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ScienceDirect
Food Chemistry 103 (2007) 1350–1357
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of *Nyctanthes arbor-tristis* leaf extract

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Received 27 March 2006; received in revised form 29 August 2006; accepted 22 October 2006

Abstract

Nyctanthes arbor-tristis (Harsingar) leaf extracts are extensively used in Indian traditional medicine. The acetone-soluble fraction of its ethyl acetate extract showed impressive antioxidant activity as revealed by several *in vitro* experiments, e.g., DPPH, hydroxyl and superoxide radicals, as well as H_2O_2 scavenging assays. Moreover, its preventive capacity against Fe(II)-induced lipid peroxidation of liposomes and γ-ray-induced DNA damage also confirmed this. The strong reducing power and high phenolics and flavonoids contents could be responsible for the antioxidant activity.

Keywords: *Nyctanthes arbor-tristis*; Leaf extract; Antioxidant activity; ROS

1. Introduction

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by-products of biological reactions, or from exogenous factors (Cerutti, 1991). *In vivo*, some of these ROS play positive roles such as energy production, phagocytosis, regulation of cell growth and intercellular signalling, or synthesis of biologically important compounds (Halliwell, 1997). However, ROS may also be very damaging as they can induce oxidation of lipids, causing membrane damage, decreasing membrane fluidity, and leading to cancer *via* DNA mutation (Cerutti, 1991, 1994; Pietta, 2000). A potent scavenger of these ROS may serve as a possible preventative against free radical-mediated diseases (Ames, Gold, & Willet, 1995).

The decoction of the leaves of *Nyctanthes arbor-tristis* L. (Oleaceae), commonly known as night jasmine, is widely used in Ayurvedic medicine for the treatment of sciatica, arthritis and malaria, as well as a tonic, and a laxative (Chopra, Chopra, Handa, & Kapur, 1958; Chopra, Chopra, & Varma, 1959; Kirtikar & Basu, 1935; Nadkarni, 1976). It has also been credited with hepatoprotective, anti-leishmanial, antiviral and antifungal activities. The water-soluble portion of the alcoholic extract of *N. arbor-tristis* leaves has been reported to possess anti-inflammatory activity in a variety of experimental models (Saxena, Gupta, Saxena, Singh, & Prasad, 1984). More recently, some of these properties, such as analgesic and antipyretic (Saxena, Gupta, Saxena, Srivastava, & Prasad, 1987), anti-allergic (Gupta, Srimal, & Tandon, 1993), antimalarial (Badam, Deolankar, Rojatkar, Nagsampgi, & Wagh, 1988; Mishra, Pal, Guru, Katiyar, & Tandon, 1991), leishmanicidal (Singh, Guru, Sen, & Tandon, 1992; Tandon, Srivastava, & Guru, 1991), amoebicidal (Chitravanshi et al., 1992) and anthelmintic (Lal, Chandra, Raviprakash, & Sabir, 1976) activities have also been verified in the laboratory. The leaf extract of the plant also shows impressive immunological activity. It strongly stimulates antigen-specific and non-specific immunity, as shown by an increase in humoral and delayed type hypersensitivity responses to sheep erythrocytes, and in macrophage migration index. Interestingly, the water-soluble fraction of its ethanol extract demonstrates a significant anti-inflammatory activity against acute inflammatory oedema produced in rats by different phlogistic agents

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doi:10.1016/j.foodchem.2006.10.048

(Saxena et al., 1984), possibly by suppressing prostaglandin formation, like the other non-steroidal anti-inflammatory drugs.

Its anti-leishmanial activity has been attributed to its constituent iridoid glucosides, arbortristosides A, B, and C and 6-β-hydroxyloganin (Tandon et al., 1991) while arbortristoside A and arbortris etoposide C are antiallergic (Gupta et al., 1993). Given some of the medicinal properties of the plant might be attributed to its ROS-scavenging ability, the antioxidant properties of different extracts and some sub-fractions of the leaves were investigated in the present study. To the best of our knowledge, this activity of the plant has not been reported so far.

2. Materials and methods

2.1. Materials

Fresh leaves of *N. arbortrists-tristis* were purchased from the local market. The plant, collected from Panchgani (Maharashtra) India, was identified and authenticated by matching with the reference specimen no. 5073 at the Blatter Herbarium, St. Xavier's College, Mumbai, India. Ascorbic acid, ferrous ammonium sulfate, 2-thiobarbituric acid (TBA), NADH, nitroblue tetrazolium chloride (NBT) and 2-deoxyribose were obtained from Himedia Lab., India. H_2O_2 (35%) was purchased from Lancaster (England), while Fe(III) chloride and trichloroacetic acid (TCA) were from Thomas Baker, India. Other materials used were ethylenediamine tetraacetic acid (EDTA) (Sarabhai Chemicals, India), phenazine methosulfate (PMS) (Aldrich, USA), potassium ferricyanide (BDH, India), phenol red solution (PRS) (S.D. Fine-Chem, India), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (both from BDH, England), horse radish peroxidase (HRPO) (SRL, India), and α-tocopherol and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (both from Sigma, USA). All solutions, including the stock solution of ferrous ammonium sulfate, were freshly prepared in triply distilled water. Stock solutions of the test extracts were prepared in ethanol or 5 mM aqueous NaOH solution followed by neutralisation with aqueous HCl. Appropriate blanks were used for the individual assays.

