Pharmacognosy Research [Phcog Res]
Vol 1, Issue 5, Sep-Oct, 2009 Page 294-298
(An Official Publication of Pharmacognosy Network Worldwide)
Received: Jul 10, 2009
Modified: Jul 22, 2009
Accepted: Aug 25, 2009

PHCOG RES.: Research article

Antioxidant Effects of Roots of *Clerodendrum serratum* Linn.

Bhujbal S.S.[1]*, Kewatkar S.M.K.[1], More L.S., and Patil M.J.[2]

[1.] Dept. of Pharmacognosy, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Science and Research, Pimpri, Pune-411018., (INDIA).
[2.] Marathawada Mitra Mandal's, College of Pharmacy, Kalewadi, Pimpri, Pune-411017, INDIA.

*** Corresponding Author:** *santoshbhujbals@yahoo.com*

ABSTRACT

Over the past decade, herbal and ayurvedic drugs have become a subject of world importance, with both medicinal and economical implications. A regular and widespread use of herbs throughout the world has increased serious concerns over their quality, safety and efficacy. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. In the present study we examined the antioxidant effects of ethanolic extract of roots of *Clerodendrum serratum* (CSR) at various concentrations in the DPPH radical scavenging assay, FRAP assay (Ferric Reducing Antioxidant Power) and the Hydrogen peroxide radical scavenging assay. The results of the present study revealed that the plant extract has significant antioxidant activity and are encouraging for further assessment to elucidate the mechanism of action and to identify the bioactive compounds implicated in the antioxidant effect and the membrane stability.

Keywords: Antioxidant effect; *Clerodendrum serratum* Linn; DPPH (1,1-diphenyl-2-picryl-hydrazil); Ferric Reducing Antioxidant Power.

INTRODUCTION

Oxidative stress is believed to be a primary factor in various diseases as well as in the normal process of aging (1–2). Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, atherosclerosis and cardiovascular diseases. Cardiovascular diseases are the most common cause of death in the industrialized countries. The beneficial effects of phytochemicals are associated with a multitude of biological activities, including antioxidant and free radical-scavenging properties (3).

Clerodendrum serratum Linn. (Verbenaceae), known as Bharangi in ayurveda and Sirutekku in siddha system of medicine, is claimed to be useful in treating pain, inflammation, rheumatism, respiratory diseases and malarial fever (4). Owing to its importance in traditional medicine the plant was investigated for its anti-inflammatory, analgesic, antipyretic (5) and hepatoprotective properties (6). The plant is reported to contain β-sitosterol, 24(S)-ethyl cholesta-5,22,25-trien-3 β-ol, 5-hydroxy-7,4'-dimethoxy flavone, luteolin, apigenin, scutellarien, ursolic acid and two iridoid glucosides namely 7 β–coumaroyloxyugandoside and 7 β-cinnamoyloxyugandoside (7). The vast ethnomedical uses of the plant inspired us to investigate the antioxidant properties.

MATERIAL AND METHODS

Plant Material and Extraction:

The plant was collected from foothills of Sinhagad Pune (India), and was authenticated from Botanical Survey of India, Pune with voucher specimen no. SSBC1. The roots were dried under shade, coarsely powdered and passed

through 40 mesh sieve. The powdered material (500g) was extracted with ethanol using Soxhlet apparatus. The extract obtained was dried in rotary vacuum evaporator at 40^0 C, yielding a dark brown colored viscous mass 50g (10.0%).

Antioxidant study of ethanolic extract of CSR:

RSA assay (DPPH Radical Scavenging Activity)

In DPPH radical scavenging activity, CSR (test) sample solution (200 µl) was added to 4 ml of 100 mM/l ethanolic DPPH, then the mixture was incubated for 10 minutes at room temperature and the absorbance at 517 nm was measured. The difference in absorbance between a test sample and a control (ethanol) was considered as activity. The activity was shown as IC_{50} value (50% of inhibitory concentration in mg/ml). Ascorbic acid was used as standard substance. All values are shown as the mean of three measurements (8-9).

