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In vitro analysis of antioxidant capacity of Indian yellow raspberry (*Rubus ellipticus* Smith.)

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Abstract

We examined for first time the ripened fruits of Indian yellow raspberry (*Rubus ellipticus* Smith) (RE), an under exploited fruit from the Nilgiris, India, for its polyphenolic compounds and in vitro antioxidant/radical scavenging ability. The fruit yielded phenolic content (TPh) of 6100 ± 0.082 mg gallic acid equivalents (GAE)/100g of fresh material (FM) and total flavonoid content (TFl) of 320 ±0.120 mg quercetin equivalents (QE)/100g of fresh material (FM). The RE extract displayed excellent scavenging capacity towards 1, 1 – diphenyl – 2-picryl hydrazyl (DPPH·) (EC_{50} 9.85 ±1.33 µg mL^{-1}), superoxide anion ($O_2^{\cdot-}$) (EC_{50} 64.65 ±0.82 µg mL^{-1}), hydroxyl ion radicals ($^{\cdot}OH$) (EC_{50} 79.98 ±1.02 µg mL^{-1}) and nitric oxide (NO) (EC_{50} 75.21 ±1.32 µg mL_{-1}). The RE also showed strong reducing capacity (OD at 700 nm -1.435), strong Fe^{2+} chelation (EC_{50} 45.24 ±1.42 µg mL^{-1}) and exhibited remarkable reduction of lipid peroxidation (EC_{50} 71.1 ±0.22 µg ml^{-1}). The antioxidant capacities of the extract were comparable butyl hydroxytoluene (BHT), ethylene diamine tetraacetic acid disodium salt (EDTA-Na_2) and catechin. Significant and positive correlations were observed between polyphenolic contents and the antioxidant capacities, indicating that the phenolics were major contributors of the antioxidant property. Further, the separation of ethyl acetate (EtOAc) soluble fraction on a silica gel column afforded ellagic acid and quercetin. The results strongly point that Indian yellow raspberry may be a promising source of natural antioxidant agents.

Introduction

Increase in formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is known to damage cellular biomolecules (DNA, proteins, lipids, amines and carbohydrates), resulting in a wide number of degenerative diseases (Sogut *et al*., 2003). In recent years, overwhelming epidemiological studies and intervention trails have consistently indicated the role of consumption of fruits and vegetables as antioxidants in the prevention of the degenerative diseases caused by free radicals (Scalbert *et al.*, 2005; Faller and Fialho, 2009; Wang *et al*., 2011). These protective effects of fruits are mostly related to the antioxidant components including vitamins, flavonoids, phenolic acids, and carotenoids (Prior, 2003). Previous research works have demonstrated the antioxidant activities and health benefits of the several fruits antioxidants (Kahkonen *et al*., 2001; Garcia-Alonso *et al*., 2004; Atawodi *et al.*, 2009). Therefore, it is of great interest in research concerning the antioxidant ability of fruits.

Rubus ellipticus Smith., commonly referred to as Indian yellow raspberry or Himalayan raspberry (Family Rosaceae), is a brambling raspberry with yellow fruits, native to tropical and subtropical India and Asia. It is found in the Nilgris (locally *"Mulli hannu"*) and Palni hills, southern India at an altitude of 1,800 m (Wealth of India, 1990). The aggregate of fruits are edible and, fruits and root are used in treating dysentery (Jain, 1991). Recent studies have confirmed that raspberries were rich repository of phenolic components and have been proved to posses 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, data on antioxidant capacity of Indian fruits is scarce. Recently, Sharma and Kumar (2011) reported *in vitro* antioxidant activity of *R. ellipticus* using DPPH scavenging capacity and reducing power. Phenolic contents and DPPH radical scavenging activity of *R. ellipticus* were reported (Karuppusamy *et al*., 2011). However, no extensive studies have so far been conducted on the antioxidant capacity of *R.*

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ellipticus. The main objectives of the present study encompass (a) to determine the total phenolic and total flavonoid contents, (b) to measure the influence of extract of *R. ellipticus* on free radicals in different *in vitro* systems and (c) to isolate antioxidant ellagic acid and quercetin from the fruits.

