Asian Journal of Chemistry Vol. 19, No. 6 (2007), 4638-4644

Free Radical Scavenging Activity of *Salvadora persica* Linn.

SAAHIL ARORA† and D. KAUSHIK*
University Institute of Pharmaceutical Science, Kurukshetra University
Kurukshetra-139 116, India
E-mail: tony_just4u@msn.com

This study comprises of *in vitro* antioxidant activity on hydro alcoholic (SPHA) and aqueous (SPW) extracts from shade dried roots of *Salvadora persica* L. by various models. Both SPHA and SPW extracts showed significantly high scavenging ability on 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical (87.60 and 72.29 % at 400 µg/mL), 2,2-azino *bis*(3-ethyl benzothiazoline-6-sulphonic acid (ABTS) radical (87.50 and 78.45 % at 500 µg/mL), superoxide nitroblue-tetrazoliun (NBT) radical (54.39 and 42.25 % at 600 µg/mL) and reduction of ferric ion solution (73.10 and 64.90 % at 500 µg/mL). Result indicates the powerful electron donating and radical scavenging activity of *S. persica* L. Hence both the extracts can be considered as effective chain breaking *in vivo* antioxidants.

Key Words: *Salvadora persica* L., Toothbrush tree, Pilu, Miswak, Salvadoraceae, Alkaloids, Antioxidants.

INTRODUCTION

Salvadora persica Linn.; also known as tooth brush tree or mustard tree is a species of Salvadoraceae family, distributed mainly in tropical and sub-tropical Asia. Plant is a large, evergreen profusely branched, shrub or a small tree up to 2-6 m tall[1,2]. *S. persica* has been used commonly as toothbrush to strengthen the gums. The fresh root-bark 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, headaches and hepatic disorders[3]. Various phytochemical studies on *S. persica* reported the presence of alkaloids-salvadorine[4], trimethylamine, salvadoricine[5], *etc.*, flavanoids (quercitin)[6] and volatile oil such as lauric acid, myristic acid, palmitic acid, sterculic acid[7] and β-caryophyllene[8].

Various pharmacological activities on *S. persica* include antimicrobial activity *in vivo* especially on *lactobacilli* and *Streptococcus mutans*[9], anti-ulcer activity in cold stress-induced ulcers with moderate secretary activity[10], protection against PTZ-induced convulsions[11], significantly high ACE inhibiting ability[12], antiplasmodial activity on *Plasmodium falciparum* NF54

†Department of Pharmaceutical Chemistry, Shri Baba Mastnath Institute of Pharmaceutical Sciences & Research, Asthal Bohar, Rohtak-124 021, India.

strain[13], hypolipidemic activity[14], mild hypoglycemic activity in glucose-loaded mice[15] and antifertility activity in male rats[16].

The present study was undertaken to explore the ascorbic acid content of plant towards effective and safe antioxidant and to evaluate *in vitro* antioxidant activity on hydro alcoholic (SPHA) and aqueous (SPW) extracts of *Salvadora persica* L. by various models.

EXPERIMENTAL

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical [100 μM; 3.9 mg in 100 mL ethanol], 2,2-azino *bis*(3-ethyl benzothiazoline-6-sulphonic acid (ABTS) radical solution [2 mM; 0.0548 g in 50 mL distilled water], phosphate buffer (pH 7.4) 20 mM, potassium persulphate solution [70 mM; 0.0189 g in 1 mL distilled water], *o*-phenanthroline 3 mM, ferric chloride 1 mM, ascorbic acid 1 mM, EDTA 1 mM, nitrobluetetrazolium (NBT) in phosphate buffer (pH 7.4), alkaline-DMSO containing 1% water and 5mM NaOH and double distilled water. The roots of *S. persica* were obtained from New Pratap Nursery and Seed store, Dehradun and specimen was verified by Dr. A.K. Sharma, Botanist, FRI, Dehradun, India.

Extraction: The hydro alcoholic extract of *S. persica* was prepared by boiling, about 200 g of the powdered, shade-dried root in 70 % ethanol (1 L), maintaining the temp 70°C. Aqueous extract was prepared by boiling, about 200 g of the powdered, shade-dried root in double distilled water (1 L). Both the extracts were filtered before the study.

