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In Vitro Antioxidant Activity of the Whole Plant of *Amaranthus spinosus* Linn.

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ABSTRACT

The antioxidant components and antioxidant activity of the whole plant of *Amaranthus spinosus* Linn. were studied. The whole sun-dried plant was powered; 100 g of this dried powder was continuously heat extracted 5 times with hydroalcholic solvent (70% ethanol +30% water). The extract was collected and distilled to recover the solvent. The antioxidant activity of the extract was compared with standards (ascorbic acid and butyl hydroxyl toluene (BHT)). The DPPH-radical scavenging and NED methods were used to measure the antioxidant activity, which was due to the presence of flavanoids and other phenolic contents. The extract showed significant scavenging activity when the IC_{50} value was compared to ascorbic acid and BHT: IC_{50} of the hydroalcoholic extract = 525.593 and 433.168 µg/ml, for ascorbic acid = 594.937 and 570.652 µg/ml, and for BHT = 29.452 and 22.499 µg/ml by the DPPH method and NED method, respectively.

Keywords: free radical, hydro alcoholic extract, scavenging
Abbreviations: BHT, butyl hydroxyl toluene; **DPPH**, 2,2-diphenyl-1-picrylhydrazyl; **NED**, *N*-(1-naphthyl) ethylene diamine

INTRODUCTION

Many diseases are due to "oxidative stress" that results from an imbalance between formation and neutralization of pro-oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Selvamathy *et al.* 2008). These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Maxwell 1995; Braca *et al.* 2002). All human cells protect themselves against free radical damage by enzymes such as superoxide dismutase (SOD) and catalase (CAT), or compounds such as ascorbic acid, tocopherol and glutathione (Niki *et al.* 1994). Sometimes, these protective mechanisms are disrupted by various pathological processes, and antioxidant supplements are vital to combat oxidative damage. Recently, much attention has been directed towards the development of ethnomedicines with strong antioxidant properties but low cytotoxicities. Some studies have suggested that antioxidants are beneficial to health, while others, mostly larger clinical trials, have concluded they have no effect on health and say in some cases that taking too many of them can be harmful (Goran *et al.* 2007).

Reactive free radicals are continuously generated in the body by both exogenous and endogenous factors such as normal aerobic respiration, stimulated polymorpho-nuclear leukocytes, microphages and exposure to various pollutants like tobacco smoke or organic solvents (Beris 1991; Yerra 2005). These free radicals, which may include hydrogen peroxide, singlet oxygen, hydroxyl radical, nitric oxide and various lipid peroxides (Murray 2000), cause cellular damage by reacting with various biomolecules such as membrane lipids, nucleic acid, proteins (Doherty 2008). Such damage is the cause of many medical disorders like cancer, hepatic ailments, cardiovascular diseases, cataracts, diabetic mellitus, inflammation, renal failure and the process of aging (Malila 2002). Antioxidants may offer resistance against oxidative stress by scavenging free radicals (Patil 2008). Antioxidants are capable of effectively neutralizing the deleterious effects of free radicals (reviewed by Hancock 2009, 2010). Various antioxidants are naturally present in the body, namely CAT or SOD, while synthetic antioxidants, including butylated hydroxyanisole (BHA) and butyl hydroxyl toluene (BHT) are suspected to be carcinogenic and hence have no use (Chung 1999). Therefore, the need for antioxidants from natural origin has been generated in recent years (Jayaprakash 2001). Indian medicinal plants are good sources of antioxidant (Utpal *et al.* 2008). Vegetables and fruits are good sources of natural antioxidants for human diet, containing many different antioxidant components which provide protection against harmful free radicals (Cao 1996; Wang 1996).

Amaranthus spinosus Linn. (Amaranthaceae) is valued for its mucilaginous properties (Perez *et al.* 1993). It increases milk production in cattle when boiled given with pulses in India while the seeds are eaten by birds (CSIR 2006).

The leaves and stems contain α-spinasterol and hentriacontane (CSIR 2006) while the roots contain α-spinasterols, octacosanoate ($C_{57}H_{102}O_2$, mp 85-86°), and saponins, namely of oleanolic acid (CSIR 2006). The whole plant is used in the treatment of snake bite (Basu 2001).