Materials and Methods

Chemicals

2-Deoxy-D-ribose, potassium ferricyanide, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), L-ascorbic acid, ellagic acid, quercetin, (+)-catechin, 1,1-diphenyl-2- picryl-hydrazyl ($DPPH^{\bullet}$), 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), trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Merck Co., Mumbai, India. Gallic acid was purchased from Riedel-de-Hahn, Germany. Solvents used for HPLC analysis were of HPLC grade. All other chemicals and solvents used were of analytical grade. Milli-Q quality water was used to prepare all reagents and solutions.

Raspberry extract

The fully ripened berries of *R. ellipticus* (Rosaceae) were collected during May 2007 and June 2007 from Doddabetta forest range, the Nilgiris (TN), India. *R. ellipticus* was identified by Dr. R. Gopalan, Taxonomist, Karpagam University, Coimbatore, India (Specimen no. KU/PROJ/2007-09-010). A voucher specimen was deposited in herbarium of Karpagam University. The berries were manually pooled and were kept in cold (-4° C) dark storage until further analysis. The raspberry fruits (100 g) were blended exhaustively and extracted with 5 times its volume of methanol (1:5 v/v) and centrifuged (3000 x g, Remi, India) for 15 min at 4ºC and the supernatant was transferred to an amber bottle. The extraction process was repeated thrice using the same conditions. The supernatants were combined and filtered over Whatman No. 1 filter paper. The filtrate was concentrated at 40 ±1°C by rotary flash evaporator under reduced pressure to obtain the dry extract (5.41 g, 54.1% w/w). The extract (RE) was kept at -4°C to protect from light until further use.

Total phenolic and flavanoid determinations

The content of total phenolics (TPh) in the RE extract was determined calorimetrically using folin-ciocalteu phenol reagent according to the method proposed by Singleton *et al.* (1974). In brief, an aliquot of the sample extract 0.1 ml were mixed and made up the volume to 3 ml with water and 0.5 mL of folin-ciocalteu reagent (1 N). After 3 min, 2 ml of Na_2CO_3 (20%) was added and mixed thoroughly. The sample was then incubated for 5 min at 50°C and cooled. The absorbance was measured at 650 nm against the reagent blank. The analyses were performed in triplicate. The TPh was expressed as mg gallic acid equivalents from a gallic acid standard curve (mg GAE/100 g fresh material, $r^2 = 0.9968$).

The determination of total flavonoid content (TFl) in the raspberry extract was based on the method described by Ordonez *et al.* (2006). The RE extract (0.1 mL) a volume of 0.5 ml of $AlCl_3$ ethanol solution (2%) was added to 0.5 ml of sample solution. After one hour incubation at room temperature, the absorbance was measured at 420 nm. Extract sample was evaluated at a final concentration of 0.1 mg/mL. The analyses were performed in triplicate. The TFl was estimated from a quercetin standard curve and the results were expressed as mg quercetin equivalents (mg QE/100 g fresh material, $r^2 = 0.9665$).

Isolation of polyphenolic compounds

The yellow raspberries were (500 g) macerated in a ice cold mortar and extracted with 2.5 L of acidified MeOH (0.1% HCl) at room temperature (27°C). The resultant extract was suspended in one liter of H_2O and kept overnight and filtered. The filtrate was then partitioned with ethyl acetate (EtOAc). The EtOAc soluble fraction was subjected over silica gel column (60 x 2.5 cm, G 60, 200 mesh) and eluted gradient with CHCl3-MeOH to give 10 pooled fractions (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100 with fraction weights 6.2, 6.25, 6.2, 7.3, 14.3, 28.5, 17.8, 18.6, 18.2 and 19.6 mg respectively). Each fraction was examined in TLC (Silica gel F254 plates). The fraction VI upon preparative thin layer chromatography (PTLC) developed with $CHCl_3$-MeOH (50:50; 28.5 mg) afforded two compounds.