Radical scavenging activity on DPPH radical: Free radical scavenging activity of *S. persica* ethanolic extract was determined using the DPPH method[17]. An aliquot (0.5 mL) of ethanolic solution containing test drug at various concentrations (10 μg to 500 μg/mL) was added to equal volume of freshly prepared ethanolic DPPH (100 μM) solution; after incubation for 20 min at room temperature, absorbance was measured at 517 nm by a spectrophotometer. The antioxidant assay was carried out in triplicate and the readings were averaged. The scavenging activity was measured as the decrease in absorbance of the samples *vs.* DPPH standard solution. Results were expressed as percentage inhibition of the DPPH by comparing with blank and mean inhibiting concentration (IC_{50}) of the DPPH.

IC_{50} parameter is defined as the concentration, in μg/mL of the substrate required to inhibit DPPH radical formation by 50 % and it was calculated by using the Litchfield and Wilcoxon test.

Radical scavenging activity on ABTS radical: Free radical scavenging activity of *S. persica* ethanolic extract was determined using the ABTS method[18]. To study the antioxidant activity, the final reaction mixture contained 0.3 mL of ABTS radical, test compound at various concentrations from 10 μg to 500 μg/mL in ethanol, phosphate buffer (pH 7.4, 20 mM;

added to obtain final volume of 2.5 mL). After 3 min, the absorbance was measured at 734 nm. The experiment was carried out in triplicate and the readings were averaged.

The scavenging activity was measured as the decrease in absorbance of the samples *vs.* ABTS standard solution. Results were expressed as percentage inhibition of the ABTS by comparing with blank and mean inhibiting concentration (IC_{50}) of the ABTS.

Superoxide scavenging activity: Superoxide scavenging activity of *S. persica* ethanolic extract was determined using the NBT reduction method[17,19]. To study the antioxidant activity, the final reaction mixture contained 0.5 mL of various concentrations of drug (50 µg to 600 µg/mL) in ethanol, 0.2 mL of NBT and 1 mL of alkaline DMSO. After 10 min, absorbance was measured at 560 nm. The experiment was carried out in triplicate and the readings were averaged.

The scavenging activity was measured as the decrease in absorbance of the samples *vs.* NBT standard solution. Results were expressed as percentage inhibition of the NBT by comparing with blank and mean inhibiting concentration (IC_{50}) of the NBT.

Reduction of ferric ions: To study the antioxidant activity by reduction of ferric ion[20], the final reaction mixture contained *o*-phenanthroline (0.9 mL), ferric chloride (0.3 mL), EDTA (0.3 mL), various concentrations of test compound ranging from 10 to 500 µg/mL dissolved in ethanol (0.5 mL) and phosphate buffer (pH 7.40, 20 mM; added to obtain final volume of 3.0 mL). After incubation for 10 min at ambient temperature, the absorbance was recorded at 510 nm against blank. In standard, ascorbic acid was added instead of test drugs in equivolume. The experiment was carried out in triplicate and the readings were averaged.

The reducing activity was measured as the decrease in absorbance of the samples *vs.* standard ascorbic acid solution. Results were expressed as percentage inhibition of the ferric solution by considering absorbance obtained from ascorbic acid standard solution as equivalent to 100 % reduction of all ferric ions and mean inhibiting concentration (IC_{50}) of the ferric ion reduction.

RESULTS AND DISCUSSION

Scavenging of DPPH free radical: SPHA and SPW at conc. range of 10-500 µg/mL scavenge the DPPH radical in a conc. dependent manner. Both the extracts at conc. 400 µg/mL showed maximum activity but at different extent. SPHA showed maximum scavenging activity (87.6 %) while SPW showed a maximum activity (72.29 %) at conc. 400 µg/mL. In both the case biphasic effect was observed at the conc. above 400 µg/mL. The IC_{50} of SPHA was found (100.60 µg/mL), while it was (150.00 µg/mL) for SPW. IC_{50} concentration of SPHA and SPW neutralized the 50 % of DPPH in 32.50 and 75.0 s, respectively (Table-1, Fig. 1).

