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Antioxidant Capacity of *Rubus niveus* Thunb. and *Elaeocarpus oblongus* Gaestn. Fruits

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ABSTRACT

In this study, antioxidant capacity of the crude methanolic extract of *Rubus niveus* and *Elaeocarpus oblongus* fruit was assessed for the first time. The methanolic extract of *R. niveus* and *E. oblongus* fruits displayed excellent scavenging capacity towards 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH•) (EC_{50} = 0.200 ± 0.76 and 0.250 ± 1.76 mg mL^{-1}), superoxide anion ($O_2^{\cdot-}$) (EC_{50} = 0. 50 ± 0.82 and 0. 50 ± 1.20 mg mL^{-1}), hydroxyl ion (•OH) (EC_{50} = 0.250 ± 0.22 and 0.500 ± 0.48 mg mL^{-1}) and nitric oxide (NO) (EC_{50} = 0.250 ± 0.22 and 0. 250 ± 0.552 mg mL^{-1}). The results also showed that *R. niveus* and *E. oblongus* extracts had a strong reductive capacity (OD at 700 nm) (EC_{50} = 0. 315 ± 1.32 and 0. 221 ± 0.52 mg ml^{-1}, strong ferric ion (Fe^{3+}) chelation (EC_{50} = 0.251 ± 1.02 and 0.251 ± 1.42 mg mL^{-1}) and remarkable reduction of lipid peroxidation (LPO) (EC_{50} = 0.502 ± 0.24 and 0.502 ± 0.38 mg mL^{-1}). The antioxidant capacity of both extracts was comparable with antioxidant standards, butyl hydroxy toluene (BHT), EDTA (iron chelation) and catechin. The results of the present study revealed that the fruits of *R. niveus* and *E. oblongus* possess potent antioxidant activity.

Keywords: antioxidant activity, DPPH scavenging, lipid peroxidation

INTRODUCTION

Reactive oxygen species (ROS), including superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (•OH), and reactive nitrogen species (RNS), nitric oxide (NO•) are known to damage cellular biomolecules (DNA, proteins, lipids, amines and carbohydrates), resulting in ageing and other degenerative diseases (Aruoma 1994; Sogut *et al.* 2003). In recent years, increasing *in vitro* studies, epidemiological studies and intervention trials have consistently indicated the role of consumption of fruits and vegetables as antioxidants in the prevention of degenerative and chronic diseases (Cox *et al.* 2000; Scalbert *et al.* 2005). These protective effects are mostly related to the antioxidant components i.e. vitamins, flavonoids, and carotenoids (Rice-Evans 2001; Prior 2003). Previous studies have demonstrated the antioxidant activities and health benefits of various fruits (Wang *et al.* 1996; Kahkonen *et al.* 2001; Garcia-Alonso *et al.* 2004).

Rubus niveus Thunb. (raspberry) and *Elaeocarpus oblongus* Gaestn are edible fruits and well flavoured. *R. niveus*, belonging to the family Rosaceae, is cultivated throughout the world for its heavy production of sweet fruit (Morton 1987). Raspberries are a rich repository of phenolic components and possess excellent antioxidant properties (Deighton *et al.* 2000; Halvorsen *et al.* 2002; Reyes-Carmona *et al.* 2005; Wolfe *et al.* 2008; Zhang *et al.* 2010). However, as far as we know, no data on antioxidant capacity of Indian *R. niveus* exists. *E. oblongus*, belonging to the family Myrtaceace, is a pearl-like edible fruit. Fruits are used as an emetic for rheumatism, pneumonia, ulcers, piles, leprosy, and dropsy disease (Wealth of India 1991). This is a prominent tree species in Nilgiris, India (Manigandan 2009). However, no studies have been identified on antioxidant properties of these under-utilized fruits from Nilgiris, Tamil Nadu State, India. The main objective of the present study was to investigate the *in vitro* antioxidant potential of the crude methanolic extracts of *R. niveus* and *E. oblongus*.

MATERIALS AND METHODS

Chemicals and reagents

2-deoxy-D-ribose, potassium ferricyanide, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), L-ascorbic acid, 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS), quercetin, (+)-catechin, 1,1-diphenyl-2- picryl-hydrazyl (DPPH•), nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), ethylene diamine tetraacetic acid disodium salt (EDTA-Na_2), hydrogen peroxide, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Merck Co., India. All other chemicals and solvents used were of analytical grade. Milli-Q quality water was used to prepare all reagents and solutions.