2.2. Instrumentation

Absorbance spectrophotometry was carried out at 25 °C using a Jasco V-550 UV–vis spectrophotometer. Wavelength scans and absorbance measurements were made in 1 ml quartz cells of 1 cm path length.

2.3. Animals

The rats were bred in the BARC Laboratory Animal House Facility and procured after obtaining clearance from the BARC Animal Ethics Committee. The rats were handled following international Animal Ethics Committee guidelines.

2.4. Preparation of the plant extracts

The dried and powdered *N. arbor-tristis* leaves (100 g) were extracted successively with petroleum ether, chloroform, ethyl acetate, methanol, and water (each 1 l) for four days using a Soxhlet apparatus. The entire process was repeated thrice, each of the combined supernatants was filtered through a nylon mesh and evaporated in vacuo, to obtain the respective extracts. These extracts were designated as NP (2.8 g), NC (2.19 g), NE (3.09 g), NM (1.78 g) and NB (2.09 g), respectively, and stored in a vacuum desiccator.

2.5. Fractionation of ethyl acetate extract (NE)

NE was sub-fractionated by successive extraction with petroleum ether, chloroform, ethyl acetate, acetone, and methanol (each 2×50 ml) at room temperature. Subsequent processing of these extracts as above furnished different sub-fractions of NE, which were designated as NEP, NEC, NEE, NEA and NEM, respectively.

2.6. DPPH scavenging assay

An ethanolic solution of DPPH (100 μM) was incubated with an ethanolic solution of each of the test samples and the absorbances monitored at 517 nm. The percentage of reduction of DPPH absorbance during a 30 min period was taken as a measure of free radical-scavenging activity (Mellors & Tappel, 1966). Amongst the test samples, NEA showed the best scavenging activity and hence was used for all the subsequent studies.

2.7. Estimation of the total phenolics contents in different extracts and sub-fractions of N. arbor-tristis leaves

The method (Singleton & Rossi, 1965) downscaled to 1 ml final volume was followed to determine the amounts of total phenolics in the extract. The test samples (each 100 μl) was mixed with 1:10 Folin-Ciocalteau's reagent (500 μl) followed by addition of aqueous Na_2CO_3 (400 μl, 7.5%). After incubating the reaction mixture at 24 °C for 2 h, the absorbance at 765 nm was recorded. Gallic acid monohydrate was used as the standard. The total phenolic content of each extract is expressed in terms of mg gallic acid equivalent (mg GAE)/g dry weight of the respective extract.

2.8. Estimation of the total flavonoids contents in NEA

The known method (Jia, Tang, & Wu, 1999) downscaled to 1 ml was followed. The sample (100 μg) was added to 0.4 ml distilled water followed by $NaNO_2$ (0.03 ml, 5%). After 5 min at 25 °C, $AlCl_3 \cdot 6H_2O$ (0.03 ml, 10%) was

added, followed by aqueous NaOH (0.2 ml, 1 M) after 6 min. The mixture was diluted with water to 1 ml and the absorbance at 510 nm was read. Epicatechin was used as the standard and the total flavonoids content of NEA is expressed as mg epicatechin equivalents (mg ECE)/g dry weight of NEA.

2.9. Anti-lipid peroxidation (LPO) assay

Lipid peroxidation of liposome was carried out as reported earlier (Patro et al., 2002) with minor modifications. Briefly, small unilamellar vesicles were prepared from phosphatidylcholine (32 mg). To a total volume (1.5 ml) containing potassium phosphate buffer at pH 7.4 (50 mM), the liposome (3× from a 10× stock solution), with or without NEA, was added Fe(II) (final concentration 50 μM) and ascorbic acid (final concentration 500 μM). After incubating the mixture at 25 °C for 30 min, TCA–TBA–HCl (1 ml, 15% w/v, TCA, 0.375% w/v, TBA, 0.25 M HCl) was added. The mixture was heated at 100 °C on a boiling water bath for 15 min, the developed chromogen extracted in equal volume of water-saturated 1-butanol, and its absorbance read at 532 nm.

2.10. Hydroxyl radicals scavenging assay

The assay was performed as described by Halliwell and Gutteridge (1981). The reaction mixture (1 ml) contained the following reagents in the final concentration stated: 2-deoxyribose (2.8 mM), Fe (III) chloride (20 μM), EDTA (100 μM) (EDTA and Fe (III) chloride were mixed prior to the addition of 2-deoxyribose) and H_2O_2 (200 μM) without or with NEA (0–1200 μg/ml) in 10 mM potassium phosphate buffer (pH 7.4). The reaction was triggered by adding ascorbic acid (final concentration 300 μM) and subsequent incubation of the mixture at 37 °C for 1 h. Solutions of Fe (III) chloride, ascorbic acid and H_2O_2 were prepared in deaerated water, just prior to use. A solution of TBA in 50 mM NaOH (1 ml, 1% w/v) and TCA (1 ml, 2.8% w/v aqueous solution) was added, the mixture heated for 15 min on a boiling water bath and the amount of chromogen produced was measured at 532 nm.