TABLE -1
IC_{50} VALUE OF THE *S. persica* EXTRACT IN DIFFERENT MODELS

Test models	SPHA (µg/mL)	SPW (µg/mL)
DPPH Scavenging	100.60	150.00
ABTS Scavenging	143.78	175.87
Ferric ion Reduction	100.65	198.90
Superoxide Radical	553.09	Not scavenged

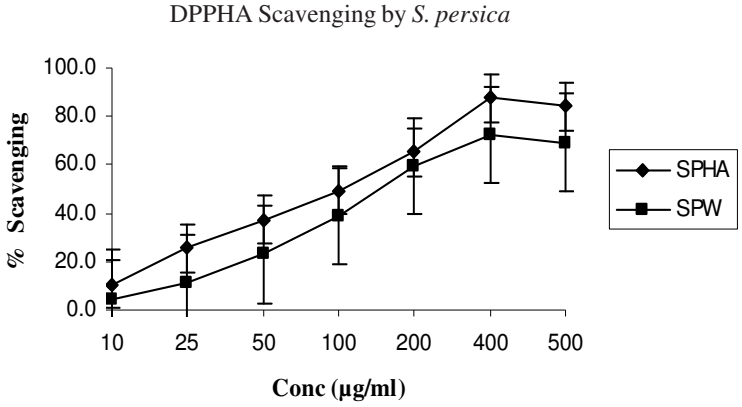

Fig. 1. Interaction of *Salvadora persica* extract with DPPH radical
Final volume 1.0 mL contain 0.5 mL of DPPH (0.1 mM) and 05 mL of different concentration of test drugs, absorbance was recorded at 517 nm after 20 min of incubation.

Scavenging of ABTS free radical: Both the extracts at the conc. range of 10-500 µg/mL scavenges the ABTS radical in a conc. dependent manner. Maximum activity was observed at the conc. of 500 µg/mL in both the extracts, SPHA showed a maximum scavenging 87.5 % while SPW showed 78.45 % scavenging. The IC_{50} of SPHA was found (143.78 µg/mL), while it was (175.87 µg/mL) for SPW (Table-1, Fig. 2).

Superoxide scavenging: SPHA and SPW scavenge the superoxide radical in a conc. dependent manner at conc. range of 50-600 µg/mL. Both the extracts showed maximum scavenging at conc. of 600 µg/mL. SPHA showed maximum scavenging 54.39 % while SPW showed maximum scavenging 45.25 % . No significant activity was observed at conc. below 50 µg/mL. The IC_{50} of SPHA was found (553.09 µg/mL). (Table-1, Fig. 3).

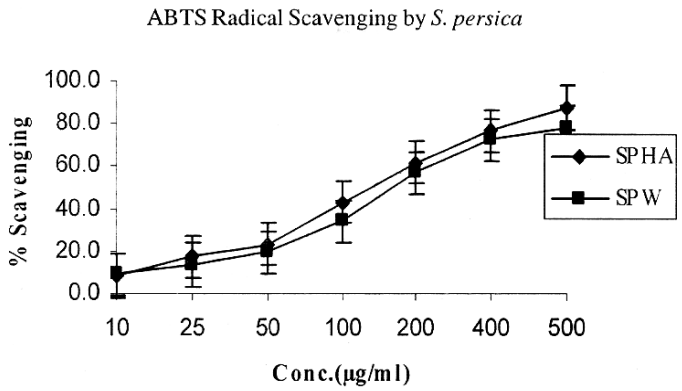

Fig. 2. Interaction of *Salvadora persica* with ABTS radical
Final volume, 2.5 mL of the reaction mixture contained 0.3 mL of ABTS radical 0.5mL of extract in different concentrations and 1.7 mL phosphate buffer pH 7.4. Absorbance was recorded at 734 nm immediate after additions of ABTS radical