Fruits and preparation of extract

The fully ripened fruits of *R. niveus* and *E. oblongus* were collected from Doddabetta forest range, Nilgiris (T. N.), India during May 2007 and June 2007. The plants were identified by Dr. R. Gopalan, Taxonomist, Karpagam University, Coimbatore, India and were authenticated in Botanical Survey of India (southern circle), Coimbatore, India. The ripened fruits were manually pooled and were kept in cold (-4°C) dark storage until further analysis. The frozen berries (100 g) were blended, exhaustively extracted with 5 times its volume of methanol (1: 5, v/v) and centrifuged (3000 × *g*, Remi, India) for 15 min at 4°C and the supernatant was transferred to an amber bottle. The extraction process was repeated three times under the same conditions. The supernatants were then combined and filtered with Whatman No. 1 filter paper. The filtered extract was concentrated *in vacuo* at 40 ± 1°C by a rotary flash evaporator (Buchi-type Rotavapor, Flawil, Switzerland) under reduced pressure to obtain the dry extract, which was re-dissolved in methanol; the stock solution was kept at -4°C in the dark to protect from light until further use. The stock solution was used to determine antioxidant capacity.

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Research Note

In vitro antioxidant analysis

1. Scavenging capacity towards DPPH· stable radical

The determination of DPPH· stable radical scavenging activity of the extracts was based on a method described previously (Singh *et al*. 2002). Briefly, 1-mL aliquots of the extract and standards (0.01-1 mg mL^{-1}) were added to MeOH solution of DPPH· (5 mL, 0.1 mM) and vortexed. After 20 min reaction at 25°C, absorbance was measured at 517 nm against a blank in a UV-Vis spectrophotometer (Elico Scanning mini spec SL 177, India). BHT and ascorbic acid were used for comparison. The control contained no extracts. The percentage quenching of DPPH· was calculated as follows:

Inhibition of DPPH· (%) = $1\text{-Sample}_{517\ nm}/\text{Control}_{517\ nm} \times 100$

where $\text{Sample}_{517\ nm}$ was absorbance of the sample and $\text{Control}_{517\ nm}$ was absorbance of the control.

The results were expressed as EC_{50}, which means the concentration at which DPPH· radicals were quenched by 50%.

2. Measurement of reductive capacity (RC)

The reducing capacity of *R. niveus* and *E. oblongus* extracts was measured using the potassium ferricyanide reduction method (Oyaizu 1986). Various concentrations of the extract and standards (0.025-1 mg mL^{-1}) were added to 2.5 ml of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide $[K_3Fe_3(CN)_6]$ (1%) solution and vortexed. After incubation at 50°C for 20 min, 2.5 ml of TCA (10%, w/v) was added to all the tubes and centrifuged (Remi, India) at 3000 × *g* for 10 min. Afterwards, the upper layer of the solution (5 mL) was mixed with deionized water (5 mL). To this, 1 mL of $FeCl_3$ (1%) was added to each test tube and incubated at 35°C for 10 min. The formation of Perl's Prussian colour was measured at 700 nm in a UV-Vis spectrophotometer (Elico Scanning mini spec SL 177, India). Increased absorbance of the reaction mixture indicated increasing reducing power. BHT and BHA were used for comparison.

3. Scavenging capacity towards super oxide anion ($O_2^{\cdot -}$)

Super oxide anion radicals ($O_2^{\cdot -}$) generated in the phenazine methosulfate-reduced form of nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium chloride (NBT) by the extract with some changes (Liu *et al*. 1997). The $O_2^{\cdot -}$ were generated in 1.25 mL of Tris–HCl (16 mM, pH 8.0), 0.25 mL of NBT (150 μM), 0.25 mL of NADH (468 μM) and different concentrations (0.025-1 mg mL^{-1}) of *R. niveus* and *E. oblongus* extracts and standards. The reaction was initiated by addition of 0.25 mL of phenazine methosulphate (PMS) (60 μM) to the mixture. Following incubation at ambient temperature for 5 min the absorbance was read at 560 nm in a UV-Vis spectrophotometer. BHT and catechin were used for comparison. The control contained no extracts. The percentage scavenging of $O_2^{\cdot -}$ was calculated as follows:

Inhibition of $O_2^{\cdot -}$ (%) = $1\text{-Sample}_{560\ nm}/\text{Control}_{560\ nm} \times 100$

where $\text{Sample}_{560\ nm}$ was absorbance of the sample and $\text{Control}_{560\ nm}$ was absorbance of control.

