ORIGINAL ARTICLE

Successive solvent extraction and free radical scavenging activity of *Azadirachta indica* A. juss

Mohammed Ibrahim, M. Kiranmai
Department of Biotechnology and Pharmaceutical Chemistry, Nizam Institute of Pharmacy, Greater Hyderabad, India

Background: Plant-based or plant-derived drugs occupied 30% of the modern system of medicine. Several trees possess a variety of biologically active compounds. Among them, *Azadirachta indica*, belonging to the family *Meliaceae,* plays a vital role as it acts as nature's pharmacy from several centuries of time. In the path of searching for potential antioxidants from plant origin, different parts of *Azadirachta indica* have been selected. **Aim:** Successive solvent extracts of leaves, seeds and root barks of *Azadirachta indica* A. Juss (neem) were studied for their free radical scavenging activity. **Materials and Methods:** Extraction capacity of different solvents based on their polarity order was compared and the extracts were subjected to 1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay. **Statistical Analysis:** All values were expressed in mean±SEM and correlation coefficient (R^2) values obtained from dose response curves were expressed for antioxidant results. **Results:** The results showed that the highest yields was found with the hydroalcoholic extract of leaves, followed by the hydroalcoholic extract of seeds and methanolic extracts of leaves and seeds, respectively. Free radical scavenging activity of various extracts was determined by measuring 1, DPPH free radical scavenging activity. The results were expressed in terms of IC_{50} values. The root bark methanol extract was found to exhibit the highest IC_{50} of 14.82-µg/mL at the concentration of 25-µg/mL. **Conclusion:** It is concluded that hydroalcohol and methanol are the best solvents to extract the antioxidant compounds from *Azadirachta indica.* The root bark methanolic extract was found to have the highest free radical scavenging potential against DPPH radical.

Key words: Antioxidant activity, *Azadirachta indica*, 1-diphenyl-2-picryl hydrazyl, neem, root bark methanol extract and leaves hydroalcoholic extract

INTRODUCTION

Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hydrochloric acid, peroxy nitrite) produced during aerobic metabolism in the body can cause oxidative damage of amino acids, lipids, proteins and DNA.[1-2] Recent development in biomedical point to the involvement of free radicals in many chronic and degenerative diseases. Antioxidants can prevent undesirable oxidation processes by reacting with free radicals, chelation of free catalytic metals and also by acting as O_2 scavengers. Restriction in the use of some synthetic antioxidants is being imposed because of their carcinogenicity.[3] There are some synthetic antioxidant compounds such as butylated hydroxyl toluene (BHT) and butylated hydroxy anisole (BHA), commonly used in processing food. However, it has been suggested that these compounds have some side-effects.[4-5] This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oil seeds, beans, fruits and vegetables has increased.[6-8]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.104939

An indigenous tree of the Indo–Pak subcontinent, *Azadirachta indica* A.Juss, known in common terms as neem, belongs to the family *Meliaceae*. Neem has received worldwide attention for different reasons. Neem is a nature's pharmacy.[9-10] Scientists of diverse fields have concentrated their attention on the therapeutic, preventive and bioactive constituents of neem.[11-12]

These compounds, either in pure form or in the form of extracts obtained from different parts of the plant, display a vast array of biological activities.[13-15] The extracts from several parts of the neem tree showed free radical scavenging activity against 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulphonicacid radicals, especially the leaf, flower and stem bark extracts showed strong activities. Leaf and flower extracts of neem tree inhibited the lipid peroxidation process.[16-17] Neem tree is an interesting plant for future

Address for correspondence: Dr. Mohammed Ibrahim, Department of Pharmacology and Biotechnology, Nizam Institute of Pharmacy, Deshmukhi, Greater Hyderabad, India. E-mail: ibrahim_cce@rediffmail.com
Received: 28-12-2011; **Accepted:** 03-08-2012

purposes related to its antioxidant activity, including medicinal agents and health supplements. Therefore, the aim of this study is to investigate the free radical scavenging activity of extracts of different parts of the neem tree from successive solvent extraction method.

