



Short Communication

Antiradical activity of *Azadirachta indica* extracts and fractions

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SUMMARY

In present study, total phenolic content of crude extract and antiradical activity of crude extract and different solvent fractions of *Azadirachta indica* were evaluated. Crude extract and most of the polar fractions showed higher radical scavenging activity. Among the crude extract and nine different fractions, the aqueous/methanol (3:1) fraction showed the highest activity.

Key words: Antiradical activity; Total phenolic content; DPPH assay; *Azadirachta indica*

INTRODUCTION

Azadirachta indica (neem) is traditionally employed as folklore remedy for a wide spectrum of diseases in Nepal. Every part of this plant is used in traditional medicine. Decoction of leaf is used in ulcers and eczema (Medicinal plants of Nepal, 1997), leaf juice orally administered for blood purification and intestinal worms and externally applied on wounds, sores, blisters and skin diseases (Siwakoti and Siwakoti, 2000), Leaf, bark, fruit and young stem mixed with sugar candy used in skin diseases, and used as antiseptic, anthelmintic in man and cattle (Yadav, 1999). In Ayurvedic medicine, Neem formulations remained as a mainstay of Ayurvedic pharmacy. More than 135 compounds having diverse structure have been isolated from various parts of Neem. In recent

time, interest in the antioxidant activity of plant extracts, or isolated substances from plants, has grown, due to the fact that free radicals have been related to some diseases, as well as to the aging process (Willcox *et al.*, 2004). Moreover, there is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) that are commonly used in lipid-containing food (Takahata *et al.*, 2001). Neem is one of the plants that have been extensively studied for its biological and chemical properties. However, there is no report on DPPH radical scavenging activity of the plant, especially Nepalese origin. Since nature does not supply products with a consistent and standardized composition, the metabolic profile of a certain species and, consequently, its bioactivity can vary depending on climatic, genetic and various environmental factors like soil, weather, nutrients etc (McChesney, 1999; Gilani and Rahman, 2005; Khan, 2006). Here, we reported

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total phenolic content of crude extract and the antiradical potency of the extracts and different solvent fractions of *Azadirachta indica* leaf.

MATERIALS AND METHODS

Plant material collection and extraction

Azadirachta indica leaves were collected from Kanchanpur district, Nepal in February 2004. The plant material was collected and identified by one of us (LR Bhatt) and further authenticated by Dr Lokendra R Sharma and Mr. Puran Pd Kurmi, Department of Plant Resources, Ministry of forest & soil conservation, Nepal. The plant was chosen based on its reported uses in the literature. The freshly picked leaves were air-dried at room temperature for 2 weeks, with no direct sunlight and 10 g of powdered leaves were extracted in ethanol, using Soxhlet extractor. The obtained extract was evaporated, using vacuum evaporator under 40°C to give the crude dried extract and stored in refrigerator until use.

Reverse phase flash chromatography

The ethanol extracts of *Azadirachta indica* leaves (3.78 g) was coated on C₁₈ (7.56 g) and packed onto a C₁₈ column (37.8 g). The column was developed in a stepwise manner starting with H₂O and followed by H₂O: MeOH, MeOH, MeOH: CHCl₃, CHCl₃ and Hexane (Table 1).

Antiradical activity

The scavenging capacity of crude extracts were determined, using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), as a stable free radical (Yen and Chen, 1995). Briefly, different concentrations of sample prepared by dissolving the dried extract in methanol were mixed with 0.12 mM methanolic DPPH solution. After 30 min incubation at room

temperature, the absorbance was measured at 517 nm against blank. The percentage DPPH radical scavenging activity was determined using following equation.

$$\text{DPPH radical scavenging (\%)} = \frac{[(\text{control abs.} - \text{sample abs.})/\text{control abs.}] \times 100}{1}$$

Kinetic behaviors of sample and standard were monitored at the interval of 1 min interval with a spectrophotometer, until ninety min.