FRAP assay (Ferric reducing antioxidant power)

In ferric reducing antioxidant power assay, one ml of CSR (test) sample was mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The reaction mixtures were incubated in a temperature-controlled water bath at 50 ^{0}C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionised water and 200 µl of 0.1% $FeCl_3$. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A700 after blank substraction (10–11).

Hydrogen peroxide (H2O2) scavenging:

In hydrogen peroxide scavenging assay, a 40 mM solution of H_2O_2 was prepared in a phosphate buffer (pH 7.4). The concentration of H_2O_2 was determined spectrophotometrically at 230 nm. Different concentrations (20–140 µg/ml) of CSR extract were added to 0.6 ml of H_2O_2 (40 mM) and absorbance of the solution was read at 230 nm after 10 min against a blank containing CSR extract in phosphate buffer without H_2O_2 (12–13). The percent scavenging of H_2O_2 by CSR was calculated using the formula:

$$\% \text{ Scavenging of H2O2} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis:

Data obtained were analyzed using the Student's t-test and a P value less than 0,05 and 0,01 was considered statistically significant. Our results are expressed as means ± SEM.

RESULTS

In DPPH radical scavenging assay, CSR at various concentrations (50, 100, 150, 200, 250 µg/ml) and ascorbic acid (50, 100, 150, 200, 250 µg/ml) showed the significant inhibitory activity with IC_{50} value 175 and 137 respectively (Table I) (Fig 1).

In reducing power assay, a linear increase in reducing power was observed over the concentration range 20 – 120 µg/ml sample, equivalent to 20 - 120 µg/ml ascorbic acid. The results indicated that CSR consist of hydrophilic polyphenolic compounds that cause the greater reducing power. Result shows that reducing power of the ethanolic extract of CSR as a function of sample concentration In hydrogen peroxide scavenging assay, the inhibitive effect of CSR extract was found to be moderate when compared to other assays (Table II). The IC_{50} values were 48 and 85 for ascorbic acid, ethanolic extract of CSR respectively. The inhibition of 73.32 ± 0.002%, and 64.49 ± 0.242% was observed for ascorbic acid (standard) and ethanolic extract of CSR (test) respectively at maximum concentrations. Nevertheless CSR proved to possess the vital antioxidant active chemical compounds (Fig 3).

DISCUSSION

DPPH (1,1-diphenyl-2-picryl-hydrazil) is a stable free radical that accepts an electron or hydrogen radical

Table I: Effect of CSR on DPPH radical scavenging activity.

Concentration µg/ml	Ascorbic acid		CSR Extract	
	% Scavenging	IC 50 Value	% Scavenging	IC 50 Value
50 µg/ml	34 ± 2.00		20 ± 2.00	
100 µg/ml	40 ± 2.00		34 ± 2.00	
150 µg/ml	54 ± 3.464	137	48 ± 3.464	175
200 µg/ml	60 ± 3.464		52 ± 2.00	
250 µg/ml	68 ± 2.00		58 ± 2.00	

