In vitro study on the radical scavenging and anti-lipidperoxidative effects of Eugenia jambolana aqueous extracts

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Received on: 28-08-2009; Revised on: 19-10-2009; Accepted on: 18-12-2009

ABSTRACT

The present study was planned to evaluate the radical scavenging and anti-lipidperoxidative activity of aqueous extracts of Eugenia jambolana seeds by three in vitro methods viz., DPPH free radical scavenging assay, reducing power and lipid peroxidation inhibition using rat liver homogenate as substrate. Further, the effect of heat treatment on the antioxidant activity was also studied. Both untreated and heat treated extracts exhibited significant radical scavenging activity comparable to that of butylated hydroxyl toluene (BHT); a synthetic antioxidant. Both the extracts exhibited similar reducing power which was significantly lower \((p=0.05)\) than that of ascorbic acid. In case of anti-lipidperoxidation assay both the extracts inhibited the formation of lipid peroxides to a significant extent \((p=0.05)\) and significantly \((p=0.05)\) restored glutathione content in the liver homogenate. These findings emphasize that Eugenia jambolana possesses a strong antioxidant activity besides, being a proven hypoglycemic agent.

Keywords: Antioxidant effect, Eugenia jambolana, lipid peroxidation, medicinal plants, oxidative stress

INTRODUCTION

Free radicals arising from either the normal metabolism or induced by environmental sources interact continuously in the biological systems. Oxidants/antioxidants must be kept in balance to minimize molecular, cellular and tissue damage\(^1\). Oxidative stress is implicated in several diseases including cardiovascular diseases, cancer and diabetes\(^2\). Potential sources of natural antioxidant have been searched in different types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices and herbs\(^3\). A number of plants such as rosemary and sage belonging to Labiateae family have provided effective antioxidative extracts, used for the protection of oils, fats and salad dressings\(^4\). Eugenia jambolana Lam (Myrtaceae) commonly called as jamun, black plum or Indian black berry is widely used in the Indian system of medicine for the treatment of various diseases and disorders\(^5\). The jamun tree is a large evergreen and is native to India but is also found in other parts of the world especially tropical countries\(^6\). Various medicinal properties of E. jambolana including its astringent, stomachic, astringent, diuretic and anti-diabetic properties have been described in traditional medicine\(^7\). E. jambolana seeds are reported to possess hypoglycemic\(^8\), anti-inflammatory\(^9\), neuropsycho-pharmacological\(^9\), anti-bacterial\(^9\), anti-HIV\(^10\), anti-diarrheal\(^11\) and anti-diabetic effects. E. jambolana seeds are reported contain several active constituents such as flavonoids, gallic acid, ellagic acid and tannins\(^12\). The present study evaluated the antioxidant potential of Eugenia jambolana seeds using three in vitro methods.

MATERIALSAND METHODS

Plant material

Eugenia jambolana seed powder was purchased from a local Ayurvedic dispensary. It was dried at 50°C for 1 hr and passed through 60 mesh sieve (BS) and stored in an air tight container at 4°C till further use.

Heat treatment

The seed powder was subjected for heat treatment in a vacuum oven at 100°C for 60 min, cooled in a decicator and used for the preparation of heat treated extract.

Preparation of extracts

Untreated and heat treated Eugenia jambolana seed powder (10 g) was extracted with distilled water (100 mL) in a mechanical shaker for 24 h at room temperature, filtered and freeze dried to yield untreated and heat treated aqueous extracts respectively.

DPHH radical scavenging assay

The hydrogen atom or electron donation ability of the extracts was measured from the bleaching of purple colored methanol solution of
DPPH. This spectrophotometric assay uses stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent. Various concentrations of the extracts in 3 ml methanol were added to 1 ml of a 0.1 mM solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank (methanol) at 517 nm. The percent RSA was calculated using the following formula:

\[
\text{% RSA} = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100
\]

Where, \( \text{Abs control} \) is the absorbance of the control reaction (containing all reagents except the test compound), and \( \text{Abs sample} \) is the absorbance of the test compound. Synthetic antioxidant butylated hydroxytoluene (BHT) was used as positive control and all tests were carried out in triplicate.

