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Evaluation of Antioxidant Activity of Root extracts of *Rubus ellipticus* (Smith)

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Abstract

The present work is targeted mainly to quantify the phenolic and flavanoid content of Rubus ellipticus root extracts. The quantitative analysis of data showed that, the methanol extract of Rubus ellipticus root contains 21 to 225mg/g of gallic acid and 16 to 29mg/g of rutin. The preliminary antioxidant activity of extracts was screened in vitro using 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH), ABTS, Hydroxyl and lipid peroxidation (LPO) free radical models. The methanol extract showed strong scavenging activity against free radical compared to all other extracts experimented.

Key words: Antioxidant activity, *Rubus ellipticus*, DPPH, ABTS, Hydroxyl and Lipid peroxide radicals.

Introduction

Free radicals are chemical species, which contains 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. Currently, there is great interest in finding antioxidants from natural sources to minimize oxidative damage to cells. Oxidative damage is caused by free radicals and reactive oxygen species (ROS)[1]. Free radical induced oxidative stress, which involve preventive mechanisms, repair mechanisms, physical defenses and antioxidant defenses[2]. In herbal products, phenolic compounds have been shown to be good antioxidant constituents. Many polyphenolics exert more powerful antioxidant effect than Vitamin E *in vitro* and inhibit lipid peroxidation by chain breaking radical scavenging[3]. Recently, a great interest has been given to naturally occurring antioxidants, which may play important roles in inhibiting both free radicals and oxidative chain reactions within tissues and membranes[4]. It's commonly accepted that in a situation of oxidative stress, ROS such as superoxide ($O^{\cdot}$, OOH), hydroxyl (OH) and peroxide ($ROO^{\cdot}$) radicals are generated whose primary targets are major intracellular and extracellular components, protein, lipids, and nucleic acids[5]. The ROS play an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation[1,6,7]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and the concomitant lipid per oxidation, protein damage and DNA strand breaking[7].

Materials and methods

Plant material

Rubus ellipticus root was collected from the government horticulture garden, Ootacamund. The plant was authenticated by Botanical Survey of India, Medicinal Plant Survey and Collection Unit, Government Arts College, Ootacamund, Tamilnadu, India. A voucher specimen has been deposited for further reference at J.S.S College of Pharmacy herbarium, Ootacamund, India.

Chemicals

DPPH and ABTS were obtained from Sigma- Aldrich Co., St. Louis, USA. Rutin and *p*- nitroso dimethyl aniline (*p*-NDA) were obtained from Acros Organics, NJ, USA. Ascorbic acid, was from SD Fine Chemicals Ltd., Mumbai, India. All chemicals used were of analytical grade.

Extraction procedure

The shade dried root was coarsely powdered and soxhleted with different solvents. The extracts were then concentrated to dryness under reduced pressure and controlled temperature to yield a dark brown semi solid consistency. The percentage yield was noted and tabulated. (Table:1)

Preparation of test and standard solutions

All the extracts of *Rubus ellipticus* root and the standard antioxidants (ascorbic acid, rutin, and α-tocopherol) were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for the *in vitro* antioxidant assay using four different methods. The stock solutions were serially

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Table 1

Extraction yield of different extracts of *Rubus ellipticus* root

Extracts	Weighto fo Plant power (g)	Total volume of solvent (ml)	Weight of the crude obtained (g)	Percentage of Yield %	Phenol content (mg/g)	Flavanoid content (mg/g)
Petroleum ether	115	900	4.20	3.65	21	18
Chloroform	111.10	1050	3.90	3.39	130	12
Ethyl acetate	108.30	1050	2.64	2.30	70	21
n-butanol	28.20	950	69.30	60.26	150	24
Methanol	99.70	1175	8.51	7.40	225	29
Water	6.20	1000	17.28	15.03	110	16

diluted with the respective solvents to obtain lower dilutions.

Total phenolic compounds estimation

1 mg/ml to 0.5 mg/ml extracts were mixed separately with 2 ml of Folin-Ciocalteu reagent and 1.6ml of sodium carbonate. After shaking, it was kept for 2hr reaction time. The absorbance was measured at 750 nm (Shimadzu UV-160 A Spectrophotometer, Shimadzu Corporation, Japan). Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 2.5 to 25µg/ ml. using the standard curve the total phenol content of extracts was obtained[7]. The total phenol content was expressed as gallic acid equivalent in mg/g or % w/w of the extracts[8].

Total flavonoid estimation[8,9,]

0.5ml of the extracts was separately mixed with 1.5ml methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415nm with a Shimadzu UV-160A Spectrophotometer (Shimadzu Corporation, Japan). Using rutin, standard curve was prepared and linearity was obtained in the range of 1-10 µg/ml. Using the standard curve the total flavonol content of extracts was obtained. The total flavonol content was expressed as rutin equivalent in mg/g or % w/w of the extracts.

***In vitro* antioxidant activity**

All the extracts were tested for their *in vitro* antioxidant activity using the standard methods. In all these methods, a particular concentration of the extract or standard solution was used which gave a final concentration of 1000-0.45µg/mL after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standard, but without the reagents. A control test was performed without the extract or standards. Percentage scavenging and IC_{50} values ± S.E.M. (IC_{50}values is the concentration of the sample required to inhibit 50% of radical) were calculated.

DPPH radical scavenging method[10,11,12]

The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37°C for 20 minutes and the absorbance of each well was measured at 490nm, using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated.

ABTS radical Scavenging activity studies [12,13]

To 0.2ml of various concentrations of the extract or standards, 1ml of distilled DMSO and 0.16ml of ABTS solution were added to make a final volume of 1.36ml. Absorbance was measured spectrophotometrically, after 20min at 734nm using ELISA reader. Blank is maintained without ABTS. IC_{50} value obtained is the concentration of the sample required to inhibit 50 % ABTS radical mono cation.