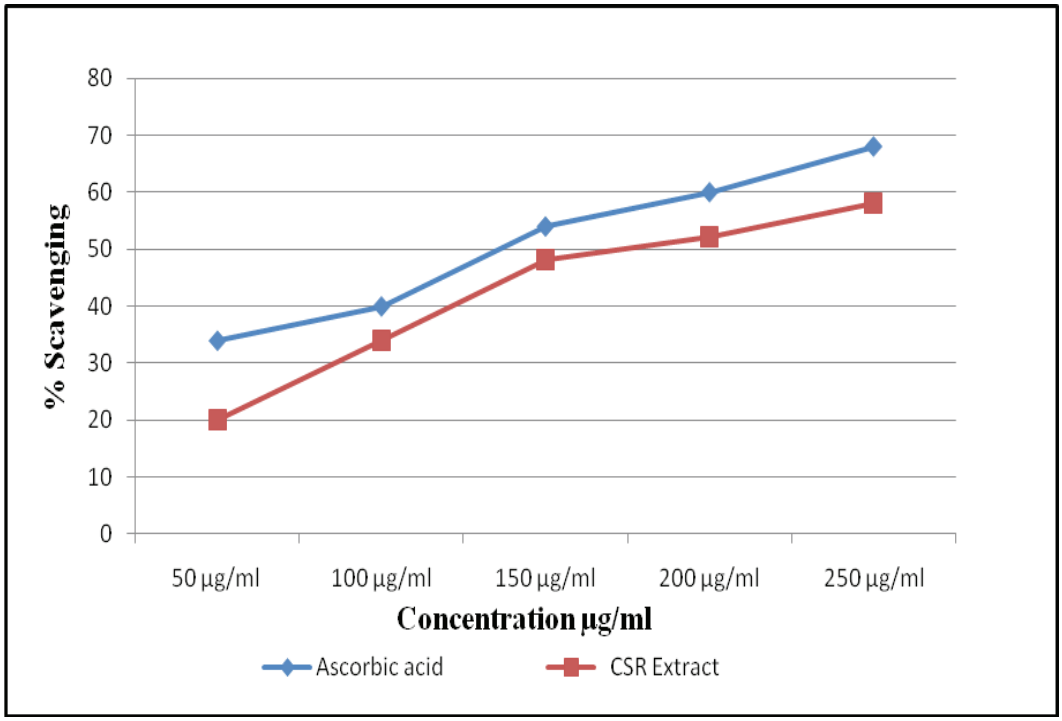

Fig 1: *Effect of CSR on DPPH radical scavenging activity*.

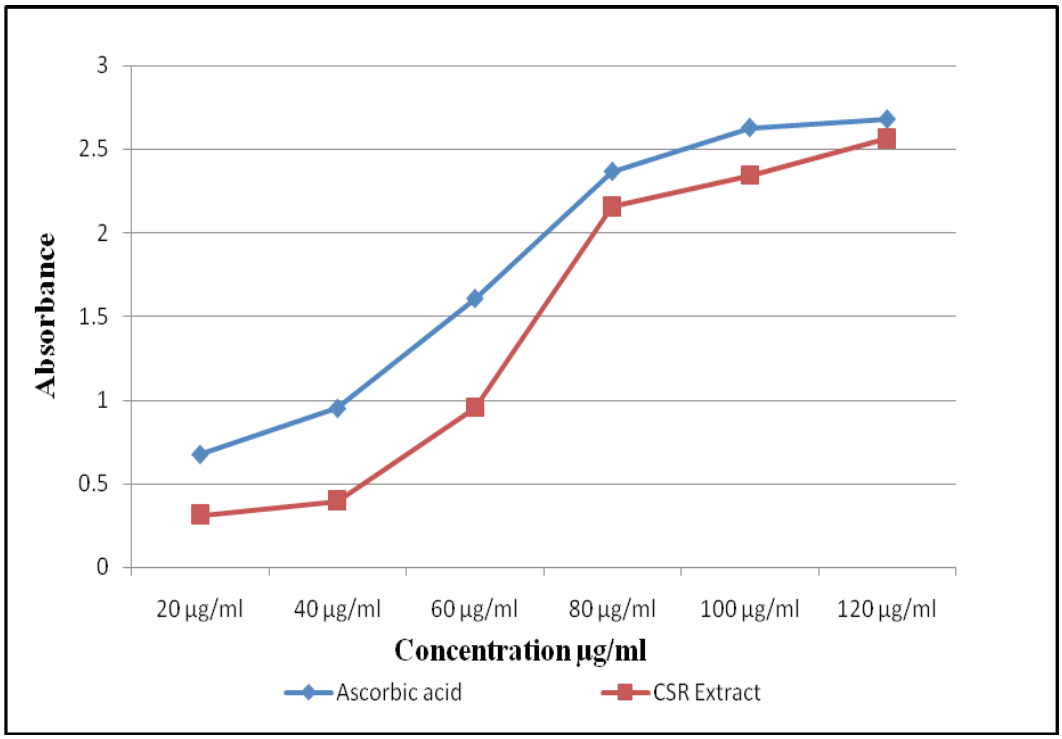

Fig 2: *Effect of CSR on FRAP assay (Ferric Reducing-Antioxidant Power)*.

to become a stable diamagnetic molecule. The model of scavenging the stable DPPH radical is widely used for relatively rapid evaluation of antioxidant activities compared to other methods (12). The reduction capability of the DPPH radical is determined by its absorbance decrease at 517 nm, as induced by natural antioxidants (3).