4. Scavenging capacity towards hydroxyl ion ($^{\cdot}OH$) radicals

Hydroxyl radicals ($^{\cdot}OH$) were generated by a Fenton reaction model system, and the scavenging capacity towards the $^{\cdot}OH$ radical was measured using deoxyribose method with minor modifications (Halliwell *et al*. 1997). To 1 mL of *R. niveus* and *E. oblongus* extracts (0.025-1 mg mL^{-1}), 1 mL of phosphate buffer (50 mM; pH 7), 0.2 mL of EDTA (1.04 mM), 0.2 mL of $FeCl_3$. $6H_2O$ (1.0 mM) and 0.2 mL of 2-deoxy-d-ribose (60 mM) were added. Following incubation in a water bath at 37°C for 60 min, 2 mL of cold TBA (in 50 mM NaOH) and 2 mL of TCA (25% w/v aqueous solution) were added to the reaction mixture. The mixture was then incubated at 100°C for 15 min. After cooling, the absorbance of the pink chromogen developed was recorded at 532 nm in a spectrophotometer. BHT and catechin were used for comparison. The control contained no extracts. The percentage scavenging of $^{\cdot}OH$ was calculated as follows:

Inhibition of $OH^{\cdot}$ (%) = $1\text{-Sample}_{532\ nm}/\text{Control}_{532\ nm} \times 100$

where $\text{Sample}_{532\ nm}$ was absorbance of the sample and $\text{Control}_{532\ nm}$ was absorbance of the control.

5. Scavenging capacity towards nitric oxide (NO)

Nitric oxide (NO) generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH was estimated by the use of Griess reaction with minor changes (Green *et al*. 1982). The reaction mixture (3 mL) containing SNP (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and the methanol extract of *R. niveus* and *E. oblongus* at different concentrations and standards (25-1000 µg mL^{-1}) were incubated at 25°C for 150 min. After incubation, 0.5 mL of the incubated solution containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of *N*-1-naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. The absorbance of pink coloured chromophore formed during diazotization was immediately measured at 540 nm in a UV-Vis spectrophotometer. BHT and catechin were used for comparison. The control contained no extracts. The percentage scavenging of NO was calculated as follows:

Inhibition of NO (%) = $1\text{-Sample}_{540\ nm}/\text{Control}_{540\ nm} \times 100$

where, Sample_{540nm} was absorbance of the sample and Control_{540nm} was absorbance of the control.

6. Measurement of iron chelating capacity

Iron chelating capacity (ICC) was investigated using the method of Singh and Rajini (2004). Briefly, different concentrations of *E. kologa* and standards (0.025-1 mg mL^{-1}) were mixed with 0.1 ml of $FeCl_2$ (2 mM) and 0.2 ml of ferrozine (5 mM). The mixture was made into 0.8 mL with deionized water. After 10 min incubation at room temperature, the optical density value of ferrous ion-ferrozine complex was measured at 562 nm in a UV-Vis spectrophotometer. EDTA and catechin were used as standards for iron chelating assay. The control contained no extracts. The percentage of inhibition of ferrozine-fe^3 complex formation was calculated as:

Chelating effect (%) = $1\text{-Sample}_{562\ nm}/\text{Control}_{562\ nm} \times 100$

where $\text{Sample}_{562\ nm}$ was absorbance of the sample and $\text{Control}_{562\ nm}$ was absorbance of the control.

7. Reduction of lipid peroxidation

Inhibition of lipid peroxidation (LPO) in rat lever homogenate was determined in terms of formation of thiobarbituric acid reactive substances (TBARS) with minor changes (Halliwell and Gutteridge 1985). In brief, different concentrations of *R. niveus* and *E. oblongus* extracts and standard (0.025-1 mg mL^{-1}) were individually added to 0.2 mL of liver homogenate (10%) extracted with KCl (15%). To the above mixture, 0.1 mL of $FeSO_4$ (10 mM) solution was added to initiate LPO. The volume of the mixtures was finally made up to 2 mL with phosphate buffer (0.1 mM, pH 7) and incubated at 37°C for 30 min. At the end of the incubation period, reaction mixture (0.3 mL) was added with 1 mL of TBA (0.8%, w/v) and 0.1 mL of TCA (20%) solution. The mixture was then heated on a water bath at 100°C for 60 min. After cooling, *n* – butanol (4 mL) was added in each tube and centrifuged at 3000 × *g* for 10 min. The absorbance of the organic upper layer was read at 532 nm in UV-Vis spectrophotometer. Catechin was used for comparison. The control contained no extracts. The percentage reduction of LPO was calculated as follows:

