ORIGINAL ARTICLE

In vitro antioxidant activity of *Rubus ellipticus* fruits

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ABSTRACT

Various studies have been done to identify antioxidants from plant sources and efforts have been taken to incorporate it in conventional therapy. In our present study, petroleum ether, ethanolic, and aqueous extracts of *Rubus ellipticus* fruits have been evaluated for *in vitro* antioxidant activity using DPPH radical scavenging and reducing power assay. BHA was used as a standard antioxidant for DPPH radical scavenging activity. The reducing power assay of extracts was carried out with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer. The results of the both assay showed that all the extracts of *R. ellipticus* fruits possess significant free radical scavenging and reducing power properties at concentration-dependent manner. Hence, it can be concluded that the *R. ellipticus* fruits could be pharmaceutically exploited for antioxidant properties.

Key words: DPPH scavenging, reducing power, *Rubus ellipticus*

INTRODUCTION

Free radicals are chemical species, which contain one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. [1]

Free radicals are generated as part of the body's normal metabolic process and play a dual role in our body as both deleterious and beneficial species. Excess production of reactive oxygen species (ROS) and/or a decrease in antioxidant levels may lead to the tissue damage and different diseases.[2] Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by ROS.[3] Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, while relatively plant-based medicines confer fewer side effects than the synthetic drug in some instances.[4]

The plant *Rubus ellipticus* (Smith) is commonly called as Indian raspberry tree and Himalayan raspberry belonging to family Rosaceae.[5,6] Different parts of the plant have been claimed to be useful in ailments like diabetes, diarrhea, gastralgia, wound healing, dysentery, antifertility, antimicrobial, analgesic, and epilepsy.[7,8]

In view of the above observation, our interest was to find out natural antioxidant from the fruits of *R. ellipticus* using *in vitro* antioxidant assays.

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MATERIALS AND METHODS

Collection and Authentication of Plant Materials

Fruits of *R. ellipticus* smith were obtained from Shivam nursery and plant supplier, Etawah, UP, India, in the month of June–July 2008. The identification and authentication were done by Dr. Harish K Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen (No. AO-101) has been submitted in herbarium department of Sir Madanlal Institute of Pharmacy, Etawah, UP, India, for future reference.

Preparation of Plant Extracts

The fully ripe and shade dried fruits of *R. ellipticus* smith

were grinded with the help of grinder and extracted successively with petroleum ether (60%), ethanol (80%), and distilled water (10 cycles). The extracts were concentrated for further studies at reduced pressure and temperature in a rotary evaporator. The dried extracts were then stored in an airtight container in refrigerator at a temperature below 10°C. The solutions of petroleum ether, ethanol, and aqueous extracts were prepared by dissolving the extracts in ethanol.

Preliminary Phytochemical Screening

The preliminary phytochemical investigation was carried out for the petroleum ether, ethanolic, and aqueous extracts of *R. ellipticus* fruits for the detection of phytoconstituents present. Test for the presence of common phytochemicals were carried out by standard methods [Table 1]. [9]

Chemicals and Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), BHA (butylated hydroxy anisole), ascorbic acid, potassium ferricyanide, $FeCl_3$, tris HCl buffer, phosphate buffer, trichloroacetic acid (TCA), and all other chemicals including solvents were of analytical grade and procured from Nice Chemicals Pvt. Ltd., Cochin.

Antioxidant Assay

The antioxidant activity of plant extracts was determined by different *in vitro* methods such as the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in ethanol at a concentration of 50–200 mg/ml. All the assays were carried out in triplicate, and average values were considered.

Dpph Radical Scavenging Activity

DPPH scavenging activity of the plant extracts was carried out according to the method of Koleva *et al.* and Mathiesen *et al.*[10,11] Ethanol solution of plant extracts (0.2 ml) at different concentrations (50–200 μg/ml) was mixed with 0.8 ml of tris HCl buffer (100 mM, pH 7.4). One milliliter DPPH (500 mM in 1.0 ml ethanol) solution was added to the above mixture. The mixture was shaken vigorously and incubated for 30 min in room temperature. Absorbance of the resulting solution was measured at 517 nm UV-Visible Spectrophotometer (Systronics 117, Japan). All the assays were carried out in triplicates. Ethyl alcoholic solution of *R. ellipticus* fruits (0.2 ml) were used as blank and DPPH ethanolic solution was (500 mM, 1.0 ml) served as control. The BHA was used (butylated hydroxy anisole) as a standard antioxidant in this method. Percentage of DPPH scavenging activity was determined as follows:

$$\% \text{ DPPH radical scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample}}{(\text{Absorbance of control}) \times 100} \times 100$$

Decreased absorbance of the reaction mixture indicates stronger DPPH radical scavenging activity. In this study, petroleum ether, ethanolic, and aqueous extracts of *R. ellipticus* fruits were used.

Table 1: Preliminary phytochemical screening of *R. ellipticus* fruit extracts

Phytoconstituents	Petroleum ether extract	Ethanolic extract	Aqueous extract
Carbohydrates	+	+	+
Alkaloids	+	++	++
Saponins	+	++	++
Tannins	+	++	+
Flavonoids	+	++	++
Triterpinoids	-	+	+

-: Absent; +: Present in low concentration; ++: Present in high concentration

Reducing Power Assay

This was carried out as per the method of Yildrim *et al.* and Lu and Foo.[12,13] One milliliter of ethyl alcoholic solution of plant extracts (final concentration 100–200 mg/l) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (10 g/l); mixture was incubated at 50°C for 20 min; 2.5 ml of trichloro acetic acid (100 g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1 g/l). Absorbance was measured at 700 nm in UV-Visible Spectrophotometer; 2.5 ml solution of ascorbic acid (concentration 5–10 mg/ml) and phosphate buffer were used as standard and control group, respectively. Ethyl alcoholic solution of plant extracts (1.0 ml) was employed as blank. Increased absorbance of the reaction mixture indicates stronger reducing power. [14]

Statistical Analysis

Results are expressed as mean±SEM of three determinants. Comparison among the groups was tested by one-way ANOVA. $P < 0.05$ values were considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical studies revealed the presence of carbohydrates, glycosides, flavonoids, alkaloids, saponins, and tannins in all the fruit extracts of *R. ellipticus* as exhibited in Table 1. *In vitro* antioxidant activity of the plant extracts was observed by DPPH radical scavenging assay and reducing power methods.