2.11. Hydrogen peroxide scavenging assay

The procedure employed was essentially the same as that of Pick and Keisari (1980). The buffered phenol red solution (PRS) used in all the assays contained the following reagents in the final concentration stated: sodium chloride (140 mM), dextrose (5.5 mM), phenol red (0.28 mM) and HRPO (8.5 U/ml) in 10 mM potassium phosphate buffer (pH 7.0). Phenol red and HRPO were added to the buffer briefly before carrying out the experiment. Different concentrations of NEA and H_2O_2 (final concentration 60 μM) were incubated at 25 °C for 30 min and the concentration of H_2O_2 remaining in each case was assayed by adding PRS followed by NaOH (10 μl, 1 N), and measuring the absorbance at 610 nm against an appropriate blank. The concentration of the H_2O_2 stock solution was calculated from its absorbance at 230 nm, using an extinction coefficient of 81 mol^{-1} cm^2. α-Tocopherol was used as a reference inhibitor.

2.12. Superoxide radicals scavenging assay

The superoxide scavenging ability of NEA was assessed by a reported method (Nishikimi, Rao, & Yagi, 1972). The reaction mixture, contained the following reagents in the final concentration stated: NEA (0–45 μg/ml), PMS (30 μM), NADH (338 μM) and NBT (72 μM) in a phosphate buffer (0.1 M, pH 7.4). It was incubated at room temperature for 5 min, and the absorbance of the solution at 560 nm was measured against an appropriate blank that did not contain any NADH. BHA was used as a reference inhibitor.

2.13. DNA strand break assay

The assay was carried out as previously described (Subramanian, Chintalwar, & Chattopadhaya, 2003). Briefly the reaction mixture (13 μl) containing pBR322 plasmid DNA (final amount 200 ng) in 10 mM phosphate buffer (pH 7.4), in the presence or absence of NEA, was irradiated with a ^{60}Co source at 25 °C up to a dose of 17 Gy (dose rate of 8 Gy/min). After irradiation the resultant open circular and supercoiled forms of the plasmid DNA were separated by electrophoresis and visualised under ultraviolet light after staining with ethidium bromide. The relative intensities of the bands were determined with a Bio-Rad gel documentation system.

2.14. Assay of reducing power of NEA

The reducing power of NEA was quantified by the method of Yen and Chen (1995). Briefly, a reaction mixture (total volume 1 ml) containing NEA (final concentration 0–30 μg) in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1% w/v) at 50 °C for 20 min. After terminating the reaction by adding TCA solution (10% w/v), the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1% w/v) solution and the absorbance at 700 nm was measured. Increased absorbance of the reaction mixture was indicative of increased reducing power.

3. Results and discussion

The herb, *N. arbor-tristis* is traditionally used for the treatment of sciatica and arthritis that are induced by free radicals. The leaves contain several well-known antioxidants such as tannic acid, methyl salicylate, mannitol, ascorbic acid and carotene, besides amorphous glucosides, resin, and traces of volatile oils (Zaheer, Prasad, Chopra,

Santapau, & Krishnan, 1966). In view of this, we studied its antioxidant activity by a series of *in vitro* protocols, using some biologically relevant models.

For this, the plant constituents were initially partitioned by extracting with solvents of increasing polarities, viz., petroleum ether, chloroform, ethyl acetate, methanol, and *n*-butanol, to furnish NP, NC, NE, NM and NB. Based on the DPPH assay results, the antioxidant activity was primarily located in NE. Subsequent fractionation of NE by solvent extraction furnished different sub-fractions. The acetone soluble sub-fraction (NEA) showed maximum DPPH scavenging activity. Consequently, its antioxidant property was assessed in detail, using a series of *in vitro* methods. The activity was subsequently correlated in terms of its total phenolics and flavonoids contents.

3.1. DPPH scavenging activities of different extracts and sub-fractions of N. arbor-tristis leaves

The bleaching of DPPH absorption (517 nm) by a test compound is representative of its capacity to scavenge free radicals, generated independent of any enzymatic or transition metal-based systems. Hence the DPPH-scavenging activities of a fixed concentration (30 μg/ml) of the extracts were taken as the parameter to check their antioxidant potential. The results shown in Fig. 1 clearly indicate that NE was the strongest radical scavenger (~81% scavenging), followed by NB (~55% scavenging). NM also showed some scavenging activity (~23%), while the less polar extracts (NP and NC) were inactive. Amongst the sub-fractions of the ethyl acetate extract (NE), NEM and, especially, NEA showed impressive scavenging activities (~15% and 20% respectively), the assay being carried out at a much lower concentration (3 μg/ml) (Table 1). A concentration dependent study with NEA and α-tocopherol as the positive control revealed their IC_{50} values as 14.89 ± 0.12 and 5.23 ± 0.02 μg/ml, respectively.