Reduction of TBARS (%) = $1\text{-Sample}_{532\ nm}/\text{Control}_{532\ nm} \times 100$

Table 1 Antioxidant profile of the methanol extract of *R. niveus* fruit and standards.[#]

	EC$_{50}$ (mg mL^{-1}) *					
Sample	**DPPH**	**NO**	**OH˙assay**	**O$_2$·$^-$ assay**	**Ion chelation**	**TBARS**
Methanolic extract	0.200 ± 0.002 a	0.250 ± 1.3 c	0.250 ± 0.22 a	0.5 ± 0.82 a	0.250 ± 1.02 a	0.502 ± 0.24 c
Catechin[1]	ND	0.0062 ± 0.04 a	0.0016 ± 0.013 c	0.0030 ± 005 b	0.0064 ± 0.06 b	0.0032 ± 0.05 a
BHT[2]	26 ± 0.005 b	0.0046 ± 0.002 b	0.0019 ± 0.01 b	0.0016 ± 0.01 c	ND	ND

[#] Each value in the table was obtained by calculating the average of three experiments (n = 3) mean ± standard deviation
* EC$_{50}$ value is defined as the concentration of the extract necessary to inhibit the radical concentration by 50%
ND - not determined
[1] Catechin - reference standard
[2] BHT- reference standard
Values in a column with a different letter indicate significant differences at $P > 0.05$ using DMRT

Table 2 Antioxidant profile of the methanol extract of *E. oblongus* fruit and standards [#]

	EC$_{50}$ (mg mL^{-1}) *					
Sample	**DPPH**	**NO**	**OH˙ assay**	**O$_2$·$^-$ assay**	**Ion chelation**	**TBARS**
Methanolic extract	0.250 ± 0.02 a	0.250 ± 0.55 a	0.500 ± 0.48 a	0.50 ± 1.20 b	0.251 ± 1.42 a	0.506 ± 0.38 a
Catechin[1]	ND	620.00 ± 0.04 d	0.0016 ± 0.013 c	0.0030 ± 005 a	0.0064 ± 0.06 d	0.0032 ± 0.05 b
BHT[2]	26 ± 0.005 c	0.0046 ± 0.002 c	0.0019 ± 0.01 d	0.0016 ± 0.01 c	ND	ND

[#] Each value in the table was obtained by calculating the average of three experiments (n = 3) Mean ± Standard Deviation
* EC$_{50}$ value is defined as the concentration of the extract necessary to inhibit the radical concentration by 50%
ND - not determined
[1] Catechin - reference standard
[2] BHT- reference standard
Values in a column with a different letter indicate significant differences at $P > 0.05$ using DMRT

where Sample$_{532\ nm}$ was absorbance of the sample and Control$_{532\ nm}$ was absorbance of control.

Statistical analysis

The experimental data were reported as mean ± SD of three parallel measurements. Linear regression analysis was performed quoting the correlation coefficient. One-way analysis of variance (ANOVA) accompanied with (DMRT) (SPSS version 10 for Windows 98, SPSS Inc.) conducted to determine significant differences ($P < 0.05$) between samples.

RESULTS AND DISCUSSION

Determination of antioxidant capacity

In our study, the fruits of Indian yellow raspberry species (*R. niveus* and *E. oblongus*) from Nilgiris, South India was evaluated for its antioxidant effects for the first time. In this study, in order to determine of antioxidant activity a series of established *in vitro* protocols were applied. The EC$_{50}$ values were obtained for tested assays and are provided in **Tables 1, 2**.