MATERIALS AND METHODS

All chemicals used were used of analytical grade. DPPH (2,2-diphenyl-1-picrylhydrazyl) and NED (*N*-(1-naphthyl) ethylene diamine) were purchased from Sigma Chemical, Bangalore. Ethanol was purchased from BCPL, Kolkata.

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Research Note

Table 1 Antioxidant activity of hydro alcoholic extract of *Amaranthus spinosus* by DPPH method (Absorbance at 517 nm) and NED method (Absorbance at 540 nm).

Amaranthus spinosus extract conc. (µg/ml)	Absorbance at 517 nm (DPPH method)	% of inhibition by DPPH method	Absorbance at 540 nm (NED method)	% of inhibition by NED method
100	0.152 ± 0.016	30.59	0.234 ± 0.028	33.11
200	0.141 ± 0.01	35.62	0.219 ± 0.021	37.42
300	0.136 ± 0.021	37.90	0.198 ± 0.015	43.42
400	0.13 ± 0.009	40.64	0.186 ± 0.017	46.85
500	0.121 ± 0.011	44.75	0.148 ± 0.016	57.71
600	0.114 ± 0.012	49.77	0.130 ± 0.016	62.85
700	0.104 ± 0.011	52.51	0.118 ± 0.012	66.28
800	0.097 ± 0.009	55.71	0.113 ± 0.013	67.71
900	0.089 ± 0.008	63.93	0.087 ± 0.021	75.14
1000	0.082 ± 0.016	68.49	0.073 ± 0.020	79.14

Values are expressed as the mean ± SEM, n=3, t-test: paired two sample for means $P < 0.01$

Plant material

The whole plants of *A. spinosus* were procured from different places of Asansol, Burdwan, West Bengal (WB) and authenticated by the Botanical Survey of India, Shibpur, Howrah, WB. A voucher specimen (NO-CHN/I-I/2008/Tech. II/) was retained in our laboratory for further references. It was also identified by the Department of Botany, B. B. College, Asansol, WB, India.

Methods

The whole sun-dried plant was powered; 100 g of this dried powder was continuously heat extracted 5 times with hydroalcholic solvent (70% ethanol +30% water). The extract was collected and distilled to recover the solvent. The antioxidant activity of the extract was compared with standards (ascorbic acid and BHT). The DPPH-radical scavenging and NED methods, *in vitro* methods, were used to measure the antioxidant activity, which was due to the presence of flavanoids and other phenolic contents.

1. DPPH radical scavenging method

The effect of extracts on DPPH radical was determined using the method of Liyana-Pathiranan (2005). A solution of 0.135 mM DPPH in methanol was prepared; 1.0 ml of this solution was mixed with 1.0 ml of hydroalcoholic extract containing 0.02–0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min (Chen *et al*. 2003). The absorbance of the mixture was measured spectrophotometrically at 517 nm (Sreejayan 1996). Ascorbic acid and BHT were used as standards. The IC_{50} value was defined as the concentration (µg/ml) of extract that inhibited the formation of DPPH radicals by 50% (Mana *et al*. 2000). The reducing power of nutraceutical herbs was determined according to the method of Oyaizu (1986).

2. NED method

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with O_2 to produce nitrite ions, which can be estimated by the use of the Griess Illusory reaction (Hazra *et al*. 2008).

In the present investigation, Griess Illusory reagent was modified by using naphthylene diamine dihydrochloride (Ambuja Intermediates Pvt. Ltd., Mumbai) (0.14%, w/v) instead of 1-napthylene (5%). Scavengers of NO compete with O_2 leading to reduced production of NO. The reaction mixture (3 ml) containing sodium nitroprosside (10 mM, 2 ml) phosphate buffer saline (0.5 ml) and extract or standard solution (0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for diazotization to be complete. Then 1 ml of 1% napthylethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min. A pink colored chromophore was measured at 540 nm against the corresponding blank solution using a spectrophotometer (Model No UV1 094022, Merck). The percentage inhibition was calculated by comparing the absorbance values of the control and test. Nitric oxide radical scavenging activity was calculated according to an equation (Illavarasana *et al*. 2005):

$$\% \text{ of Inhibition} = (A_0 - A_1)/A_0 \times 100$$

where A_0 = absorbance of the control; A_1= absorbance in the presence of extract.

Statistical analysis

The DPPH and NED free radical scavenging activity of the plant extract was compared by a paired two-sample *t*-test using Microsoft Excel 2002. The difference between means was considered significant when one-tail and two-tail *P* values were < 0.01.