**Reducing power assay**

Reducing power of the extracts was determined by the method of Yildirim et al. Various concentrations of the extracts (25-100 µg) in 1 ml of distilled water were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml (10 g/L) potassium ferricyanide. The mixture was incubated at 50 °C for 30 min followed by addition of 2.5 ml trichloroacetic acid (100 g/l) and centrifugation at 1650 xg for 10 min. From the upper layer solution 2.5 ml was taken and mixed with 2.5 ml ferric chloride (1 g/L). The absorbance was read at 700 nm against reagent blank. Higher absorbance indicates higher reducing power. Ascorbic acid was used as reference compound. All tests were carried out in triplicate.

**Lipid peroxidation inhibition assay**

This assay was carried out using rat liver homogenate as substrate. Male rat of Wistar strain was sacrificed by cervical dislocation and liver was immediately excised, and a homogenate (5:1 w/v) was prepared using phosphate buffered saline (PBS) in cold condition. It was centrifuged at 200 g for 10 min. The supernatant was collected and finally suspended in PBS so as to contain 10 mg protein in 1 mL suspension to perform in vitro experiment. Protein content was estimated by using diagnostic kit (Span diagnostics, India).

Various concentrations of the extracts dissolved in 1 ml of PBS were mixed with 5 ml of homogenate. Lipid peroxidation was initiated by adding 100 µl of \( \text{H}_2\text{O}_2 \) (10 mM), gently mixed and incubated at 37°C for 30 min. After 30 min the thio barbituric acid-reactive substances (TBARS) were estimated by the method of Okhawa et al. and reduced glutathione was estimated according to the method of Ellman. A control is run without the addition of extract and the percent inhibition of lipid peroxidation was calculated by the following formula:

\[
\text{% inhibition} = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100
\]

Where, \( \text{Abs control} \) is the absorbance of the control reaction (containing all reagents except the test compound), and \( \text{Abs sample} \) is the absorbance of the test compound. BHT was used as positive controls and all tests were carried out in triplicates.

**Statistical analysis**

The data was analyzed by ANOVA followed by Tukey’s multiple comparisons test for significant differences using SPSS 14.0 computer software.

**RESULTS AND DISCUSSION**

A body of evidence suggests that free radicals play an important role in the development of tissue damage and pathological events in living organisms. Oxidative stress is found to implicate in several cardiovascular diseases, including septic shock, ischemic reperfusion injury, heart failure, atherosclerosis, hypertension and diabetes. In
the present investigation the antioxidant effect of untreated and heat treated aqueous extracts was evaluated using three in vitro methods. Both untreated and heat treated extracts exhibited significant dose dependent radical scavenging activity ranging between 40-65%. However, the radical scavenging activity of both untreated and heat treated extracts were significantly lower (p=0.05) than that of BHT (Fig 1). Similar trend was seen in case of reducing power wherein although the extracts exhibited reduced capacity it was significantly lower (p=0.05) than that of ascorbic acid (Fig 2).

The peroxidative property of hydrogen peroxide can be justified as the formation of free radicals with ferrous iron and with oxygen radicals [Haber Weiss reaction]. These radicals could further attack the phospholipids of cell membrane causing lipid peroxidation. Generation of free radicals eventually cause depletion of antioxidants and glutathione (GSH) and increases TBARS. Hence, estimation of reduced glutathione may serve as a better marker of antioxidant status. The inhibition of lipid peroxidation by the extracts is presented in Figure 3. Both the extracts exhibited a dose dependent inhibition of lipid peroxidation. However, the antilipoperoxidative activity of both the extracts was significantly lower (p=0.05) than that of BHT. Both the extract restored glutathione concentration to near normal comparable with that of BHT. Heat treatment did not cause any significant difference in their antioxidant activity. The antioxidant activity of Eugenia jambolana could be attributed to the presence of phenolic compounds as they are believed to be the major phytochemicals responsible for antioxidant activity of plant materials.

CONCLUSION
From the results of the present study, it is concluded that Eugenia jambolana possesses good antioxidant activity in vitro and heat treatment does not decrease its antioxidative property.

REFERENCES

Source of support: Nil, Conflict of interest: None Declared