DPPH· quenching capacity

The DPPH· assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products (Molyneux 2003). With regard to DPPH· stable scavenging activity or H-donor activity of *R. niveus* and *E. oblongus* a dose dependent inhibition was observed (**Fig. 1**). The RN and EO exhibited impressive DPPH· scavenging with 68.33% at 1 mg mL^{-1} concentration while BHT was able to scavenge 88.87% at 1000 µg mL^{-1} respectively. Based upon the measured EC$_{50}$ values, the DPPH· quenching ability of RN and EO (0.200 ± and 0.250 ± mg mL^{-1} and BHT (0.0026 ± 0.04 mg mL^{-1}) (**Tables 1** and **2**). DPPH· scavenging activity was with that of commonly consumed fruits including *Syzygium cumini* (IC$_{50}$ 168 µg/ml) (Banerjee *et al*. 2005). DPPH· activity was also higher than its activity against other radical scavenging activities of the extracts.

Reducing capacity

The reduction of ferrous ion (Fe^{3+}) is measured by the intensity of the resultant Prussian blue colour complex which absorbs at 700 nm. In the RC assay, the RN and EO were able to convert the oxidized form of Fe^{2+} into Fe^{3+} (**Fig. 2**). The higher absorbance at high concentration indicates the strong reducing capacity. The *R. niveus* and *E. oblongus* caused significant elevation of reducing power with OD value of 0.732 ± 0.28 and 0.768 ± 0.56, which was more pronounced than that of BHT (1.022 ± 0.42) at 1 mg mL^{-1}. The RC of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing properties are generally associated with the presence of reductones. The antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom (Gordon 1990). The result indicated that the marked reducing power of the fruit extract seems to be due to polyphenols which may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them into more stable products and terminate free radical chain reaction.

$O_2^{\cdot -}$ scavenging capacity

The $O_2^{\cdot -}$ radical is one of the most dangerous free radicals in humans (Schlesier *et al*. 2002) and also the source of hydroxyl radical (OH˙). In the PMS/NADH-NBT system, The $O_2^{\cdot -}$ radicals derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. In the present work, the dose dependent inhibition of $O_2^{\cdot -}$ generation by RN and EO was found (**Fig. 3**). The extracts exhibited 56.78% and 54.02% of $O_2^{\cdot -}$ scavenging at 1 mg mL^{-1}. The RN and EO exerted noticeable scavenging effect on $O_2^{\cdot -}$ radicals though the activity was lower than the BHT (**Tables 1, 2**) and *S. cumini* (Banerjee *et al*. 2005).

˙OH scavenging capacity

The hydroxyl radical (˙OH), which is the most reactive free radical, has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity (Naidu *et al*. 2008). In our study, as illustrated in **Fig. 4**, the extracts were capable of inhibiting OH˙ radical formation in concentration dependent manner. Their scavenging activity towards ˙OH was 59.18% and 46.77% at the concentration of 1 mg/mL^{-1}. However, this value was lower than the values of positive controls BHT (88.1% at 1 mg/mL^{-1}). The EC$_{50}$ values of *R. niveus* (0.250 ± 0.22 mg/mL^{-1}) and *E. oblongus* (0.5 ± 0.48 mg/mL^{-1}) were significantly lower when compared to BHT (**Tables 1, 2**). The ˙OH scavenging ability of *R. niveus* was more efficient as compared with commonly consumed fruit in India, *S. cumini* (IC$_{50}$ 428 µg/ml) (Banerjee *et al*. 2005).