The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts,

Table II: Effect of CSR on hydrogen peroxide (H2O2) scavenging assay.

Concentration µg/ml	Ascorbic acid		Ethanolic extract	
	% Scavenging	IC 50 Value	% Scavenging	IC 50 Value
20 µg/ml	41.04 ± 0.006		40.85 ± 0.660	
40 µg/ml	46.66 ± 0.004		42.66 ± 0.330	
60 µg/ml	56.33 ± 0.002	48	45.75 ± 0.408	85
80 µg/ml	61.33 ± 0.002		48.97 ± 0.209	
100 µg/ml	67.66 ± 0.0007		54.60 ± 0.627	
120 µg/ml	69.33 ± 0.006		62.31 ± 0.406	
140 µg/ml	73.32 ± 0.002		64.49 ± 0.242	

decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging (13). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (14).

Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems (15). Scavenging of H_2O_2 by antioxidants may be due to donation of electrons to H_2O_2, thus neutralizing it to water (14).

According to phytochemical investigation it is observed that the ethanolic extract of CSR contains phenolic compounds such as flavonoids, tannins. It has been recently shown that quercetin and its glycosides exert inhibitory activity against lipid peroxidation (9, 16). Since luteolin and its derivates, along with quercetin and rutin, belong to the same group of compounds – flavonoids, the following antioxidative mechanism can be proposed: conjugation of the double bound in position 2,3 with C4- -carbonyl group, and also the existence of a free OH group on C5 and C7, enable the formation of chelate complexes with d-elements (Fe2+, Cu2+, Zn2+). The formation of a complex with Fe2+ prevents the production of OH_ radicals (Fenton's reaction), which was used to evaluate the inhibitory effects.

The antioxidant activity of this extract can be linked up to the high polyphenols and flavonoids content. Divers studies mentioned an implication of the polyphenols and flavonoids in the antioxidant activity of different plants extracts (17–18). Phenolics have been shown to possess an important antioxidant activity toward these radicals, which is principally based on the redox properties of their phenolic hydroxyl groups and the structural relationships between different parts of their chemical structure (19–20). It have been established a highly positive relationship between total phenols and antioxidant activity in many plant species (21).

CONCLUSION

The results of the present study shows that the ethanolic extract of the roots of *Clerodendrum serratum* Linn possess antioxidant activity through the DPPH free radical scavenging activity, reducing power assay and scavenging of hydrogen peroxide. The preliminary phytochemical investigation indicates the presence of flavonoids in the plant. Polyphenols like flavonoids and tannins are the well known natural antioxidants (22). So, the antioxidant potential of the plant may be attributed to the presence of flavonoids. The separation and identification of flavonoids present in the roots can help researchers find new molecules which can be used as natural antioxidants. Further studies are currently infact underway to isolate and characterize the active constituents responsible for its antioxidant activity.

ACKNOWLEDGEMENT

The authors are thankful to the Director and Principal, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, for providing necessary facilities and also to the Botanical survey of India for the authentication of the plant.