High performance liquid chromatography (HPLC) analysis

Identification of the compounds was achieved by comparing the retention time of the compounds with corresponding standards. The analytical HPLC system (P-40000, Thermo separation products, USA) employed consisted of a quaternary HPLC pump, photodiode array detector (UV 6000 LP) and a recorder. HPLC analysis was performed using a water prevail C18 analytical column (15 cm x 4.6 mm id, 5 µm particle size; ALTech, IL, USA) was used. The auto-injection system (spectra system-AS

3000) consisted of a 20 µl sample loop. The mobile phase consisted of 20% acetonitrile in water (Solvent A) (v/v), 0.5% methanol (Solvent B) in water after injection of 20 µl of the sample. The peak responses were detected at 280 nm using UV detector. A flow rate of the mobile phase was maintained at 1.0 mL /min. Identification and peak assignment of compounds was based on comparison of its retention times with those of authentic standards.

Quenching capacity towards 1, 1-diphenyl-2- picryl-hydrazyl (DPPH·) radical

The determination of DPPH· stable radical scavenging activity of the RE extract was based on the method described by Singh *et al*. (2002). Briefly, methanol solutions of RE extract (5-1000 µg mL^{-1}) were added to MeOH solution of DPPH· (5 mL, 0.1 mM) and vortexed. After 20 min reaction at 25°C, the absorbance was measured at 517 nm. BHT and ascorbic acid were used for comparison. The percentage quenching of DPPH· was calculated as follows: % inhibition of DPPH· = [(Control517nm-Sample517nm)/Control517nm] x100, where, Sample517nm was absorbance of the sample and Control517nm was absorbance of control. The results were expressed as EC_{50}, which means the concentration at which DPPH· radicals were quenched by 50%.

Measurement of reductive potential

The reductive potential of the RE extract was measured using the potassium ferricyanide reduction method (Oyaizu, 1986). Various concentrations of the extract and standards (25-1000 µg 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 x g for 10 min. Afterwards, upper layer of the solution (5 mL) was mixed with deionized water (5 mL). To this, one millilitre 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. Increased absorbance of the reaction mixture indicated increasing reducing power. BHT was used for comparison.

Scavenging capacity towards superoxide radical

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) (Kakkar *et al*., 1984) with some changes. 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 (25-1000 µg mL^{-1}) of RE extract and standards. The reaction was initiated by addition of 0.25 mL of PMS (60 µM) to the mixture. Following incubation at ambient temperature for 5 min, the absorbance was read at 560 nm. BHT and catechin were used for comparison. The percentage scavenging of $O_2^{\cdot -}$ was calculated as follows: Inhibition of $O_2^{\cdot -}$ (%) = [(Control517nm-Sample517nm)/Control517nm] x100, where, Sample560nm was absorbance of the sample and Control560nm was absorbance of control.

Scavenging capacity towards hydroxyl ion radicals

Hydroxyl radicals ($^{\cdot}OH$) were generated by a fenton reaction system, and the scavenging capacity towards the $^{\cdot}OH$ radical was measured using deoxyribose method (Halliwell *et al*., 1987) with minor modifications. To one millilitre of RE extract (10-1000 µg 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 incubated at 100°C for 15 min and cooled. The absorbance of the pink chromogen developed was recorded at 532 nm. BHT and catechin were used for comparison. The percentage scavenging of $^{\cdot}OH$ was calculated as follows: Inhibition of $OH^{\cdot}$ (%) = [(Control517nm- Sample517nm)/Control517nm] x100 x100, where, Sample532nm was absorbance of the sample and Control532nm was absorbance of control.

Scavenging capacity towards nitric oxide

Nitric oxide (NO) generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH was estimated by the use of Griess reaction (Garrat, 1964) with minor changes. The reaction mixture (3 mL) containing SNP (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and the RE at different concentrations and standards (25-1000 µg mL^{-1}) were incubated at 25º 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 at 25ºC for 30 min. The absorbance of pink coloured chromophore formed during diazotization was measured at 540

nm. BHT and catechin were used for comparison. The percentage scavenging of NO was calculated as follows: Inhibition of NO (%) = [(Control517nm-Sample517nm)/Control517nm] x100, where, Sample540nm was absorbance of the sample and Control540nm was absorbance of control.