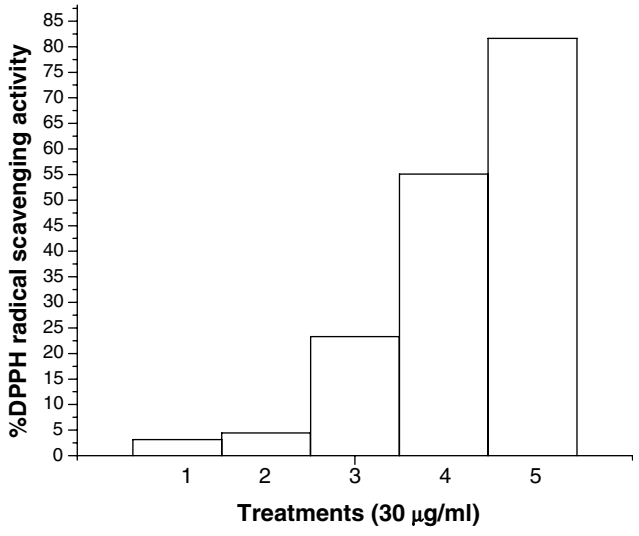

Fig. 1. DPPH radical-scavenging activities of *Nyctanthes arbor-tristis* leaf extracts at 30 μg/ml concentration: (1) NP; (2) NC; (3) NM; (4) NB and (5) NE. The values are mean ± SE ($n = 4$).

Table 1
DPPH radical-scavenging activities of the sub-fractions of NE

Sample	% DPPH radical scavenging activity[a]
NEP	0.1 ± 0.03
NEC	0.01 ± 0.09
NEE	15.1 ± 0.25
NEM	15.3 ± 0.21
NEA	19.6 ± 0.13

[a] The values are mean ± SE ($n = 4$).

3.2. Polyphenolic and flavonoids contents of NEA

It is well-known that plant phenolics, in general, are highly effective free radical scavengers and antioxidants. Consequently the antioxidant activities of plant/herb extracts are often explained with respect to their total phenolics and flavonoids contents, with good correlation. We also observed similar correlation between the DPPH radical-scavenging activities of the plant extracts and sub-fractions in the present study. The total phenolics in the extracts (NP, NC, NE, NM and NB) and the sub-fractions of NE (NEA and NEM) were determined spectrophotometrically by the Folin-Ciocalteu method and expressed as mg GAE/g of the test samples (Table 2). As revealed by the data, the total phenolics contents of NE, NM and NB (~297, 231 and 223 mg GAE/g of sample) were significantly higher than those of NP and NC (198 and 60 mg GAE/g of sample). Overall, the order of the phenolic contents of the test samples was NE > NM > NB > NP > NC, which was broadly similar to their DPPH radical-scavenging abilities. Fractionation of NE led to enrichment of the phenolics contents, NEA being more enriched than NEM. The total phenolics and flavonoids contents of NEA were found to be ~330 mg GAE/g and 368.7 ± 0.5 mg ECE/g. Thus, NEA contains high levels of total phenolics and flavonoids, which may account for its impressive antioxidant activity.

3.3. Anti-LPO activity of NEA

Owing to the high levels of unsaturation and the increased consumption of oxygen, mitochondrial lipids

Table 2
Total phenolics contents of various extracts and sub-fractions

Test sample	Polyphenolic content (mg/g gallic acid equivalents (GAE)[a])
NP	198 ± 4.22
NC	60 ± 1.41
NE	297 ± 3.11
NM	231 ± 0.81
NB	223 ± 3.14
NEA	330 ± 2.88
NEM	285 ± 1.37

[a] The values are mean ± SE ($n = 4$).

are susceptible to oxidative damage. Lipid peroxidation can inactivate cellular components and play a major role in oxidative stress in biological systems. Further, several toxic byproducts of the peroxidation can damage other biomolecules including DNA away from the site of their generation (Box & Maccubbin, 1997; Esterbauer, 1996). It is well-established that transition metal ions like iron and copper stimulate lipid peroxidation through various mechanisms (Halliwell & Gutteridge, 1984). These may either generate hydroxyl radicals to initiate the lipid peroxidation process and/or propagate the chain process *via* decomposition of lipid hydroperoxides (Braughler, Chase, & Pregenzer, 1987). Hence the protective capacity of NEA against liposomal LPO was studied.

The LPO of liposomes was triggered with Fe(II)-ascorbic acid, and the end-products of the process were measured in terms of the thiobarbituric acid reactive substrates (TBARS) formed. In unstimulated experiments, the amount of TBARS was marginal, the absorption at 532 nm (A_{532}) being only 0.20 ± 0.02 ($n = 4$). In control experiments, where lipid peroxidation was stimulated by Fe(II) (50 μM) and ascorbic acid (200 μM), the A_{532}value increased to 0.89 ± 0.01 ($n = 4$). NEA and the positive control, α-tocopherol, inhibited lipid peroxidation in a concentration dependent manner (Fig. 2). For example, the protective activities of 3, 6, and 12 μg/ml of NEA were 39.6 ± 2.4, 52.3 ± 1.35, and 64.3 ± 1.64, respectively. From this, the IC_{50} value of its anti-LPO activity was found to be 5.88 ± 0.68, while that of the positive control, α-tocopherol, showed an IC_{50} value of 3.73 ± 0.40 μg/ml.