MATERIALS AND METHODS

Collection of Plant Materials

Neem (leaf, seed and root bark) was collected from the institutional medicinal garden. The young leaves, ripened seeds and old root barks were collected, cleaned, washed under running tap water and shade dried. The plant sample was authenticated at the botany division, Osmania University and the voucher specimen was deposited at the same place (0125).

Preparation of Plant Extracts

Plant material was extracted successively with petroleum ether, chloroform, ethyl acetate and methanol in the increasing order of their polarity using soxhlet apparatus and the solvent was removed under reduced pressure to obtain the extracts. The yield of the extract in each solvent was recorded.

1-diphenyl-2-picryl hydrazyl Method

The free radical scavenging activity of extracts and standard vitamin C were determined by the method of Lamaison *et al*., which depends on scavenging of coloured free radical (DPPH) in methanol solution by the test samples. The reaction mixture contains DPPH and test drug in a final concentration of 3-mL. Absorbance of DPPH at its absorption maximum of 516-nm is inversely proportional to the concentration of scavenger (test sample). For all successive extracts, dimethyl sulphoxide (DMSO) and for hydroalcoholic extracts, distilled water was used as the solvent. The activity was expressed as inhibitory concentration 50 (IC_{50}), i.e., the concentration of the test solution required to give 50% reduction in absorbance of the test solution as compared with that of blank solution.[18]

$$\% \text{ Inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}}$$

RESULTS

The yield of the crude extracts and free radical scavenging activity of the extracts are given in Tables 1 and 2, respectively. Comparison of extraction capacity of various solvents is given in Figure 1. Antioxidant activity results were subjected to statistical analysis. The correlation coefficient (R^2) values were determined from the dose response curves.

DISCUSSION

Leaf hydroalcoholic, aqueous; seed hydroalcoholic, methanol; root bark methanol and chloroform showed highest yield when compared with the other extracts of the same part of the neem tree. From the comparison of solvent capacity in extracting active constituents from the neem tree, it is found that methanol and 80% ethanol (hydroalcoholic) are superior solvents than the others. In the case of leaves, water (maceration) is the suitable solvent when compared with the other solvents used in successive extraction procedure.

Root bark methanol, root bark hydroalcoholic and root bark ethyl acetate extracts showed highest free radical scavenging activity as determined by the DPPH method. After root bark leaf methanol, leaf hydroalcoholic and seed methanol extracts exhibited significant free radical scavenging activity. Root bark methanol extract showed highest antioxidant activity against DPPH free radical monocation at the concentration of 25 µg/mL. Root bark

Table 1: Successive solvent extracts of different parts of neem and % yield w/w

Extract	Leaves	Seeds	Root bark
Pet ether	1.64±0.22	12.60±0.15	2.50±0.03
Chloroform	2.25±0.10	1.78±0.11	3.80±0.19
Ethylacetate	1.60±0.01	2.40±1.21	1.72±0.22
Methanol	9.98±0.77	5.61±1.09	4.70±0.36
Hydroalcoholic	19.77±1.01	15.25±0.98	1.29±0.01

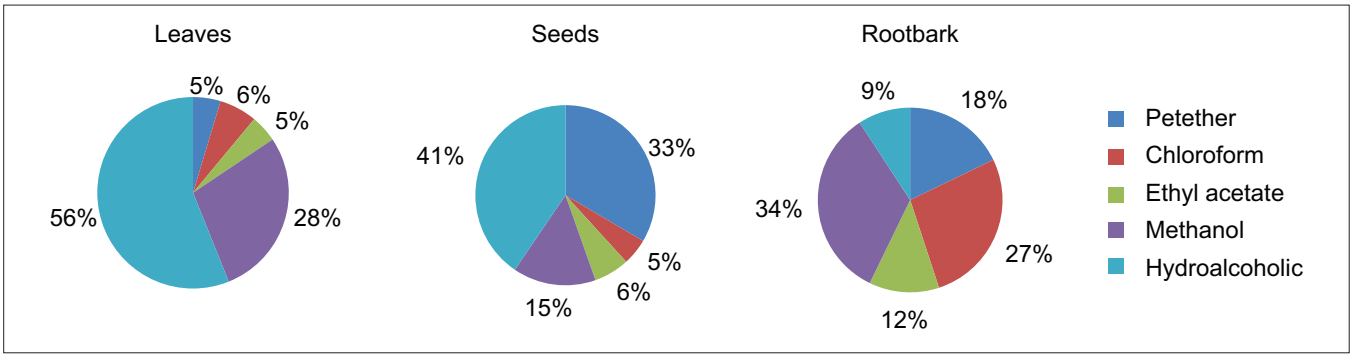

Figure 1: Various solvents used for extraction and their extraction capacity based on % yield