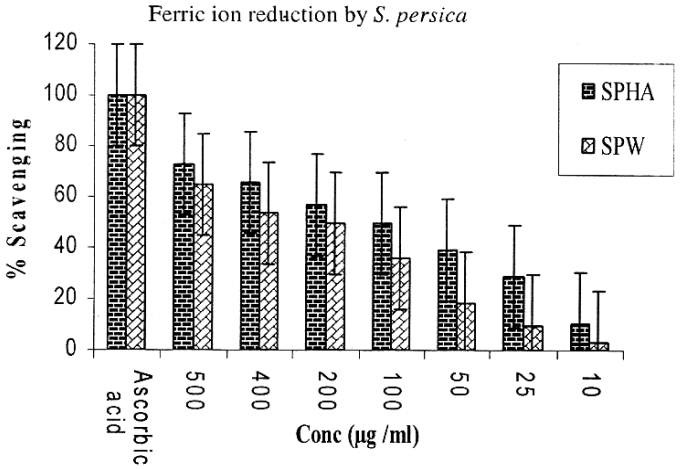

Fig. 3. Reduction of ferric ions by *Salvadora persica* extract
Final volume (3 mL) of reaction mixture containing 0.9 mL *o*-phenanthroline, 0.3 mL of 1 mM ferric chloride, 0.3 mL of EDTA (1 mM), 0.5 mL of various conc. of extract, 1 mL of phosphate buffer pH 7.4 (20 mM) was incubated for 10 min at ambient temperature, absorbance at 510 nm was recorded. Ascorbic acid (300 mM) was added instead of test compound and absorbance obtained taken as equivalent to 100 % reduction. Blank was carried out without drug/ascorbic acid

Reduction of ferric ion: The Fe(II)-Fe(III) couple is known to be involved in various free radical reactions. Both SPHA and SPW reduce Fe(III) into Fe(II) at pH 7.4. In this study, reduction of Fe(III) into Fe(II) by ascorbate was taken as 100 % . The SPHA (500 µg/mL) caused a significant reduction of Fe(III) (73.10 %), while SPW (500 µg/mL) produced a reduction of 64.9 % . The IC_{50} of SPHA was found (100.65 µg/mL), while it was (198.90 µg/mL) for SPW. (Table-1, Fig. 4).

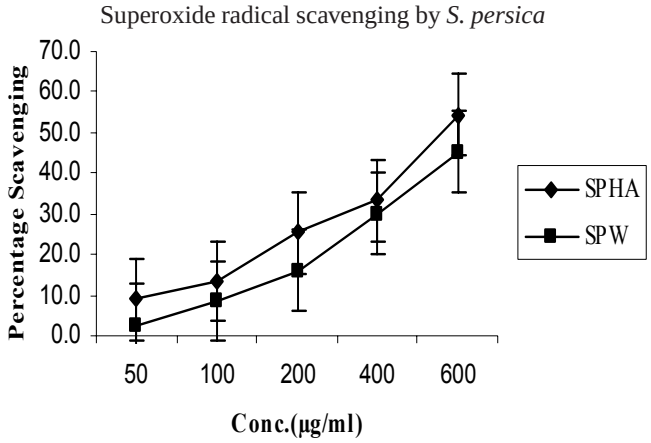

Fig. 4. Scavenging of superoxide radical by *Salvadora persica* extract
To the reaction mixture containing 0.5 mL of various concentrations of drug (50 mg to 600 mg), 0.2 mL of NBT and 1 mL of alkaline DMSO were added. Blank was carried out with only DMSO. Absorbance was recorded at 560 nm

A generic definition of an antioxidant is not experimentally constructive unless it is associated with the notion of the oxidant that has to be neutralized. Critically thinking on this point, series of *in vitro* antioxidant screening tests were performed in this study. Scavenging the 87.6 % of DPPH at conc. of 400 µg/mL by SPHA and 72.29 % by SPW shows reliable electron donating and *in vitro* antioxidant properties. Similar pattern was also observed in scavenging of ABTS radical. Both the extracts are more active in scavenging the ABTS radical than DPPH. Scavenging more rapidly ABTS radical than DPPH support the presence of more lipophilic compounds in SPHA. Comparing the IC_{50}, SPHA is much more potent and fast acting (Table-1). The SPHA reduces the ferric ion 70 % at conc. of 500 µg/mL, which shows it's powerful electron donating and radical scavenging activity. It is the general principle that most of free radicals produce cellular and molecular toxicity by initiating the cascading and chain reaction by stealing the hydrogen atom from neighboring

compounds[21,22]. Electron donors are considered to be effective chain breaking antioxidants by supplying pair of electrons to active oxidant and rendering them inactive[23]. Both the extracts having effective electron donating capability and can be considered as an effective chain breaking *in vivo* antioxidants.

