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IN VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF *EUGENIA BRACTEATA* ROXB

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ABSTRACT

The role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes, etc. *Eugenia bracteata* Roxb also known as Jaman has been useful in the treatment of diabetes. There is no detailed study on free radical scavenging activity on *Eugenia bracteata*. Hence, a detailed study was carried out on the methanolic extract of *Eugenia bracteata* for scavenging activity of superoxide radical, hydroxyl radical, DPPH radical, hydrogen peroxide, nitric oxide, and hypochlorous acid scavenging activities and lipid peroxidation inhibition. In the present study *Eugenia bracteata* showed free radical scavenging activity on wide range of free radicals. The results are statistically comparable with standard reference ascorbic acid. The mean IC_{50} values for scavenging activity by methanolic extract of *Eugenia bracteata* were found to be 198.406 µg (superoxide), 538.336 µg (hydroxyl), 1145.076 µg (lipid peroxidation), 22.3725 µg (DPPH), 123.276 µg (nitric oxide), 11.7 µg (hypochlorous acid), and 88.148 µg (hydrogen peroxide). *Eugenia bracteata* showed potent scavenging activity on superoxide; equal effect on hydrogen peroxide and hypochlorous acid; moderate effect on DPPH; low activity on hydroxyl radical; inhibition of lipid peroxidation and nitric oxide compared to standard ascorbic acid.

Key words: *Eugenia bracteata,* Free radical, Methanolic extract, Ascorbic acid, Antioxidant.

INTRODUCTION

The genus Eugenia is of trees or shrubs, distributed throughout coastal districts and also in the warmer parts of the world. *Eugenia bracteata* Roxb Syn *Syzygium bracteatum* (Willd) family: Myrtaceae[1] commonoly known as jaman is a shrub upto 6 mts tall resembling myrtle with pretty white flowers and red berries, bark yellowish grey smooth, wood grey hard, and close grained distributed along the hilly areas and most commonly in the coastal regions of India[2]. The plant is reported to have antibacterial and antifungal

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activity and is also reported to have hypoglycemic activity[3]. So an attempt is made to find its antioxidant activity as there are no reports available.

EXPERIMENTAL

Plant material

The leaves of *Eugenia bracteata* Roxb were collected from Kailash hills of Visakhapatnam, Andhra Pradesh, South India. Dr. K Hemadri, Taxonamist, Regional Research Institute, Botanical Survey of India, Vijayawada, identified the herb. A voucher specimen (EB-03) was deposited in the herbarium of our department.

Preparation of plant extract

Freshly collected leaves of *Eugenia bracteata* Roxb were shade dried. The dried leaves were powdered in Wiley mill. The powdered leaves (2500 g) were extracted with methanol by process of continuous extraction (Soxhlation). The crude extract was evaporated to dryness in a rotary film evaporator (Roteava, Equitron, Medica instrument, India). 1 g of methanolic extract was equivalent to 24.3 g of crude drug.

Antioxidant activity

Table 1. Mean IC_{50} values of alcoholic extract of *Eugenia bracteata* and ascorbic acid in micrograms (µg) free radicals, reactive oxygen species and reactive nitrogen species of extract/ ascorbic acid

Antioxidant activity	Methanolic extract of *Eugenia bracteata*	Standard Ascorbic acid
Superoxide	198.40	198.97
Hydroxyl	538.33	304.62
Lipid peroxidation	1145.07	920.50
DPPH	22.37	16.55
Nitric oxide	123.27	98.99
Hypochlorous acid	11.7	10.55
Hydrogen peroxide	88.14	84.55

Inhibition of superoxide radical by riboflavin photo reduction method

Superoxide scavenging activity of the plant extract was determined by nitro blue tetrasolium reduction method[4] .The reduction mixture contained EDTA (6 µM) containing (3 µg) NACN, riboflavin (2 µM), nitroblue tetrazolium (50 µM), various concentrations of extract and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL.The tubes were uniformly illuminated with an incandescent lamp for 15 min, and the optical density was measured at 530 nm before and after illumination .

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/ EDTA/ H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS)[5]. Fenton reaction mixture consisting of 10 mM ferrous sulphate ($FeSO_4.7H_2O$), 10 mM EDTA and10 mM of 2-deoxyribose and was mixed with 0.1 M phosphate buffer (pH 7.4) and to different concentrations of plant extract.Thereafter, 10 mM of H_2O_2 was added before the incubation at 37ºC for 4 h. Then, 1 mL of this fenton reaction mixture was treated with 8.1% sodium dodecyl sulphate, 0.8% thiobarbituric acid and 20 % of acetic acid. The total volume was made to 5 mL by adding distilled water and kept in an oil bath at 100ºC for 1 hour. After the mixture had been cooled, 5 mL of 15 : 1 v/ v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm.