REFERENCES

1. Austin M.A., Hokanson J.E. and Brunzell J.D. Characterization of low density lipoprotein subclasses: methodologic approaches and clinical relevance. *Current Opinion in Lipidology*. **5**: 395–403 (1994).
2. Liao L., Starzyk R.M. and Granger D.N. Molecular determinants of oxidized low-density lipoprotein-induced leukocyte adhesion and microvascular dysfunction. *Arterioscler. Thromb. Vasc. Biol.* **17**: 437–44 (1997).
3. Ramchoun M., Harnafi H., Alem C., Benlyas M., Elrhaffari L. and Amrani S. Study on antioxidant and hypolipidemic effects of polyphenolrich extracts from Thymus vulgaris and Lavendula multifida. *Pharmacognosy Research*. **1**(3): 106–112 (2009).
4. Nadkarni K. M., Indian Materia Medica, (Popular Prakashan Private Limited Publication, India, 1954) 354.
5. Vishwanathan S., Narayanan N., Thirugnanasambantham P., Vijayasekaran V. and Sukumar E. Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendrum Serratum* in Experimental animals. *Journal of Ethanopharmacology*. **65**: 237–241 (1999).
6. Vidya S.M., Krishna V., Manjunatha B.K., Mankani, MAnzoor Ahmed K.L., Jegadeesh singh S.D. Evaluation of hepatoprotective activity of *Clerodendrum serratum* Linn. *Indian journal of experimental biology*. **45**: 538–542 (2007).
7. Jaya G., Babu K., Naidu C., Ganapaty S. *Indian drugs*. **34**: 208–210 (1997).
8. Tagashira M. and Ohtake Y. A new antioxidative 1,3-benzodioxole from *Melissa officinalis*. *Planta Med.* **64**: 555–558 (1998).

9. Panovska T.K., Kulevanova S. and Stefova M. In vitro antioxidant activity of some Teucrium species (Lamiaceae). *Acta Pharm.* **55**: 207–214 (2005).
10. Oyaizu M. Studies on product on browning reaction prepared from glucose amine. *Jpn. J. Nutr.* **44**: 307–315 (1986).
11. Banerjee D., Chakrabarti S., Hazra A.K., Banerjee S., Ray J. and Mukherjee B. Antioxidant activity and total phenolics of some mangroves in undarbans. *African Journal of Biotechnology*. **7**: 805–810 (2008).
12. Yokozawa T., Chen C.P., Dong E., Tanaka T., Nonaka G.I. and Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical. *Biochem. Pharmacol.* **56**: 213–22 (1998).
13. Diplock A.T. Will the 'good fairies' please proves to us that vitamin E lessens human degenerative of disease?. *Free. Rad. Res.* **27**: 511-532 (1997).
14. Bhalodi M., Shukla S. and Saluja A.K. In-vitro Antioxidant activity of the flowers of *Ipomoea aquatica* Forsk. *Pharmacognosy Magazine*. **4**: 226–230 (2008).
15. Elmastas M., Gulcin I., Isildak O., Kufrevioglu O.I., Ibaoglu K. and Aboul-Enein H.Y. Radical scavenging activity and antioxidant capacity of Bay leaf extracts. *J. Iran. Chem. Soc.* **3**: 258–266 (2006).
16. Cook N. C. and Samman S. Flavonoids – chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.* **7**: 66–76 (1996).
17. Yanagimoto K., Ochi H., Lee K.G. and Shibamoto T. Antioxidative activities of fractions obtained from brewed coffee. *J. Agric. Food Chem.* **52**: 592–96 (2004).
18. Zhu Q.Y., Hackman R.M., Ensunsa J.L., Holt R.R. and Keen C.L. Antioxidant activities of oolong tea. *J. Agric and Food Chem.* **50**: 6929–34 (2002).
19. Bors W. and Saran M. Radical scavenging by flavonoids antioxidants. *Free Radical Res. Comm.* **2**: 289–94 (1987).
20. Visioli F., Bellomo G. and Galli C. Free-radical-scavenging properties of olive oil polyphenols. *Biochem. Biophys. Res. Comm.* **247**: 60–64 (1998).
21. Velioglu Y.S., Mazza G., Gao L. and Oomah B.D. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.* **46**: 4113–17 (1998).
22. Dreosti I.E. Antioxidant polyphenols in tea, cocoa and wine. *Nutr.* **16**: 692–694 (2000).