Total phenolic content

The total phenolic content was determined following the Folin-Ciocalteu method¹² (Iqbal and Bhangar, 2006). The reaction mixture containing 200 µL of sample, 750 µL of the Folin-Ciocalteu reagent was mixed thoroughly. After 1 min, 2 ml of 7.5% sodium carbonate solution was added. The final mixture was diluted to 7 ml with deionized water. After 2 h incubation in dark at room temperature the absorbance was measured at 765 nm against the blank (solution contained all the reaction reagents except the sample). Gallic acid (0 - 500 mg/l) was used for calibration of a standard curve. Total phenolic content was determined as Gallic acid equivalents (GAE) and values were expressed as mg of acid/g of plant material (in GAE).

Statistical analysis

The data are results of triplicate experiments. Microsoft Excel was used to compute means, standard deviation, correlation and regression.

RESULTS AND DISCUSSION

Antiradical activity of extract and different solvent fractions was carried out using DPPH assay. The chemical investigations of leaves of *Azadirachta*

Table 1. Yield, antiradical activity and total phenolic content of *Azadirachta indica* crude extract

Extract	Yield (%)	IC ₅₀ (µg/mL)	TPC (mg GA/g)
<i>Azadirachta indica</i>	16.13	13.39 ± 1.86	246.85 ± 10.64

Table 2. DPPH radical-scavenging activities of ethanol extract and different solvent fractions of *Azadirachta indica*

Extract and Fractions	^a Antioxidant activity (%)		
	5 (µg/ml)	10 (µg/ml)	15 (µg/ml)
Ethanol crude extract	22.34 ± 2.53	47.53 ± 1.12	69.85 ± 1.44
H ₂ O fraction	27.24 ± 1.30	52.11 ± 1.42	77.12 ± 2.29
H ₂ O -MeOH (9:1) fraction	37.64 ± 2.21	65.81 ± 0.52	86.05 ± 0.79
H ₂ O -MeOH (3:1) fraction	57.29 ± 0.07	87.53 ± 0.65	92.39 ± 0.68
H ₂ O -MeOH (1:1) fraction	53.97 ± 0.10	83.65 ± 1.23	87.47 ± 2.72
H ₂ O -MeOH (1:3) fraction	22.99 ± 0.82	39.78 ± 0.80	56.97 ± 0.45
H ₂ O -MeOH (1:9) fraction	11.53 ± 1.03	28.46 ± 0.10	39.66 ± 2.33
MeOH fraction	1.8 ± 0.07	14.93 ± 0.50	17.71 ± 0.16
MeOH-CHCl ₃ (3:1) fraction	10.15 ± 0.02	25.73 ± 1.44	30.49 ± 0.55
CHCl ₃ fraction	-	-	-
Hexane fraction	-	-	-
BHA	42.21 ± 0.82	72.03 ± 1.01	85.18 ± 1.95
α - tocopherol	23.96 ± 1.16	50.82 ± 0.69	83.55 ± 1.67

-: No activity at given concentration; ^a: The values represent the mean ± standard deviations for triplicate experiments.

indica resulted in isolation of several putative pharmacophores *viz.* nimbidin, azadirachtin, flavonoids like quercetin and rutin, phytosterol like β-sitosterol (Rastogi, 1993; Bramley and Pridham, 1995). Quercetin is a bioflavonoid with potent antioxidant property (Bramley and Pridham, 1995). In present study, aqueous/methanol fractions were active among the fractions evaluated. Aqueous/methanol (3:1) and aqueous/methanol (1:1) fraction of *Azadirachta indica* showed higher activity than that of BHA at all tested concentrations and aqueous/methanol (3:1) fraction was the most active (Table 2).