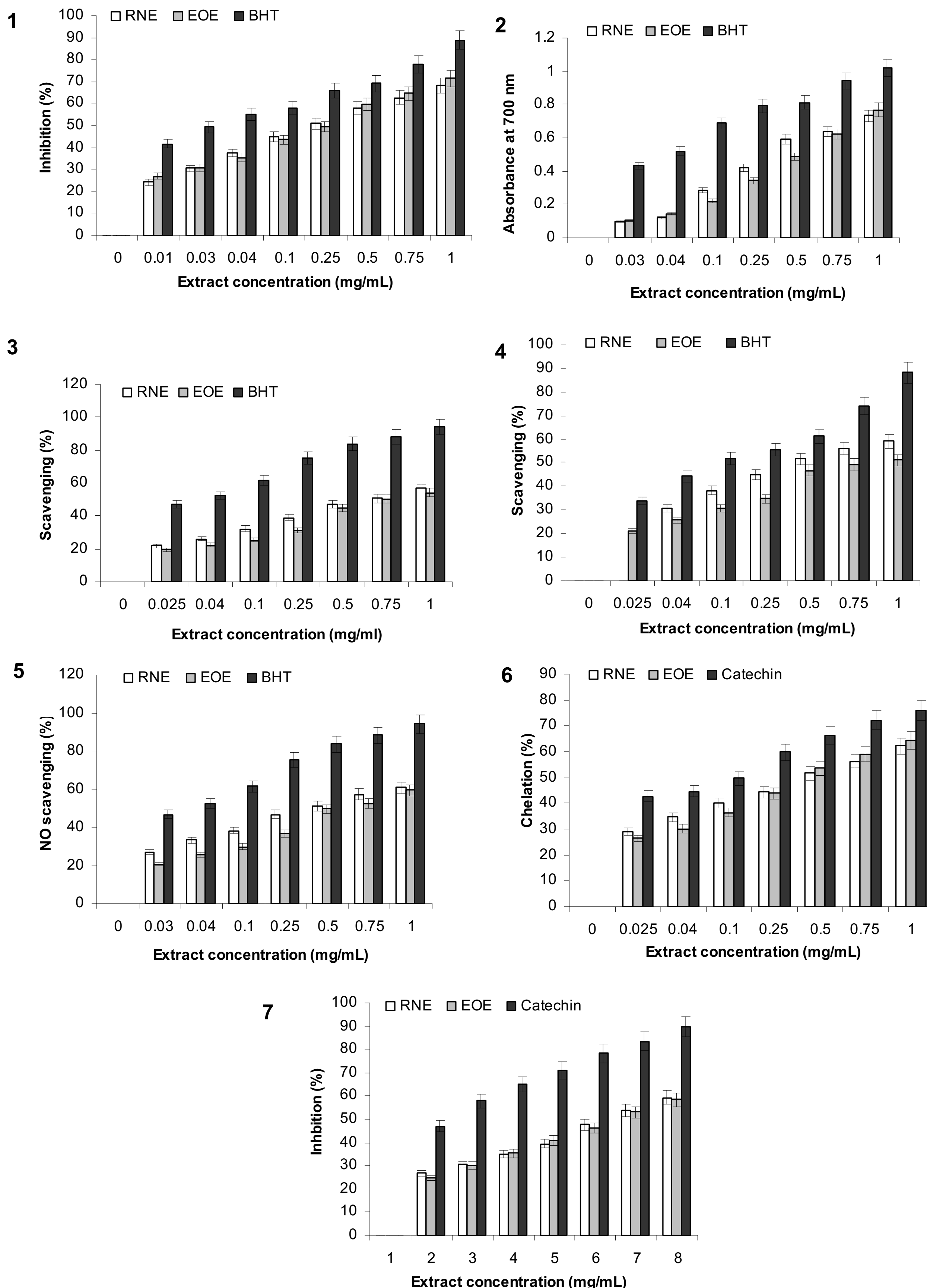

Fig. 1 DPPH· scavenging activity fruit extracts. RNO – *R. niveus* extract, EOE – *E. oblongus* extract, BHT – butyl hydroxy toluene. Results are Mean ± STD of three experiments (n =3). **Fig. 2 Reducing capacity fruit extracts.** RNO – *R. niveus* extract, EOE – *E. oblongus*, BHT – butyl hydroxy toluene. Results are Mean ± STD of three experiments (n = 3). **Fig. 3 Superoxide anion ($O_2^{\cdot -}$) scavenging activity fruit extracts.** RNO – *R. niveus* extract, EOE – *E. oblongus* extract, BHT – butyl hydroxy toluene. Results are Mean ± STD of three experiments (n = 3). **Fig. 4 Hydroxyl ion ($^{\cdot}OH$) scavenging activity fruit extracts.** RNO – *R. niveus* extract, EOE – *E. oblongus* extract, BHT – butyl hydroxy toluene. Results are Mean ± STD of three experiments (n = 3). **Fig. 5 Nitric oxide (NO) scavenging activity fruit extracts.** RNO – *R. niveus* extract, EOE – *E. oblongus* extract, BHT – butyl hydroxy toluene. Results are Mean ± STD of three experiments (n = 3). **Fig. 6 Metal chelating activity fruit extracts.** RNO – *R. niveus* extract, EOE – *E. oblongus* extract, BHT – butyl hydroxy toluene. Results are Mean ± STD of three experiments (n = 3). **Fig. 7 Inhibition of lipid peroxidation (LPO) by fruit extracts.** RNO – *R. niveus* extract, EOE – *E. oblongus* extract, BHT – butyl hydroxy toluene. Results are Mean ± STD of three experiments (n = 3).

