an important role in the pathogenesis of certain diseases and ageing[5]. Reducing the formation of TBARS improves many pathological conditions. Hence SP could be a promising plant to neutralizing free radicals and reducing the lipid per-oxidation in many pathological conditions. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases. Numerous plant constituents have shown free radical scavenging or antioxidant activity.

CONCLUSIONS

Our study showed that hydro alcoholic root extract of Salvadora persica is most effective antioxidant when compared to other extracts in various *in vitro* assay systems. SP has been reported to contain indole alkaloids, flavonoids, the sulphur-containing compounds, tropaedoin, triterpenes and phytosterols,[29,30] of these compounds, the alkaloids and flavonoids are probably responsible for its free radical and reducing property observed in this study. As compared to previous reports, our study showed that *Salvadora persica* exerted a potent effect. Further studies are required to better understand these compounds and their

effects on cellular function. Antioxidant properties of *Salvadora persica* could be beneficial in pathological condition involving oxidative stress.

REFERENCES

[1] Halliwell B. The wanderings of a free radical. Free Radic Biol Med. 46: 531-542 (2009).

[2] Toyokuni S. Reactive oxygen species-induced molecular damage and its application in pathology. Pathol. Int. 49: 91-102 (1999).

[3] Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. Trends in oxidative ageing theories. Free Radic. Biol. Med. 43**:** 477-503 (2007)

[4] Ischiropoulos H, al-Mehdi AB, Fisher AB. Reactive species in ischemic rat lung injury**:** contribution of peroxynitrite. Am. J. Physiol. 269: L158-L164 (1995).

[5] McCord J M. Oxygen-derived free radicals in post ischemic tissue injury. N. Engl. J. Med. 312: 159-163 (1985).

[6] Finkel T, Holbrook, N.J. Oxidants, oxidative stress and the biology of ageing. Natur**e** 408: 239-247 (2000).

[7] Pryor WA, Porter NA. Suggested mechanisms for the production of 4-hydroxy-2-nonenal from the autoxidation of polyunsaturated fatty acids. Free Radic. Biol. Med. 8: 541-543 (1990).

[8] Clardy J, Walsh C. Lessons from natural molecules. Nature 432: 829-837 (2004).

[9] Schmidt BM, Ribnicky DM, Lipsky PE, Raskin I. Revisiting the ancient concept of botanical therapeutics. Nat. Chem. Biol. 3: 360-366 (2007).

[10] Sofrata AH, Claesson RL, Lingstrom PK, Gustafsson AK. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. J. Periodontol. 79: 1474 -1479 (2008).

[11] Kirtikar KR, Basu BD, Blatter E. Indian Medicinal Plants: Periodical Experts, Vol.-2 Delhi. 1537-1539 (1975).

[12] Malik S, Ahmad SS, Haider SI, Muzaffar A. Salvadoricine: A new indole alkaloid from the leaves of *Salvadora Persica*. Tetrahedron Letters 28: 163-164 (1987).

[13] Bader A, Flamini G. The composition of the root oil of *Salvadora persica Linn*. J. Essent. Oil Res. 14: 128-129 (2002).

[14] Basavaraj V Chivde, Karnakumar V Biradar, Rajabhau Shiramane, Kamshetty V Manoj. In-vitro antioxidant activity studies on the flower of Tagetes Erectal L. Int. J. Pharma and Bio Sci. 2(3): 223-229 (2011).

[15] Almas K, Al-Zeid Z. The immediate antimicrobial effect of a toothbrush and miswak on cariogenic bacteria: A clinical study. J. Contemp. Dent. Pract. 5: 105-114 (2002).

[16] Darmani H, Al-Hiyasat AS, Elbetieha AM, Alkofahi A. The effect of an extract of *Salvadora persica* (miswak, chewing stick) on fertility of male and female mice. Phytomedicine. 10: 63-65 (2003).

[17] Parihar VK, Ganesh S, Kumar S, Mehrotra A, Reddy M, Sreedhara Pai KR, Unnikrishnan MK, Rao MC. Analgesic, anti-inflammatory and *in-vitro* antioxidant activities of *Salvadora persica Linn*. XXXIX Annual Conference of Indian pharmacological Society, held at NIPER Mohali Chandigarh, Nov. 1-3. 2007; PP-14.

[18] Geetha K, Manavalan R, Venkappayya D. Control of urinary risk factors of stone formation by *Salvadora persica* in experimental hyperoxaluria. Methods Find Exp Clin. Pharmacol. 32: 623-629 (2010).

[19] Hooda MS, Parihar VK, Mor V. Antioxidant and anti-inflammatory activity of crude root extract of *Salvadora Persica. Linn.* Indian J. Pharm. 38: AA-6:S18-19 (2006).

[20] Narla RS, Rao MNA. Scavenging of free radicals and inhibition of lipid

peroxidation by 3-phenyl syndone, J, Pharm. Pharmacol. 47: 623-625. (1995).

[21] Parihar VK, Dhawan J, Kumar S, Manjula SN, Subramanian G, Unnikrishnan MK, Rao MC. Free radical scavenging and radioprotective activity of dehydrozingerone against whole body gamma radiation in Swiss albino mice. Chemico biological Interactions. 170: 49-58 (2007).

[22] Rajakumar DV, Rao MNA. Antioxidant properties of dehydrozingerone and curcumin in rat brain homogenates. Molecular and cellular Biochemistry 140: 73-79 (1994).

[23] Maxwell SR. Prospects for the use of antioxidant therapies. Drugs 49: 345-361 (1995).

[24] Jovanovic SV, Simic MG. Antioxidants in nutrition. Ann. N. Y. Acad. Sci. 899: 326-634 (2000).

[25] Veerapur VP, Prabhakar KR, Parihar VK, Kandadi MR, Ramakrishana S, Mishra B, Satish Rao SB, Srinivasan KK, Priyadarsini KI, Unnikrishnan MK. *Ficus racemosa* stem bark extract: a potent antioxidant and a probable natural radioprotector. Evid. Based Complement Alternat. Med. 6: 317-324 (2009).

[26] Parihar VK, Prabhakar KR, Veerapur VP, Sudheer KM, Rosi Reddy Y, Joshi R, Unnikrishnan MK, Rao MC. Effect of sesamol on radiation induced cytotoxicity in Swiss albino mice. Mutation Research 611: 1-9 (2006).

[27] Pratt DA, Tallman KA, Porter NA. Free radical oxidation of polyunsaturated lipids: New mechanistic insights and the development of peroxyl radical clocks. Acc. Chem. Res. 44: 458-467(2011).

[28] Prabhakar KR, Veerapur VP, Parihar VK, Priyadarsini KI, Rao BSS, Unnikrishnan MK. Evaluation and optimization of radioprotective activity of *Coronopus didymus Linn*. in gamma-irradiated mice. Int. J. Radiat. Biol. 82: 525-536 (2006).

[29] Kamel MS, Ohtani K, Assaf MH, Kasai R, El-Shanawany MA, Yamasaki K. Lignan glycosides from stems of *Salvadora persica*. Phytochemistry 31: 2469-2471 (1992).

[30] Akhtar MS, Ajmal M. Significance of chewing-sticks (miswak) in oral hygiene from a pharmacological view- point. J. Pak Med. Assoc. 31: 89-95 (1981).