Similarly, aqueous and most of aqueous/methanol fractions showed higher activity than that of crude extracts while methanol and non polar fractions were less active. The leaf aqueous, flower ethanol and stem bark ethanol extracts of sesame neem exhibited significantly strong free radical scavenging activity by DPPH assay where as corresponding methanol extracts were less active Pongtip *et al.* (2005). In present study also aqueous and aqueous-methanol fractions showed strong antiradical activity where as methanol fraction was even less active than that of MeOH-CHCl₃ fraction (Table 2). The specific activity of

almost all the antioxidant related enzymes including glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase were reported to be elevated by leaf extract of neem above the control basal values either in dose-dependant or dose-independent manner in mice (Trisha *et al.*, 2004). Similarly glutathione activity was increased in above basal level in the liver as well as all the extrahepatic organs showed the protection of the cell against free radical-induced damage by Neem leaf extract (Trisha *et al.*, 2004). Moreover, oxidative membrane damage by hydroxyl radical (OH) as measured by lipid peroxidation in stress ulcer is significantly blocked by leaf extract. It also prevents [•]OH-mediated mucosal DNA damage *in vitro* by scavenging the [•]OH. Neem leaf extract, thus, offers anti-ulcer activity by blocking acid secretion through inhibition of H⁺-K⁺-ATPase and by preventing oxidative damage and apoptosis (Chattopadhyay *et al.*, 2004).

The kinetic behavior of ethanol extract, active fractions and that of standard antioxidants was studied. Crude extract and aqueous -methanol fractions showed higher radical scavenging activity (Fig. 1). The kinetic classification, according to the

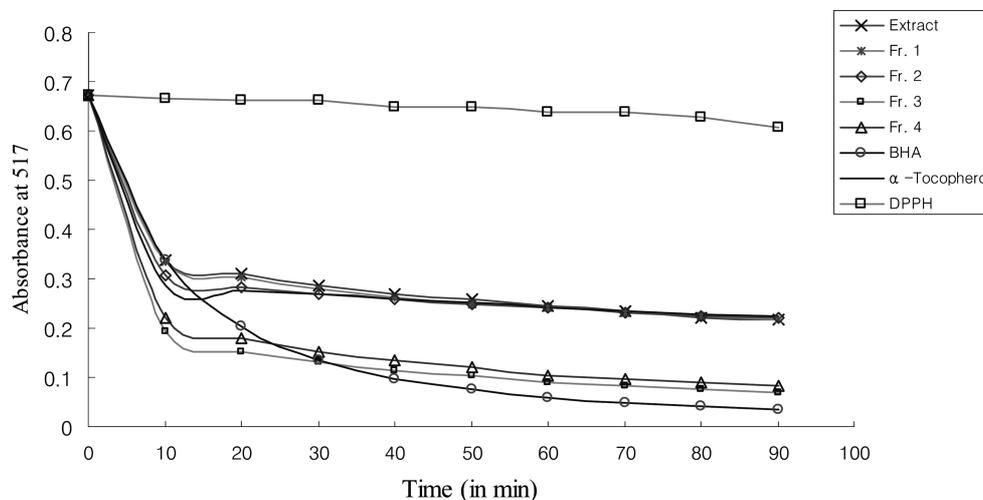


Fig. 1. Kinetics of *Azadirachta indica* extract and active fractions.

time at the steady state, has been reported as Rapid < 5 min, intermediate 5 - 30 min and slow > 30 min Sanchez-Moreno *et al.* (1998). In present study, the reaction rate of BHA was slow while that of crude extract, polar fractions and α -Tocopherol as intermediate.

The total phenolic content of extract was reported as gallic acid equivalent concentration (mg/g). Crude extract contained high phenolic content. Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free radical-scavenging, lipid peroxidation and chelating of metal ions (Shahidi, 1992). The results of the present study in part indicated the contribution of phenolics towards antioxidant capacity, besides other factors.

In conclusion, aqueous/methanol (3:1) fraction of *Azadirachta indica* leaf showed the highest radical scavenging activity. Moreover, ethanol extract and most of the aqueous-methanol fractions showed prominent activity. The activity of the extract and fractions might be in part due to phenolic compounds and /or by synergic effect.

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