3.4. Hydroxyl radical scavenging activity of NEA

Among the ROS, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism (Waling, 1975). Due to their high reactivity, the radicals have a very short biological half-life. Thus, an effective scavenger of them must be present at a very high concentration or possess very high reactivity towards these radicals. Consequently, the ability of NEA to scavenge these radicals was evaluated by the 2-deoxyribose assay.

In a reaction mixture containing Fe(III)–EDTA–H_2O_2 and 2-deoxyribose, very little TBARS were formed ($A_{532} = 0.09 \pm 0.01$, $n = 4$), while addition of ascorbic acid triggered production of TBARS ($A_{532} = 1.84 \pm 0.08$, $n = 4$). As shown in Fig. 3, NEA inhibited the degradation of 2-deoxyribose in a concentration dependent manner with high efficiency (IC_{50} 526 ± 2.55 μg/ml). Its hydroxyl radical-scavenging activities were 38.9 ± 1.16 and 61.4 ± 1.17 at 250 and 700 μg/ml, respectively. The positive control, mannitol (1.02 mM) showed $19 \pm 1\%$ protection under the same conditions. Thus, NEA was found to be a significantly better hydroxyl radical scavenger than mannitol.

3.5. H_2O_2 scavenging activity of NEA

Although not a radical species, H_2O_2 is an important ROS, contributing to oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes are believed to react with H_2O_2 *in vivo* to generate highly reac-

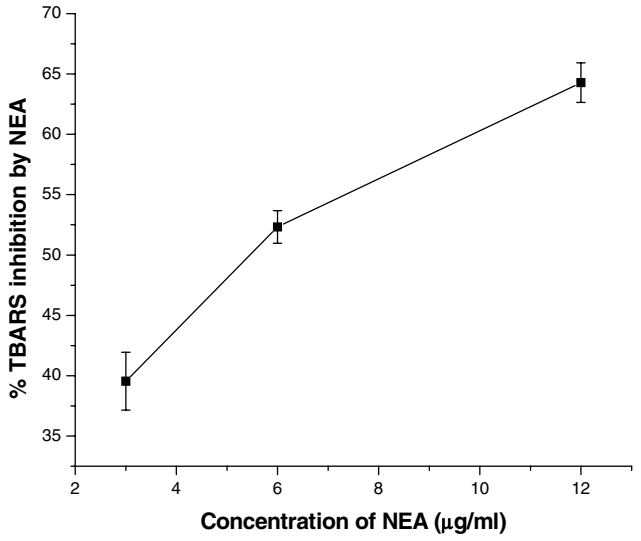

Fig. 2. Concentration dependent protective activities of NEA against Fe(II) (50 μM)-ascorbic acid (500 μM) mediated lipid peroxidation of liposome, measured in terms of TBARS formed. The values are mean ± SE ($n = 4$).

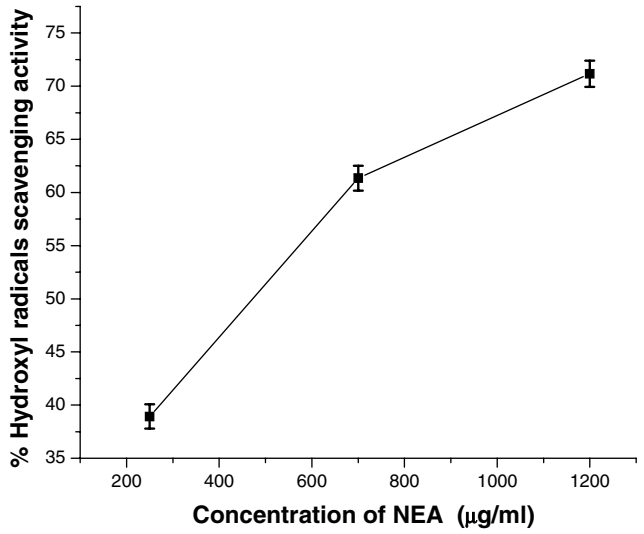

Fig. 3. Concentration dependent hydroxyl radicals scavenging activities of NEA. The assays were carried out by measuring the TBARS formed by the oxidation of 2-deoxyribose (2.8 μM) with Fe(III) (20 μM), EDTA (100 μM), ascorbic acid (300 μM), H_2O_2 (200 μM). The values are mean ± SE ($n = 4$).

tive hydroxyl radicals in a superoxide-driven Fenton reaction. Hence, the efficacy of NEA for degrading H_2O_2 was analysed following the procedure of Pick and Keisari (1980). NEA showed good activity in depleting H_2O_2, with an IC_{50} value of 29.5 ± 0.23 μg/ml (Fig. 4), which was similar to that of the positive control α-tocopherol (IC_{50} value of 29.8 ± 0.45 μg/ml).

3.6. Superoxide radicals scavenging activity of NEA

Despite involvement in many pathological processes, the superoxide radicals by themselves are not as reactive as the hydroxyl radicals. But they can give rise to toxic hydroxyl radicals, damaging biomacromolecules directly or indirectly (Cotelle et al., 1992) with severe consequences. The superoxide radicals have been implicated as playing crucial roles in ischemia-reperfusion injury (Radi, Beckman, Bush, & Freeman, 1991). Thus, scavenging of these radicals would be a promising remedy for this disease.