DPPH radical scavenging activity of different fruit extracts of *R. ellipticus* and BHA is presented in Tables 2–4. Reducing powers of different fruit extracts of *R. ellipticus* and ascorbic acid are presented in Tables 5–7.

Fruit extracts of *R. ellipticus* have got profound antioxidant activity. Both methods have proven the effectiveness of the

Table 2: DPPH radical scavenging activity of petroleum ether extract of *R. ellipticus* fruits

Concentration	OD 517 nm			% antioxidant activity	
	Sample	Standard		Sample	Standard
50 µl	1.202	2 µl	1.011	21.99*	34.39*
100 µl	1.134	4 µl	0.970	26.41*	37.05*
150 µl	1.100	6 µl	0.901	28.61*	41.53*
200 µl	1.001	8 µl	0.751	35.04*	51.26**

Control OD at 517 nm 1.541. *P<0.05, **P<0.01, and ***P< 0.001 (vs. control), n=3.

Table 3: DPPH radical scavenging activity of ethanolic extract of *R. ellipticus* fruits

Concentration	OD 517nm			% antioxidant activity	
	Sample	Standard		Sample	Standard
50 µl	1.112	2 µl	1.022	22.40*	28.68*
100 µl	1.101	4 µl	0.921	23.16*	35.72*
150 µl	0.930	6 µl	0.547	35.10*	61.82**
200 µl	0.561	8 µl	0.449	60.85**	68.66**

Control OD at 517 nm 1.433, *P<0.05, **P<0.01, and ***P< 0.001 (vs. control), n=3.

Table 4: DPPH radical scavenging activity of aqueous extract of *R. ellipticus* fruits

Concentration	OD 517 nm			% antioxidant activity	
	Sample	Standard		Sample	Standard
50 µl	1.130	2 µl	1.110	21.58*	22.97*
100 µl	1.032	4 µl	0.872	28.38*	39.48*
150 µl	0.988	6 µl	0.539	31.438*	62.59**
200 µl	0.761	8 µl	0.384	47.18*	73.35***

Control OD at 517 nm 1.441, *P<0.05, **P<0.01, and ***P<0.001 (vs. control), n=3.

Table 5: Reducing power of petroleum ether extract of *R. ellipticus* fruits

Sample	Concentration (mg/l)	Absorbance (700 nm)	% antioxidant activity
Control	0	0.09	–
Petroleum ether extract of *R. ellipticus*	100	0.65	86.00***
	150	0.79	88.61***
	200	0.90	92.54***
Ascorbic acid (standard)	5	0.88	89.60***
	10	0.97	91.00***
	15	1.21	93.00***

Control was test sample without plant extract. High absorbance indicates high reducing power. *P<0.05, **P<0.01, and ***P<0.001 (vs. control), n=3.

Table 6: Reducing power of ethanolic extract of *R. ellipticus* fruits

Sample	Concentration (mg/l)	Absorbance (700 nm)	% antioxidant activity
Control	0	0.08	–
Ethanolic extract of *R. ellipticus*	100	0.43	81.00***
	150	0.88	90.00***
	200	1.11	93.00***
Ascorbic acid (standard)	5	0.85	91.00***
	10	1.01	92.00***
	15	1.34	94.00***

Control was test sample without plant extract. High absorbance indicates high reducing power. *P<0.05, **P<0.01, and ***P<0.001 (vs. control), n=3.

Table 7: Reducing power of aqueous extract of *R. ellipticus* fruits

Sample	Concentration (mg/l)	Absorbance (700 nm)	% antioxidant activity
Control	0	0.11	–
Aqueous extract of *R. ellipticus*	100	0.65	82.76**
	150	0.93	88.00***
	200	1.02	89.00***
Ascorbic acid (standard)	5	0.99	88.19***
	10	1.03	89.00***
	15	1.22	91.00***

Control was test sample without plant extract. High absorbance indicates high reducing power. *P<0.05, **P<0.01, and ***P<0.001 (vs. control), n=3.

petroleum ether, ethanolic, and aqueous extracts compared to the reference standard antioxidant BHA and ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. All the fruit extracts of *R. ellipticus* exhibited a significant dose-dependent inhibition of DPPH activity.

The reducing ability of a compound generally depends on the presence of reductants, which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants (i.e., antioxidants) in *R. ellipticus* fruit extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. [15]

The reducing power of *R. ellipticus* fruits extracts was significant and the power of the extract was increased with quantity of sample. This study has the similarity with previous investigation.[16] Finally all the extracts of *R.*

ellipticus fruits exhibited significant antioxidant activities against DPPH radical scavenging activity and reducing power assay; however, activity shown by ethanolic extracts was maximum. The reported antioxidant activity may be due to the presence of phytochemicals in titled plant. [17]

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that an ethanolic extract of *R. ellipticus* fruits, which contains large amounts of phytoconstituents (flavanoids, tannins, etc.), exhibits high scavenging and reducing power activities compared to petroleum ether and aqueous extracts. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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