Inhibition of ferrozine-Fe^{2+} complex

The Fe^{2+} chelation was investigated using the method described by Dinis *et al.* (1994). Briefly, different concentrations of RE and standards (5-1000 µg mL^{-1}) were mixed with 0.05 ml of $FeCl_2$ (2 mM). The reaction was initiated by addition of 0.2 ml of ferrozine (5 mM) and the mixture was made into 0.8 mL with deionized water. After incubation at room temperature for 10 min, the absorbance of ferrous ion-ferrozine complex was measured at 562 nm. EDTA-Na_2 and catechin were used as reference standards. The percentage ratio inhibition of ferrozine/Fe^{2+} complex formation was calculated as follows: Ferrous ion chelating capacity (%) = [(Control517nm- Sample517nm)/Control517nm] x100, where, Sample562nm was absorbance of the sample and Control562nm was absorbance of control.

Reduction of lipid peroxidation

Inhibition of lipid peroxidation (LPO) in rat liver homogenate was determined in terms of formation of thiobarbituric acid reactive substances (TBARS) (Halliwell and Guttridge, 1989) with minor changes. In brief, different concentrations of RE extract and standard (25-1000 µg 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 x g for 10 min. The absorbance of the organic upper layer was read at 532 nm. Catechin was used for comparison. The percentage reduction of LPO was calculated as follows: Reduction of TBARS (%) = [(Control517nm- Sample517nm)/Control517nm] x100 where, Sample532nm was absorbance of the sample and Control532nm was absorbance of control.

Statistical analysis of data

The experimental data were expressed as mean ± SD (n = 3). Linear regression analysis was used to calculate EC50 values. One way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were carried out. The *P* values of less than 0.05 were adopted as statistically significant. Regression analysis was used to establish correlation between the parameters.

Results and Discussion

Extract yield, total phenolics and flavonoids

Methanol extract prepared from 100 g of *R. ellipticus* fruits gave a yield of 5.41 g. The content of TPh and TFl in the analysed yellow raspberry was equal to 610 mg GAE/100 g of FM and 92 mg QE/100g of FM respectively. The TPh content was found 19-20 times higher than the TFl content of *R. ellipticus*. From our study, it was observed that these values were higher than that of other *Rubus* species (Deighton *et al*., 2000; Wang and Lin, 2000; Kohkonen *et al.* 2001; Siriwoharn *et al*., 2004; Reyes-Carmona *et al.*, 2005) and *R. ellipticus* fruits from Palni hills, India (Karuppusamy *et al*. 2011). Other yellow raspberry varieties (*Anne* and *Fallgold*) were found to contain lower levels of phenolic content than *R. ellipticus* (Liu *et al*., 2002; Pantelidis *et al.*, 2007). It was also observed that the yellow raspberry had higher TPh than common fruits consumed in India (Vijaya Kumar Reddy *et al*., 2010). Hence, the berries of *R. ellipticus* can be considered as good source of phenolics.

Identification of ellagic acid and quercetin

Identification of individual polyphenolic compounds was performed by HPLC-UV in the fruits of *R. ellipticus*. Two polyphenols were successfully appeared at 4.55 and 5.45 min retention time and were proposed to be ellagic acid (EA), an ellagitannin and quercetin, a known flavonol, respectively. From the results, it was found that in the yellow raspberry, EA occurred in high content, whereas quercetin was present in the very less amount. The presence of EA in various fruits and nuts was determined for the purpose of botanical classification, and was identified in strawberries, blackberries and walnuts (Haslam, 1977). Previous studies showed that raspberries were rich in ellagitannins (Kohkonen *et al.*, 2001; Mertz *et al*., 2007). The significant finding of this study was that the EA content of yellow raspberry fruits was higher than that the accounted for other species of *Rubus* (Zhang *et al*., 2010). High and moderate levels of EA were found in strawberries and pomegranate (Daniel *et al.*, 1989, Srinivasan *et al*., 2002). Previous works also revealed the antioxidant activities of ellagic acid (Hatano *et al*., 1989; Indira Priyadarsini