Consequently, the scavenging activity of NEA for superoxide radicals generated by a PMS/NADH system was assessed by measuring the absorbance at 560 nm (Nishikimi et al., 1972). Fig. 5 shows the scavenging property of NEA (0–40 μg/ml) against superoxide radical anions. NEA could scavenge superoxide radicals effectively, the extent of scavenging being 44.5 ± 0.28 and $56.4 \pm 0.79\%$ at 30 and 45 μg/ml, respectively. NEA showed a higher potency than BHA under the chosen conditions. The comparative IC_{50} values of NEA and BHA were 37.3 ± 0.65 and 180 ± 0.38 μg/ml.

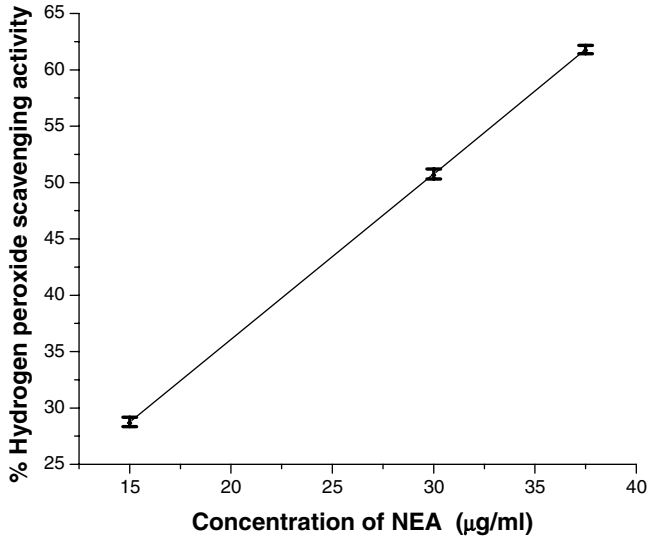

Fig. 4. Concentration dependent hydrogen peroxide scavenging activity of NEA. The assays were carried out by measuring the decrease in absorbance of hydrogen peroxide (60 μM) at 610 nm, after addition of NEA. The values are mean ± SE ($n = 4$).

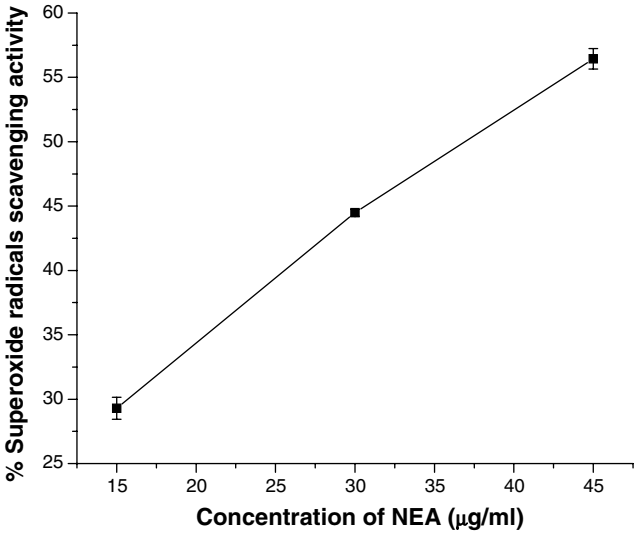

Fig. 5. Concentration dependent scavenging activity of NEA for the superoxide radicals generated by PMS (30 μM), NADH (338 μM) and NBT (72 μM) in phosphate buffer (0.1 M pH 7.4). The values are mean ± SE ($n = 4$).

3.7. Protective activity of NEA against γ-ray-induced DNA damage

It is well-established that the hydroxyl and superoxide radicals play major roles in various cellular damage, such as DNA cleavage due to exposure to ionising radiation. Given that NEA showed excellent scavenging properties for both these radicals, its radioprotective activity was also studied. For this, its preventive potential against the γ-ray induced nicking of pBR 322 plasmid DNA was assessed.

Exposure of pBR322 plasmid DNA to γ-radiation, as a function of dose, resulted in a significant increase in the single strand break of DNA (data not shown). A linear increase in single strand breaks, assessed as average strand breaks per DNA molecule, was observed up to a dose of 17 Gy (dose rate 8 Gy/min). The radiation dose of 17 Gy was sufficient to convert most of the supercoiled DNA to the open circular form. Hence, this dose was chosen for further studies. Fig. 6 shows the agarose gel electrophoresis

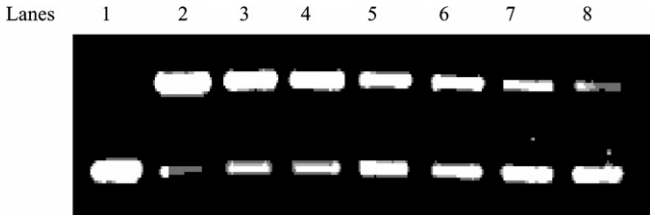

Fig. 6. Gel electrophoresis pattern of plasmid pBR 322 exposed to γ-ray in the presence and absence of different concentrations of NEA. Plasmid DNA (200 ng) samples in 10 mM potassium phosphate buffer, pH 7.4 in a 20 μl volume were irradiated at 25 °C up to a dose of 17 Gy using a ^{60}Co source. 1 – DNA sample without γ-irradiation; 2 – DNA sample after γ-irradiation; 3–8 – DNA samples after γ-irradiation in the presence of 5, 10, 25, 50, 100 and 200 μg/ml NEA, respectively.