NO scavenging capacity

In addition to reactive oxygen species, NO is also implicated in chronic inflammation, cancer and other pathological conditions. NO and $O_2^{\bullet -}$ react to prevent reaction peroxynirite ($ONOO^-$), which leads to serious toxic reactions with biomolecules such as proteins, lipids and nucleic acids (Monkada *et al*. 1991). The NO generated from SNP at physiological pH reacts with oxygen (O_2) to form nitrite ions. From the results it is apparent that the *R. niveus* and *E. oblongus* competed with O_2 to react with nitrite ions and thus inhibit the NO generation. The scavenging capacity depended on concentration (**Fig. 5**). The extracts were potent in scavenging NO by 60.75% at the concentration of 1 mg/mL^{-1}, while BHT showed a scavenging activity of 94.19% (**Tables 1, 2**).

Metal chelation

The Fe^{2+} chelation or deactivation of transition metals is claimed as one of the important mechanisms of antioxidant activity and it reduces the concentration of the catalyzing transition metal in lipid peroxidation (Duh *et al*. 1999). In this study, the ferrous ion chelating activity of *R. niveus* and *E. oblongus* was assessed by the inhibition of formation of red-coloured ferrozine and ferrous complex. The formation of the red complex was inhibited concentration dependently by the fruit extracts (**Fig. 6**) and it strongly chelated Fe^{2+} ions at 1 mg/mL^{-1} (64.34%) while catechin had considerably lower effect (76.1%) (**Tables 1, 2**). The positive control in this assay EDTA exerted the strongest chelating activity and at 1000 µg mL^{-1} there was a 96.08 ± 1.64% chelating effect, which was higher than that of *R. niveus* and *E. oblongus* (not shown in the Tables). Iron chelating ability of EDTA was also higher than that of phenolic compounds (Andjekovic *et al*. 2006). The data obtained from this assay revealed that the fruit extracts demonstrated as an effective capacity for metal-binding, suggesting that the raspberry extract may play a protective role against oxidative damage by sequestering Fe^{2+} ions.

Inhibition of lipid peroxidation (LPO)

The damage caused by LPO, is highly detrimental to the functioning of the cell (Devasagayam *et al*. 2003). It plays an important role in causing oxidative damage to biological systems and its carbonyl product, malondialdehyde (MDA) induces cancer and age related ailments. In order to evaluate the effect of the *R. niveus* and *E. oblongus* extracts on LPO, we measured the ability of the extract to inhibit the LPO induced by $FeCl_2$ in liver homogenate. From the result, it was found that the extracts from the fruits were able to inhibit the concentration of MDA generation in a significant way (**Fig. 7**). With regard to the EC_{50}, the values of the fruit extracts were 0.502 ± 0.24 and 0.506 ± 0.38 mg mL^{-1} whereas the EC_{50} value of catechin was 0.0032 ± 0.05 mg mL^{-1} (**Tables 1, 2**).

This investigation was performed to elucidate the nutraceutical potential and to develop products of added value of underutilized fruits of *R. niveus* and *E. oblongus* which are potentially valuable dietary resource. The results obtained in this study demonstrate that the methanol extracts of *R. niveus* and *E. oblongus* fruits are endowed with very interesting antioxidant capacity in all the assay models. Our findings also indicate that the *R. niveus* and *E. oblongus* fruits are competitive as free radical scavenging agents to BHT and catechin.

CONCLUSIONS

Summing up, *R. niveus* and *E. oblongus* from India demonstrated, for the first time, promising antioxidant activities as it was able to scavenge the ROS and reactive nitrogen species (RNS) such as DPPH, $O_2^{\bullet -}$, $OH^{\bullet}$ and NO and, was effective against LPO. In conclusion, the results presented in this work indicate that *R. niveus* and *E. oblongus* extract efficiently scavenged DPPH, $O_2^{\bullet -}$, NO, $OH^{\bullet}$ free radicals, chelated ferrous ion and inhibited TBARS *in vitro*. The outstanding antioxidant capacity of these fruits is reported for the first time. Obviously, the fruits may be used in the development of functional food and raw materials of medicine. Currently, *in vivo* investigations of this fruit are in progress in our lab.

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