Table 1. EC_{50} values of *R. ellipticus* in DPPH·, NO, $O_2^{\cdot -}$, $OH^{\cdot}$ scavenging, Fe^{3+} chelation and LPO inhibition assays

	EC_{50} (µg mL^{-1})					
Samples	**DPPH·** (R^2 >0.82)	**NO** (R^2 > 0.84)	**$O_2^{\cdot -}$** (R^2 > 0.65)	**$OH^{\cdot}$** (R^2 >0.92)	**Fe^{3+} chelation** (R^2 > 0.78)	**LPO** (R^2 > 0.86)
RE	9.85 ± 1.33[a]	75.21 ± 1.32[c]	64.65 ± 0.82[c]	79.98 ± 1.02[b]	45.24 ± 1.42[b]	71.1 ± 0.22[b]
BHT	26.12 ± 0.4[b]	46.34 ± 0.8[a]	16.05 ± 0.2[a]	16.44 ± 0.4[a]	ND	ND
Catechin	ND	62.20 ± 0.4[b]	30.3 ± 0.5[b]	19.2 ± 0.2[a]	64.1 ± 006[c]	32.2 ± 0.5[a]
EDTA-Na_2	ND	ND	ND	ND	9.62 ± 0.86[a]	ND

The data are presented as mean value ± SD of at least three independent tests (n = 3).
Values with different letters in same column were significantly different at $P<0.05$. ND – Not determined

et al., 2002; Han *et al.*, 2006).

Quercetin has been proven as a potent antioxidant (McAnlis *et al.*, 1999, Zhang *et al.*, 2001, Iacopini Baldi *et al.*, 2008). Furthermore, the combination of EA and quercetin found in raspberry has contributed to the health-enhancing efficacy of strawberry and Muscadine grapes (Pastrona-Bonilla *et al.*, 2003; Ren *et al.*, 2003). Thus, ellagic acid and quercetin identified in the *R. ellipticus* fruit could be responsible for the antioxidant activity observed in our work.

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 radical quenching activity or H-donor activity of RE extract, a concentration dependent inhibition was observed (Figure 1). The RE extract exhibited greater inhibitory effect on DPPH· with 96.1% at the concentration of 1000 µg mL^{-1} while BHT and ascorbic acid were able to scavenge 88.87% and 98.48% at 1000 µg mL^{-1} respectively.

Based upon the measured EC_{50} values (Table 2), the DPPH· quenching ability of RE extract (EC50 9.85 ±1.33 µg mL^{-1}) was significantly ($p<0.05$) more potent than that of ascorbic acid (EC_{50} 11.24 ± 0.02 µg mL^{-1}) and BHT (EC_{50} 26.12 ± 0.04 µg mL^{-1}). DPPH· scavenging activity of the RE extract was significantly higher than its activity against other radical scavenging activities (Table 1). The DPPH· quenching activity of RE extract was in consonance with the work of Zhang *et al*. (2010) who reported that other species of raspberry quenched DPPH· effectively. The DPPH scavenging capacity was also higher than that of *R. ellipticus* from Palni hills (Karuppusamy *et al*., 2011)

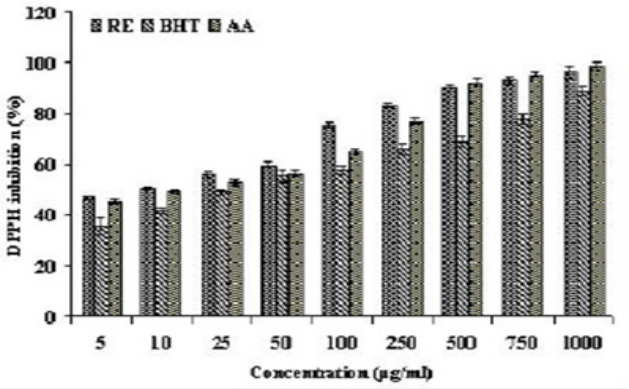

Figure 1. The DPPH· quenching capacity of methanolic extract of *R. ellipticus*. BHT and ascorbic acid were used as antioxidant standards. Data are mean ± SD (n = 3).