pattern of plasmid DNA irradiated at 17 Gy in the absence and presence of NEA at five different concentrations (5, 10, 25, 50, 100 and 200 μg/ml). Compared to the unirradiated DNA (control, lane 1), exposure of DNA to γ-radiation (17 Gy) led to extensive conversion of the supercoiled form to the open circular form (lane 2). Addition of NEA in increasing concentrations to the DNA, prior to irradiation, progressively reduced the intensity of the band due to the open circular form (lanes 3–8). Thus, NEA showed very good radioprotective activity with an IC_{50} value 31.1 ± 0.23 μg/ml. The quantification data on the extent of radioprotection by NEA are presented in Table 3.

3.8. Reducing power of NEA

The antioxidant activity of certain plant extracts have been correlated with their reducing powers (Pin-Der-Duh, 1998; Tanaka, Kuie, Nagashima, & Taguchi, 1988). The reducing properties are generally associated with the presence of reductones (Pin-Der-Duh, 1998), which exert antioxidant action by breaking the free radical chains, *via* hydrogen atom donation (Gordon, 1990). Reductones are also reported to prevent peroxide formation, by reacting with certain precursors of peroxide.

In this study, the reducing powers of NEA and BHT were assayed and the comparative results are shown in Table 4. Under similar conditions, NEA showed a marginally higher reducing power than BHT over the entire concentration range tested. This factor is likely to contribute significantly towards the observed antioxidant effects of NEA.

Table 3
Concentration dependent protective activity of NEA against γ-ray induced pBR322 plasmid DNA damage

Conc. of NEA (μg/ml)	% protection by NEA[a]
5.0	18.5 ± 1.74
10.0	24.7 ± 0.24
25.0	47.3 ± 0.68
50.0	70.0 ± 0.98
100.0	73.9 ± 0.39
200.0	80.2 ± 1.6

[a] The values are mean ± SE ($n = 4$).

Table 4
Comparative reducing powers of NEA and BHT

Concentration of the test samples (μg/ml)	Absorbance at 700 nm[a]	
	NEA	BHT
3.0	0.88 ± 0.001	0.80 ± 0.002
7.5	1.14 ± 0.002	1.07 ± 0.003
15.0	1.53 ± 0.008	1.46 ± 0.004
30	2.17 ± 0.012	1.92 ± 0.02

[a] The values are mean ± SE ($n = 4$).

4. Conclusions

Overall, NEA showed impressive antioxidant activity, due to its ability to scavenge various biologically relevant ROS and inhibit lipid peroxidation and DNA strand breaks. The impressive antioxidant activity could be attributed to its superior reducing power, possibly originating from its high contents of polyphenolics and flavonoids. Earlier, some extracts of the plant have been reported to be cytotoxic (Khatune, Mosaddik, & Haque, 2001) and induce stomach ulceration (Saxena et al., 1987). However, these adverse effects are manifested at a significantly higher concentration than used in the present study. Thus, the plant might be a good candidate for further investigation in developing new antioxidants/radioprotectants.

References

Ames, B. N., Gold, L. S., & Willet, W. C. (1995). The causes and prevention of cancer. *Proceedings of the National Academy of Sciences USA, 92*, 5258–5265.

Badam, L., Deolankar, R. P., Rojatkar, S. R., Nagsampgi, B. A., & Wagh, U. V. (1988). In vitro antimalarial activity of medicinal plants of India. *Journal of Medical Research, 87*, 379–383.

Box, H. C., & Maccubbin, A. E. (1997). Lipid peroxidation and DNA damage. *Nutrition, 13*, 920–921.

Braughler, J. M., Chase, R. L., & Pregenzer, J. F. (1987). Oxidation of ferrous ion during peroxidation of various lipid substrates. *Biochimica Biophysica Acta, 921*, 457–464.

Cerutti, P. A. (1991). Oxidant stress and carcinogenesis. *European Journal of Clinical Investigation, 21*, 1–11.

Cerutti, P. A. (1994). Oxy-radicals and cancer. *Lancet, 344*, 862–863.

Chitravanshi, V. C., Singh, A. P., Ghoshalm, S., Prasad, B. N. K., Srivastava, V., & Tandon, J. S. (1992). Therapeutic action of *Nyctanthes arbor-tristis* against caecal amoebiasis of rat. *International Journal of Pharmacognosy, 30*, 71–75.

Chopra, R. N., Chopra, I. C., Handa, K. L., & Kapur, L. D. (1958). *Chopra's indigenous drugs of India* (2nd ed.). Calcutta, India: UN Dhar and Sons (p. 480).

Chopra, R. N., Chopra, I. C., & Varma, B. S. (1959). *Supplement to glossary of Indian medicinal plants*. New Delhi: CSIR (pp. 63–64).