Table 2: Antioxidant activity of *Azadirachta indica* extracts towards the 1-diphenyl-2-picryl hydrazyl radical monocation

Extract	Dose (µg/mL)	Percent inhibition	R^2 value from dose response curve	IC_{50} µg/mL
Leaf chloroform	25	14.30	0.9774	100.91
	50	15.44		
	100	20.21		
Leaf ethylacetate	25	20.76	0.9932	102.22
	50	27.38		
	100	37.04		
Leaf methanol	25	31.31	0.9993	74.97
	50	41.23		
	100	59.07		
Seed chloroform	25	18.67	0.9981	101.20
	50	22.81		
	100	29.79		
Seed ethylacetate	25	34.66	0.9981	122.45
	50	37.20		
	100	43.30		
Seed methanol	25	25.21	0.9973	83.01
	50	37.18		
	100	56.76		
Root bark chloroform	25	13.90	0.9729	110.09
	50	29.26		
	100	46.00		
Root bark ethylacetate	25	22.80	0.9924	62.97
	50	43.83		
	100	74.04		
Root bark methanol	5	23.75	0.9995	14.82
	10	34.25		
	25	79.35		
Leaf hydroalcoholic	25	21.45	0.9992	80.08
	50	33.53		
	100	60.69		
Seed hydroalcoholic	25	12.66	0.973	109.87
	50	14.97		
	100	17.50		
Root bark hydroalcoholic	10	22.81	0.9844	35.30
	25	42.58		
	50	63.65		
Leaf aqueous	25	18.66	0.9881	122.32
	50	23.15		
	100	37.93		
Standard	Dose (µg/mL)	Percent inhibition	0.9939	IC_{50} µg/mL
Vitamin-C	1	20.39		4.52
	2.5	30.84		
	5	54.69		

hydroalcoholic extract exhibited significant antioxidant activity at the concentration of 50 µg/mL. Apart from the above mentioned extracts, other extracts of leaf, seed, root bark of neem exhibited nonsignificant free radical scavenging activity. The extracts showing % inhibition more than 50% only considered here for discussion of results.

Chemical constituents associated with *Azadirachta indica* include isoprenoids and nonisoprenoids. The isoprenoids includes diterpenoids, triterpenoids, valasinine type of compounds, limonoids and its derivatives, C-secomeliacins. The nonisoprenoids include proteins, polysaccharides, sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins and aliphatic compounds.[19] In our previous reports, the extracts from root bark of neem showed significant total flavonoid content.[20]

There has been no report regarding successive solvent extracts of different parts of the neem tree and comparison of their free radical scavenging activity. Ethanol has been proven as an effective solvent for extracting antioxidant

compounds from neem.[21-22] In our present study, methanol and 80% ethanol were proven as effective solvents to extract antioxidant compounds from neem. A phytochemical screening by TLC showed that root bark methanol and hydroalcoholic extracts presented flavonoids.[20] Therefore, flavonoids and polyphenolics compounds might play a role in the antioxidant activity of the root bark of neem tree.

CONCLUSION

In conclusion, the root bark of *Azadirachta indica* (neem) proved to be of higher antioxidant potential in comparison with other parts of the same plant. Methanol, 80% ethanol and ethyl acetate were found to be suitable solvents for extracting the biologically active constituents from neem. Further experiments are required to isolate and identify the antioxidant components, both qualitatively and quantitatively and assess the mechanisms of their activity.