and Korean raspberry, *Rubus coreanus* (140.32 µg mL) (Yoon *et al.*, 2010). DPPH· scavenging activity was also more pronounced than that of commonly consumed fruits including *Syzygium cumini* (IC_{50} 168 µg/ml) (Banerjee *et al.*, 2005) and other fruits commonly consumed in (Vijaya Kumar Reddy *et al*., 2010) in India. DPPH· scavenging of RE extract was linearly correlated with TPh (r^2 = 0.965) and TFl contents (r^2 = 0.957).

Reductive capacity (RC)

The reduction of $[Fe^{2+}(CN)_6]^{3-}$ to $[Fe^{2+}(CN)_6]^{4-}$ was measured by the intensity of the resultant Prussian blue colour complex which absorbs at 700 nm. In the RC assay, the RE extract was able to convert the oxidized form of Fe^{3+} into Fe^{2+} in dose dependent manner. The higher absorbance at high concentration indicates the strong reducing capacity. The RE extract caused significant elevation of reducing power with OD value of 1.435 ± 0.021, which was significantly more pronounced than that of BHT (1.022 ± 0.42) and BHA (0.683) ($P<0.05$) at the concentration of

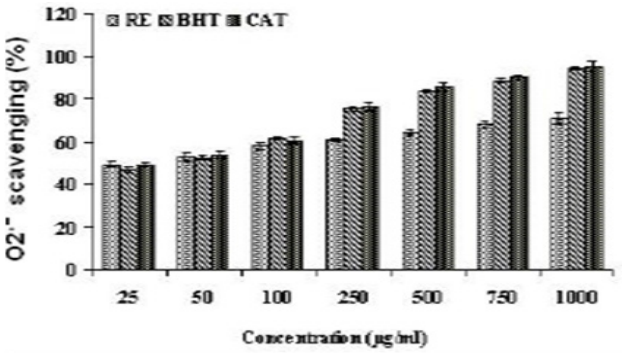

Figure 2. $O_2^{\cdot -}$ scavenging capacity of methanolic extract of *R. ellipticus*. BHT and catechin were used as antioxidant standards. Data are m ean ± SD (n = 3).

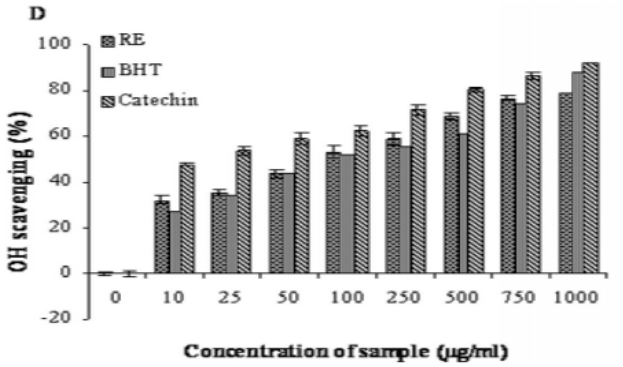

Figure 3. $OH^{\cdot}$ scavenging capacity of methanolic extract of *R. ellipticus*. BHT and catechin were used as antioxidant standards. Data are mean ± SD (n = 3)