In this study it is found that both extracts, SPHA and SPW at conc. of 600 µg/mL scavenge the super oxide (54.39 µg/mL) and (45.25 µg/mL), respectively. Super oxide radical is the principal mediator in the inflammation and oxidative stress[24]. From the in vitro study it is clear that SPHA and SPW are effective scavenger of superoxide radical that indicate *S. persica* as the promising antiinflammatory agents.

REFERENCES

1. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, published by Lalit Mohan Basu, 49, Leader Road, Allahabad, India, Vol. 2, pp. 1537-1539 (1935).
2. K.M. Nadkarni, Indian Materia Medica, Popular Book Depot Bombay, Vol. 1, edn. 3, pp. 1092-1093 (1954).
3. R.N.Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants CSIR, New Delhi, pp. 194-195 (1956).
4. M.I.H. Farooqui and J.G. Srivastav, *Quart. J. Crude Drug Res.*, **8**, 1297 (1968).
5. A. Bader, G. Flamini and P.L. Cioni, *J. Essent. Oil Res.*, **14**, 128 (2002).
6. F. Alali and T. Al-Lafi, *Nat. Prod. Res.*, **7**, 189 (2003).
7. S.T. Ezmirly and M.S. El-Nasr, *J. Chem. Soc. Pak.*, **3**, 9 (1981).
8. H.F. AbdEL Rehman, N. Skaug and A.M. Whyatt, *Pharm. Bio.*, **41**, 399 (2003).
9. I.A. Darout, A.A. Chirsty and N. Skaug, *Indian J. Pharmacol.*, **32**, 11 (2000).
10. R. Sanogo, M.T. Monforte and A. Rossitto, *Phytomedicine*, **6**, 363 (1999).
11. M.T. Monforte, A. Trovato and A. Rossitto, *Phytotherapy Res.*,**16**, 395 (2002).
12. U. Nyman, P. Joshi and L.B. Pedersen, *J. Ethnopharmacol.*, **60**, 247 (1998).
13. H. Ali, G.M. Konig, S.A. Khalid, A.D. Wright and R. Kaminsky, *J. Ethnopharmacol.*, **83**, 219 (2002).
14. E.M. Galati, M.T. Monforte, A.N. Forestieri, N. Miceli, A. Bader and A. Trovato, *Phytomedicine*, **6**, 181 (1999).
15. N.P. Shetgiri and S.S. Ambadekar, IUPAC International Conference on Biodiversity and Natural Products Chemistry and Medical Applications, New Delhi, pp. 5, 26-31 (2004).
16. H. Darmani, A.S. Al- Hiyasat, A.M. Elbetieha and A. Alkofahi, *Phytomedicine*, **10**, 62 (2003).
17. N. Sreejayan and M.N.A. Rao, *Drug Res.*, **46**, 169 (1996).
18. R. Narala and M.N.A. Rao, *J. Pharm. Pharmacol.*, **47**, 621 (1995).
19. E. Kunchandy and M.N.A. Rao, *Int. J. Pharmaceut.*, **58**, 237 (1990).
20. S.M Kumar, B.S. Reddy, S.K Babu, P.M. Bhilegaonkar, A. Shirwaikar and M.K. Unnikrishnan, *Nahrung. Food*, **47**, 126 (2003).
21. O.I. Aruoma, *Food Chem. Toxicol.*, **32**, 671 (1993).
22. K.I. Priyadarsini, G.H. Naik, D.K. Maity, M.S. Kumar, M.K. Unnikrishnan, J.G. Satav and H. Mohan, *Biol. Med.*, **35**, 475 (2003).
23. O.I. Aruoma and B. Halliwell, *Xenobiotica*, **18**, 459 (1988).
24. W.F. Petrone, D.K. English, K. Wong and J.M. McCord, *Proc. Natl. Acad. Sci., USA* **77**, 1159 (1980).

(*Received*: 8 July 2006; *Accepted*: 21 April 2007) AJC-5601