Scavenging of hydroxyl radical by p-NDA method[14]

To a reaction mixture containing ferric chloride (0.1mM, 0.5 mL), EDTA (0.1mM, 0.5mL), ascorbic acid (0.1mM, 0.5mL), H_2O_2 (2mM, 0.5mL) and p-NDA (0.01mM, 0.5mL) in phosphate buffer pH 7.4 (20mM) various concentrations of extracts or standards (0.5mL) were added to give a final volume of 3mL. Sample blank was prepared by adding 0.5mL sample and 2.5mL of phosphate buffer pH 7.4. Absorbance was measured at 440nm, percentage scavenging was calculated from the control, where no extract, instead DMSO was present. Ascorbic acid and rutin was used as reference standards in this study.

Lipid peroxidation (LPO) inhibitory assay[15]

The test samples (100 µl) of different concentrations were added to 1 ml of egg lectin mixture, control was without test sample. Lipid peroxidation was induced by adding 10 µl $FeCl_3$ (400 mM) and 10 µl L-ascorbic acids (200 mM). After incubation for 1 hour at 37°C, the reaction was stopped by adding 2ml of 0.25 N HCl containing 15% TCA and 0.375% TBA and the reaction mixture was boiled for 15min then cooled, centrifuged and absorbance of the supernatant was measured at 532nm. α-Tocopherol was used as reference standards in this study.

Statistical analysis

Results are expressed as mean ± S.E.M. Comparisons among

the groups were tested by one-way ANOVA using Graph Pad Prism, Version 4.0 (Graph Pad Software, San Diego, CA, USA).

Results

Total phenolic and flavonoid content

Phenolic compounds have been proved to be responsible for the antioxidant activity. The amounts of total phenolics, flavonoids in root extracts of *Rubus ellipticus* were measured in this study. These extracts were found to have various phenolic levels ranging from 21 to 225 mg/g of gallic acid. The methanol extract had the highest content of total phenolics, followed by the n-butanol. The lowest content was observed in the petroleum ether. The flavonoid contents were measured as rutin equivalents. These extracts were found to have various flavonoids ranging from 16 to 29 mg/g of rutin. The methanol extract had the highest content of total phenolics, followed by the n-butanol. The lowest content was observed in the Chloroform extract.

In vitro antioxidant activity studies

Among the extracts tested, the methanol extracts had the strongest radical scavenging activity (IC_{50} 12.2 ± 0.90 µg/ml) while the n- butanol extract exhibited lowest radical scavenging activity (IC_{50} 49.5 ± 0.45 µg/ml) against DPPH radical. The methanol extracts had the strongest radical scavenging activity (IC_{50} 2.5 ± 0.11 µg/ml) while the ethyl acetate extract exhibited lowest radical scavenging activity (IC_{50} 10.9 ± 0.16 µg/ml) and petroleum ether extract was found to be inactive (IC_{50} >1000 µg/ml) against ABTS free radical. The highest ability of chelating ferrous ion was observed in the methanol extract (IC_{50} 203 ± 0.91 µg/ml), while the ethyl acetate extract had the lowest activity (IC_{50} 900 ± 1.02µg/ml) against hydroxyl radicals. The n-butanol extracts had the strongest radical scavenging activity (IC_{50} 46.7 ±1.01 µg/ml) while the chloroform extract exhibited lowest radical scavenging activity (IC_{50} 180.2 ± 0.12 µg/ml) in lipid peroxidation inhibitor assay.

Conclusion

The contents of phenolics in relation to antioxidant activities of roots extract of *Rubus ellipticus* were analysed. Among these extracts tested, the methanol extract had significantly higher content of total phenolics and flavonoids than other samples. Furthermore, all of these extracts of *Rubus ellipticus* root had antioxidant activity except petroleum ether. Methanol extracts exhibited quite strong antioxidant activity and radical scavenging activity in comparison to the reference compounds ascorbic acid and rutin. n- butanol extracts exhibited strong antioxidant activity in LPO method when compared with standard α-Tocopherol (IC_{50} 91.66 ± 1.67 µg/ml). The above results indicated that region could lead to significant differences both in the content of bioactive compounds and their bioactivities. *Rubus ellipticus* root can be a source of plant antioxidants, with a potential use in gastralgia, wound healing, dysentery, ant fertility. The phenolics and flavonolic might be the major active components responsible for the strong antioxidant activity. However, a more detailed investigation on the individual phenolic compounds and flavonoid compounds present in *Rubus ellipticus* root and the antioxidants activities needs to be carried out.

Table 2

***In vitro* antioxidant activity of different solvent extracts of *Rubus ellipticus* root**

Extract	IC_{50} values ± S. E. M.[a](µg/mL)			
	DPPH	ABTS	p- NDA	LPO
Petrolium ether	>1000	>1000	>1000	>1000
Chloroform	44.6 ± 0.27	15.4 ±0.11	>1000	180.2 ± 0.12
Ethylacetate	23.5 ±1.22	10.9 ±0.16	900 ± 1.02	77.3 ± 0.33
n-butanol	49.5 ±0.45	16.5 ± 0.09	>1000	46.7 ±1.01
Methanol	12.2 ±0.90	2.5 ± 0.11	203 ± 0.91	101.2 ± 0.12
Water	31 ± 0.01	13.6 ± 0.01	>1000	172.4 ± 0.99
Standards				
Ascorbic acid	14.97 ± 0.67	11.25 ± 0.49	>1000	-
Rutin	18.91 ± 0.15	0.51 ± 0.26	233.63 ± 3.25	-
α- Tocopherol				
-	-	-	91.66 ± 1.67	

(-) Means not done.

a-Average of three determinations.

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