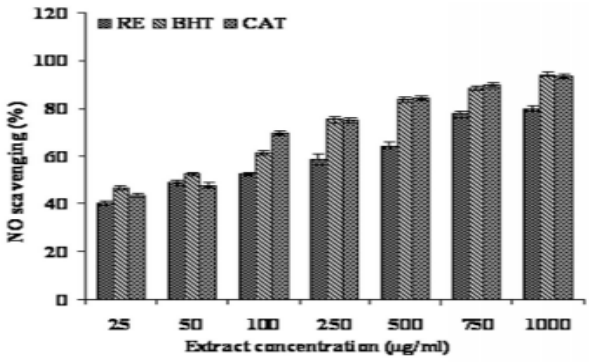

Figure 4. NO scavenging capacity of methanolic extract of *R. ellipticus*. BHT and catechin were used as antioxidant standards. Data are mean ± SD (n = 3)

1000 µg mL^{-1}. A previous study showed that ethanol extract of R. ellipticus exhibited the reducing power with 1.11 (Sharma and Kumar, 2011). 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 (Pin-Der-Duh, 1998). The antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. The result of RC imply that the marked antioxidant activity of the RE extract seems to be due to presence of 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. Significant correlations were observed between RC and, TPl (r^2 = 0.993) and TFl (r^2 = 0.993).

$O_2^{\cdot -}$ scavenging capacity

The $O_2^{\cdot -}$ is one of the most dangerous free radicals in humans (Schlesier *et al*., 2002) and also the source of hydroxyl radical ($OH^{\cdot}$). The $O_2^{\cdot -}$ scavenging activity of RE extract was measured using PMS-NADH/NBT system and the results were expressed as the inhibition of rate of $O_2^{\cdot -}$ activity. In our study, the dose dependent inhibition of $O_2^{\cdot -}$ generation by RE extract is illustrated in Figure 2. The extract exhibited 71.03% of $O_2^{\cdot -}$ scavenging at the concentration of 1000 µg mL^{-1} in comparison with BHT (94.19 %) and catechin (95.32%). As reported in Table 1, the RE extract exerted noticeable scavenging effect on $O_2^{\cdot -}$ radicals (EC_{50} 64.65 ±0.82 µg mL^{-1}) though the activity was significantly lower than BHT (EC_{50} 16.05 ± 0.2 µg mL^{-1}) and catechin (EC_{50} 30.3 ± 0.5 µg mL^{-1}) ($P<0.05$). The RE showed much higher $O_2^{\cdot -}$ scavenging activity than Korean raspberry (SC_{50} 818.30 µg mL) (Yoon *et al.*, 2010). The present finding is more efficient when compared with the values (IC_{50} 260 µg/ml) obtained from methanol extract of *S. cumini* (Banerjee *et al*., 2005). The $O_2^{\cdot -}$ scavenging and was in well correspondence with TPh (r^2 = 0.994) and TFl (r^2 = 0.994).

$^{\cdot}OH$ scavenging capacity

Hydroxyl radical ($^{\cdot}OH$) 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 present study, the RE extract was evaluated for its ability to scavenge $^{\cdot}OH$ radicals using 2-deoxyribose degradation assay. According to Figure 3, the extract was capable of inhibiting $^{\cdot}OH$ radical formation in concentration-response manner, which may be attributed to the combined effects of reducing capacity and donation of H atoms. The $^{\cdot}OH$ scavenging activity of RE extract was 78.63% at the concentration of 1000 µg mL^{-1}. However, when compared with the $^{\cdot}OH$ scavenging activity of standards BHT and catechin, RE extract exhibited a weaker radical scavenging activity. .

The EC_{50} value of RE extract (EC_{50} 79.98 ±1.02 µg mL^{-1}) was significantly ($P<0.05$) lower when compared to BHT (EC_{50} 16.44 + 0.04 µg mL^{-1}) and catechin (EC_{50} 19.2 ± 0.01 µg mL^{-1}) (Table 1). However, the percentage deoxyribose protection of RE extract was better than that of other yellow raspberry species (65.8%) (Pantelidis *et al*., 2007).

The ·OH scavenging ability of RE extract was also more efficient as compared with commonly consumed fruit in India, *S. cumini* (IC_{50} 428 µg/ml) (Banerjee *et al.*, 2005). The extract also exhibited much higher scavenging activity than Korean raspberry (SC_{50} 280.87 µg/mL) (Yoon *et al*., 2010). A positive correlation was observed between OH scavenging and TPl ($r^2 = 0.976$) and TFl ($r^2 = 0.94$).