RESULTS AND DISCUSSION

Several concentrations ranging from 100-1000 µg/ml of the hydroalcholic extract of *A. spinosus* were tested for their antioxidant activity in two different *in vitro* models (**Table 1**). Free radicals are scavenged by the test compounds in a concentration-dependent manner in all the models (Krishnamoorthy *et al*. 2009). The percentage of inhibition in the DPPH and NED methods are shown in **Figs. 1-3**. The IC_{50} values of the hydroalcoholic extract = 525.593 and 433.168 µg/ml, for ascorbic acid = 594.937 and 570.652 µg/ml, and for BHT = 29.452 and 22.499 µg/ml by the DPPH method and NED method, respectively.

Amaranthus viridis Linn., *A. lividus* Linn. and *A. paniculatus* Linn. also showed antioxidant activity (Yadav 2004; Ozsoy *et al* 2009; Kumar *et al.* 2009). The antioxidant activity was due to the presence of flavonoids and other phenolic contents. The extract showed significant scavenging activity when the IC_{50} value was compared to ascorbic acid and BHT. The reducing power of the extract was dose-dependent (**Figs. 4, 5**). There was a statistically significant difference between the free radical scavenging activity of the plant extract measured by DPPH and NED methods at $P < 0.01$. Using non-enzymatic haemoglycosylation, Kumar *et al.* (2010) showed the antioxidant activity of *A. spinosus*.

The free phenols and phenolic acids are best considered together, since they are usually identified together during plant analysis. This plant can be consider as a model herbal drug for experimental studies, including free radical-induced disorders like cancer, diabetics, atherosclerosis, etc.

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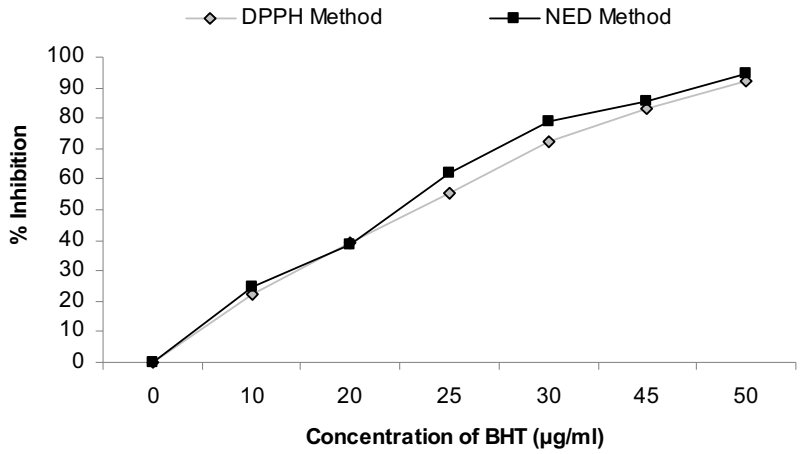

Fig. 1 Antioxidant activity of BHT by DPPH and NED method.

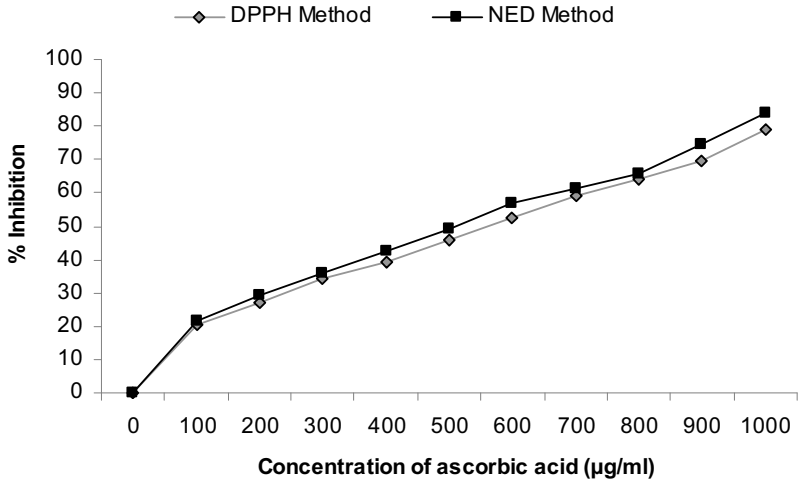

Fig. 2 Antioxidant activity of ascorbic acid by DPPH and NED method.