REFERENCES

1. Gutteridge JM. Free radical in disease process: A compilation of cause and consequence. Free Radic Res Commun 1995;19:141-58.
2. Halliwell B. How to characterize an antioxidant: An update. Biochem Soc Symp 1995;61:73-101.
3. Shyama Gowri S, Vasantha K. Free radical scavenging and antioxidant activity of leaves from Agathi (Sebania grandiflora, L.). Pers Ameri Euro J Sci Res 2010;5:114-119.
4. Branien AL. Toxicology and biochemistry of butylated hydroxyl anisol and butylated hydroxyl tolune. J Am Oil Chem Soc 1975;52:29-63.
5. Ito N, Fukushima S, Hassegawa A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyl anisol in F344 rats. J Natl Cancer Inst 1983;70:343-52.
6. Deiana M, Aruoma OI, Bianchi ML, Spencer JP, Kaur H, Halliwell B, *et al*. Inhibition of peroxynitrite dependent DNA base modification and tyrisin nitration by the extra nirginoline oil derived antioxidant hydroxytyrosol. Free Radic Biol Med 1999;26:762-9.
7. Lee KG, Shibamoto J. Antioxidant properties of the aroma compounds isolated from soyabean and mung beans. J Agri Food Chem 2000;48:4290-3.
8. Jiao H, Wang SY, Jiao H. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in black berry. J Agric Food Chem 2000;48:5672-6.
9. Manjunath BL. The wealth of India. Council of scientific and industrial research, New Delhi, India 1948;1:140-142.
10. Vietmeyer ND. Neem: A Tree for Solving Global Problems. Report of an ad hoc panel of the Board on Science and Technology for International Development, National Research Council, Washington, DC, USA: National Academy Press; 1992. p. 71-2.
11. Vander Nat JM, Vander Sluis WG, Desilva KT, Labadie RP. Ethanopharmacognostical survey of *Azadirachta indica* A Juss. J Ethanopharmacol 1991;35:1-24.
12. Jacobson M. Neem-A tree for solving global problems. Am Chem Soc Sympho Ser 1986;296:221.
13. Prakash AO, Tewari RK, Mathur R. Non-hormonal post-coital contraceptive action of neem oil in rats. J Ethnopharmacol 1988;23:53-9.
14. Roa DVK, Singh K, Chopra P, Chabra PC, Ramanujula G. *In vitro* antibacterial activity of Neem oil. Indian J Med Res 1986;84:314.
15. Jones JW, Denholm AA, Ley SV, Lovell H, Wood A, Sinden RE. Sexual development of malaria parasites is inhibited *in vitro* by the neem extract azadirachtin, and its semi-synthetic analogues. FEMS Microbiol Lett 1994;120:267-73.
16. Sithisaran P, Supabphol R, Gritsomapan W. Antioxidant activity of Siamese neem tree. J Ethnopharmacol 2005;99:109-12.
17. Sithisarn P, Supabphol R, Gritsomapan W. Comparison of free radical scavenging activity of Siamese neem tree leaf extracts prepared by different methods of extraction. Med Princ pract 2006;15:219-22.
18. Lamaison JL. [Medicinal Lamiaceae with antioxidant properties, a potential source of rosmarinic acid]. Pharma Acta Helv 1991;66:185-8.
19. Subapriya R, Nagini S. Medicinal properties of neem leaves: A review. Curr Med Chem Anticancer Agents 2005;5:149-56.
20. Kiranmai M, Mahender kumar CB, Ibrahim MD. Comparision of Total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. Res J Pharma Biolog Chemi Sci 2011;2:254-61.
21. Karadeniz F, Burdurulu HS, Koca N, Soyer Y. Antioxidant activity of selected fruits and vegetables grown in Turkey. J Agric Food Chem 2005;29:297-303.
22. Gayatri Nahak, Sahu RK. *In vitro* antioxidative activity of *Azadirachta indica* and *Melia azedarach* leaves by DPPH scavenging assay. J Am Sci 2010;6:123-8.

How to cite this article: Ibrahim M, Kiranmai M. Successive solvent extraction and free radical scavenging activity of *Azadirachta indica* A. juss. Int J Green Pharm 2012;6:237-40.

Source of Support: Nil, **Conflict of Interest:** None declared.