NO scavenging capacity

In addition to reactive oxygen species, NO is also implicated in chronic inflammation, cancer and other pathological conditions. The NO and $O_2^{\cdot -}$ 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 RE extract competed with O_2 to react with nitrite ions and thus inhibits the NO generation concentration dependently (Figure 4). The RE was potent in scavenging NO by 79.99 % at the concentration of 1000 mg/ml, while BHT and catechin showed scavenging activity of 94.19 % and 93.6 % respectively. With regard to the EC_{50} values, the scavenging capacity of RE extract towards NO (EC_{50} 75.21 ± 1.32 µg mL^{-1}) was significantly ($p < 0.05$) lower than that of BHT (EC_{50} 46.34 ± 0.8) and catechin (EC_{50} 62.20 ± 0.4) (Table 1). The NO scavenging was in well correspondence with TPh ($r^2 = 0.963$) and TFl ($r^2 = 0.976$).

Fe^{2+} 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 our study, the formation of the red Fe^{2+}-ferrozine complex was inhibited concentration dependently by the RE extract and it strongly chelated Fe^{2+} ions at 1000 mg/ml concentration (80.68 ± 2.35 %) whilst catechin had considerably lower effect (76.1%). In this assay, the reference standard EDTA exerted the strongest chelating activity (96.08 ± 1.64 %) at 60 µg mL^{-1}, which was higher than that of RE extract. Earlier, it was observed that chelating ability of EDTA was higher than that of phenolic compounds (Andjekovic *et al*., 2006).

From the EC_{50} value of RE extract it was seen that the chelating ability (EC_{50} 45.24 ±1.42 µg mL^{-1}) was significantly ($P<0.05$) higher than that of catechin and considerably less effective ($P<0.05$) chelator compared to EDTA (Table 1). The data obtained from this assay revealed that the RE extract 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 RE extract on LPO, we measured the ability of the extract to inhibit the LPO induced by $FeCl_2$ in liver homogenate. Our results showed that the RE extract was effective in reducing lipid peroxidation. From the estimated EC_{50}, the value of RE extract (EC_{50} 71.1 + 0.22 µg mL^{-1}) was significantly ($P<0.05$) less active inhibitor of lipid peroxidation than catechin (EC_{50} 32.2 + 0.052 µg mL^{-1}) (Table 1). However, higher activity was observed in RE when compared with *S. cumini* (IC_{50} 222 µg/ml) (Banerjee *et al*., 2005). The lipid peroxidation-inhibition activity of RE was lower than that of Korean raspberry (SC_{50} 1.70 µg mL) (Yoon *et al.* 2010). Significant correlation was found between TBARS assay and TPl ($r^2 = 0.993$) and TFl ($r^2 = 0.93$).

Summing up, the Indian yellow raspberry 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, $O_2^{\cdot -}$, $OH^{\cdot}$ and NO and, was effective against LPO. It exerted better activity than other species of Rubus from environmentally different locations. Antioxidant activity varies from species to species and with in species due to environmental effects (Al-mamun *et al*., 2007). It displayed good antioxidant properties comparing to standards and other *Rubus* fruits and commonly consumed fruits in India; therefore it is conceivable that the fruit could be used as one of the possible sources of antioxidant ingredient. This report constitutes the first demonstration of the antioxidant capacity of underexploited yellow raspberry from India.

The antioxidant properties and polyphenolic content of Indian yellow raspberry *(Rubus ellipticus)* was examined, by using established in vitro assay models. From the results it was observed that the fruit extract possesses significant scavenging capacity towards ROS and contained considerable total phenolics content. DPPH· quenching activity of the extract is superior to that of the synthetic antioxidants BHT and catechin. From the results, it is suggested that this fruit may be exploited for development of

functional food and raw materials of medicine for human health.

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