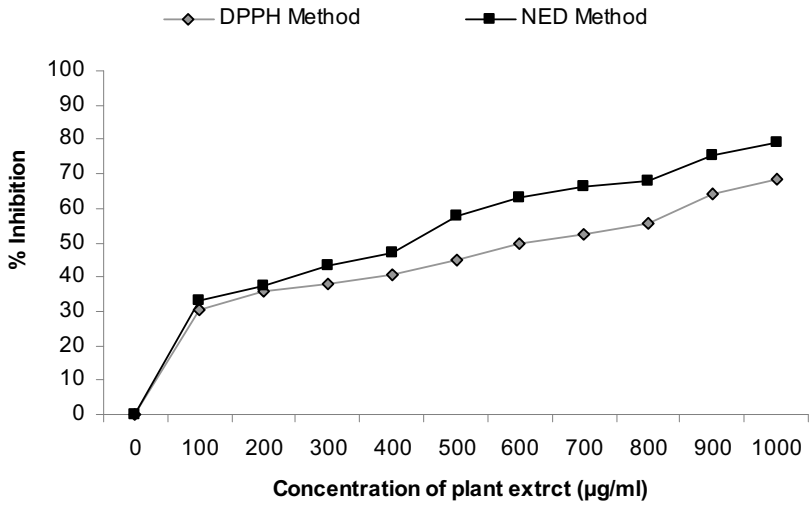

Fig. 3 Effect of hydroalcoholic extract in different *in vitro* free radical models.

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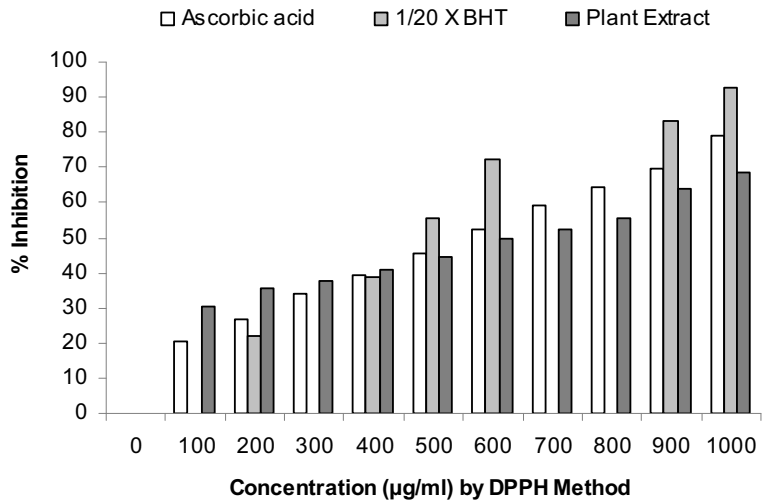

Fig. 4 Comparison of antioxidant activity of plant extract with control by DPPH method.

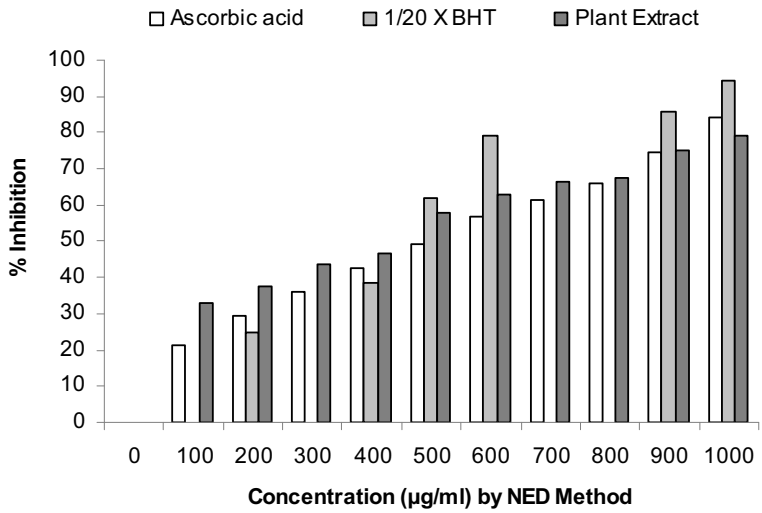

Fig. 5 Comparison of antioxidant activity of plant extract with control by NED method.

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