Cotelle, N., Bernier, J. L., Henichart, J. P., Catteau, J. P., Gaydou, E., & Wallet, J. C. (1992). Scavenger and antioxidant properties of ten synthetic flavones. *Free Radical Biology & Medicine, 13*, 211–219.

Esterbauer, H. (1996). Estimation of peroxidative damage: a critical review. *Pathologya Biologie Paris, 44*, 25–28.

Gordon, M. H. (1990). The mechanism of antioxidant action in vitro. In B. J. F. Hudson (Ed.), *Food antioxidants* (pp. 1–18). London: Elsevier Applied Science.

Gupta, P. P., Srimal, R. C., & Tandon, J. S. (1993). Antiallergic activity of some traditional Indian medicinal plants. *International Journal of Pharmacognosy, 31*, 15–18.

Halliwell, B. (1997). Antioxidants and human diseases: a general introduction. *Nutrition Review, 55*, S44–S52.

Halliwell, B., & Gutteridge, J. M. C. (1981). Formation of thiobarbituric-acid-reactive substances from deoxyribose in the presence of iron salts: the role of super oxide and hydroxyl radicals. *FEBS Letters, 128*, 347–352.

Halliwell, B., & Gutteridge, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal, 219*, 1–14.

Jia, Z., Tang, M., & Wu, J. (1999). The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555–599.

Khatune, N. A., Mosaddik, M. A., & Haque, M. E. (2001). Antibacterial activity and cytotoxicity of *Nyctanthes arbor-tristis* flowers. *Fitoterapia, 72*, 412–414.

Kirtikar, K. R., & Basu, B. D. (1935). *Indian medicinal plant* (2nd ed.). Allahabad, India: LM Basu Publ. (pp. 1526–1528).

Lal, J., Chandra, S., Raviprakash, V., & Sabir, M. (1976). In vitro anthelmintic action of some indigenous medicinal plants on *Ascaridia Galli* worms. *Indian Journal of Physiology & Pharmacology, 20*, 64–68.

Mellors, A., & Tappel, A. L. (1966). The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *Journal of Biological Chemistry, 241*, 4353–4356.

Mishra, P., Pal, N. L., Guru, P. Y., Katiyar, J. C., & Tandon, J. S. (1991). Antimalarial activity of traditional plants against erythrocytic stages of *Plasmodium berghei*. *International Journal of Pharmacognosy, 29*, 19–23.

Nadkarni, K. M. (1976). *Indian materia medica* (Vol. 1). Bombay, India: Bombay Popular Prakashan (p. 857).

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochemical Biophysical Research Communication, 46*, 849–864.

Patro, B. S., Rele, S., Chintalwar, G. J., Chattopadhyay, S., Adhikari, S., & Mukherjee, T. (2002). Protective activities of some phenolic 1,3-diketones against lipid peroxidation: possible involvement of the 1,3-diketone moiety. *ChemBioChem, 3*, 364–370.

Pick, E., & Keisari, Y. (1980). A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. *Journal of Immunological Methods, 38*, 161–170.

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products, 63*, 1035–1042.

Pin-Der-Duh, X. (1998). Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free-radical and active oxygen. *Jorunal of the American Oil Chemists Society, 75*, 455–461.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991). Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics, 288*, 481–487.

Saxena, R. S., Gupta, B., Saxena, K. K., Singh, R. C., & Prasad, D. N. (1984). Study of antiinflammatory activity in the leaves of *Nyctanthes arbor-tristis* Linn, an Indian medicinal plant. *Journal of Ethnopharmacology, 11*, 319–330.

Saxena, R. S., Gupta, B., Saxena, K. K., Srivastava, V. K., & Prasad, D. N. (1987). Analgesic, antipyretic and ulcerogenic activity of *Nyctanthes arbor-tristis* leaf extract. *Journal of Ethnopharmacology, 19*, 193–200.

Singh, U. K., Guru, P. Y., Sen, A. B., & Tandon, J. S. (1992). Antileishmanial activity of traditional plants against *Leishmania donovani* in golden hamsters. *International Journal of Pharmacognosy, 30*, 289–295.

Singleton, V. L., & Rossi, J. A. Jr., (1965). Colorimetry of total phenolics with phosophomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Subramanian, M., Chintalwar, G. J., & Chattopadhaya, S. (2003). The radioprotective property of *Tinospora cordifolia* polysaccharide. *Indian Journal of Biochemistry and Biophysics, 40*, 22–26.

Tanaka, M., Kuie, C. W., Nagashima, Y., & Taguchi, T. (1988). Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi, 54*, 1409–1414.

Tandon, J. S., Srivastava, V., & Guru, P. Y. (1991). Iridoids: a new class of leishmanicidal agents from *Nyctanthes arbor-tristis*. *Journal of Natural Products, 54*, 1102–1104.

Waling, C. (1975). Fenton's reagent revisited. *Accounts of Chemical Research, 8*, 125–131.

Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural Food Chemistry, 43*, 27–32.

Zaheer, S. H., Prasad, B., Chopra, R. N., Santapau, H., & Krishnan, M. S. (1966). *The wealth of India* (vol. 7). New Delhi, India: CSIR (pp. 69–70).