Inhibition of lipid peroxide formation induced by Fe^{2+} ascorbate system

Inhibition of lipid peroxidation was determined by the method developed by Ohkawa et al.[6]. Rat liver tissues weighing 10 g were homogenized with a polytron homogenizer in ice-cold Tris-HCl buffer to give 25% w/ v homogenate. The homogenate was centrifuged at 4000 rpm for 10 min. An aliquot of supernatant was mixed with plant extract of different concentrations, followed by addition of potassium chloride (30 mM), ascorbic acid (0.06 mM) and ammonium ferrous sulphate (0.16 mM) and these were incubated for 1 hour at 37°C. The reaction mixture was treated with sodium dodecyl sulphate (8.1%), thiobarbituric acid (0.8%) and 20 % acetic acid (pH 3.5). The total volume was made upto 4 mL by adding distilled water and kept in an oil bath at 100°C for 1 hour. After the mixture was cooled, 1 mL of distilled water and 5 mL of 15 : 1 v/v

butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured at 532 nm.

Reduction of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca et al.[7] An aliquot of 0.004% DPPH solution in ethanol and plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm.

Nitric oxide scavenging activity

The nitric oxide scavenging activity was measured according to the method of Marcocci et al.[8]. To different concentrations of plant extract, 25 mM of sodium nitroprusside solution were added in the test tubes and incubated at 37ºC for 3 h. An aliquot of incubation solution (0.5 mL) was taken into a test tube and then Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed during the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately measured at 570 nm.

Hypochlorous acid scavenging activity

Hypochlorous acid scavenging activity was determined by the method described by Long and Halliwell[9]. Plant extract of different concentrations was taken into a test tube and 100 µM pyrogallol red and 0.5 mL of distilled water were added and shaken vigorously and left at room temperature for 10 min. To this, 125 µM hypochlorous acid was added and the sample was diluted with water in a ratio 1 : 4. The decrease in absorbance was measured immediately at 542 nm.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured by the modified method of Bahorun et al.[10] The reaction mixture containing plant extract of different concentrations, 0.002% H_2O_2 and 0.8 mL of phosphate buffer were preincubated for 10 min at 37^0C. To this reaction mixture, 1 mL of phenol red dye containing horse radish peroxidase solution was added. After 15 min., 50 µL of 1 M NaOH was added and absorbance was measured at

610 nm immediately.

The percentage inhibition was calculated using the formula

$$\text{Inhibitary ratio} = \frac{(\Delta A_0 - \Delta A_1) \times 100}{\Delta A_0}$$

Where, ΔA_0 is change in the absorbance of control and ΔA_1 is change in the absorbance with plant extract /ascorbic acid.

RESULTS AND DISCUSSION

In the present study, *Eugenia bracteata* showed free radical scavenging activity on wide range of free radicals. The results are statistically comparable with standard reference ascorbic acid. The mean IC_{50} values for scavenging activity by alcoholic extract of *Eugenia bracteata* were found to be 198.406 µg (superoxide), 538.336 µg (hydroxyl), 1145.076 µg (lipid peroxidation), 22.372 µg (DPPH), 123.2763 µg (nitric oxide), 11.7 µg (hypochlorous acid), and 88.148 µg (hydrogen peroxide), when compared to the standard ascorbic acid 198.97 µg (superoxide), 304.62 µg (hydroxyl), 920.50 µg (lipid peroxidation), 16.55 µg (DPPH), 98.99 µg (nitric oxide), 10.55 µg (hypochlorous acid); and 84.55 µg (hydrogen peroxide). *Eugenia bracteata* showed potent scavenging activity on superoxide; equal effect on hydrogen peroxide and hypochlorous acid; moderate effect on DPPH; low activity on hydroxyl radical; inhibition of lipid peroxidation and nitric oxide as compared to standard ascorbic acid.

Recently, intensive research has been carried out to characterize antioxidant properties of extracts from several plant materials as plants often contain substantial amounts of antioxidants. It suggests that antioxidant action may be an important property of many plants medicines associated in treatment of several diseases. The present work has given us a plant with good antioxidant activity. Further work is required to isolate the active constituents present and *in vivo* studies has to be conducted to prove its potent antioxidant activity.

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