ORIGINAL ARTICLE

Phytochemical screening and antioxidant activity of *Dillenia bracteata*

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Abstract

Introduction: Oxidative stress may lead to a number of diseases such as atherosclerosis, nephrotoxicity, liver cirrhosis, cancers, diabetes, and Alzheimer disease. Medicinal plants are an important source of antioxidants. Therefore, the antioxidant potential of *Dillenia bracteata* was evaluated in the present study. **Materials and Methods:** The coarse powder of leaf of *Dillenia bracteata* (*Dilleniaceae*) was extracted in Soxhlet apparatus, with ethanol (90%) and ethanolic extract of *D. bracteata* (EEDB) was further processed for phytochemical screening, total phenol content, total flavonoid content, and various *in vitro* antioxidant assays. **Results and Discussion:** The phytochemicals present in EEDB were glycosides, carbohydrates, triterpenoids, proteins and amino acids, gums and mucilages, and flavonoids. The total phenolic and flavonoid contents of EEDB were found to be 168.67 mg gallic acid equivalents per gram of dry extract and 61.67 mg quercetin equivalents per gram of dry extract, respectively. In 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, EEDB exerted an inhibition of 74% and ascorbic acid exerted 83% at 500 µg/ml. In the ferric reducing antioxidant power assay, the absorbance of EEDB increased with increasing concentration. Hydroxyl radical scavenging activity of EEDB was 67% and ascorbic acid reached 74% at 500 µg/ml. The superoxide radical scavenging activity of EEDB was 87%, and ascorbic acid reached 89% at 1000 µg/ml. Nitric oxide radical scavenging activity of EEDB reached 69.21% and ascorbic acid reached 84.01% at 500 µg/ml. **Conclusion:** The antioxidant effect of *D. bracteata* leaf may be due to the phenolics and flavonoids present in it. The present study suggests that this extract could be of great importance for the treatment of free radical-related diseases.

Key words: Antioxidant activity, *Dillenia bracteat,* flavonoids, phenolic compounds, phytochemical screening

INTRODUCTION

Imbalance between the production of reactive oxygen species and detoxifying capability of the biological system produces oxidative stress in the body.[1] A reducing environment is maintained within the cells of all the living organisms. Enzymes through constant input of metabolic energy preserve this reducing state. Any disturbance in the redox potential generates free radicals and peroxides which damages the living cells by destroying the lipids, proteins, and DNA of the cells, which may lead to a number of diseases such as atherosclerosis, nephrotoxicity, liver cirrhosis, cancers, diabetes, parkinsonism, Alzheimer disease, and rheumatoid arthritis.[2-4] The living system has different antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which play an important role in scavenging the free radicals and protecting cell membrane from injury.[5] Oxidative stress in the cells is lowered by antioxidants by two mechanisms: (1) Blocking the generation of free radicles and (2) detoxifying the reactive oxygen/nitrogen species through upregulation of CAT, GPx and SOD.[6] Hence, antioxidants help to improve natural defense mechanisms in the body.

Medicinal plants are an important source of antioxidants.[7] A large number of medicinal plants have been studied and

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proved to possess antioxidant property.[8] Hence, the objective of the study was to evaluate phytochemicals present in *Dillenia bracteata* belonging to the family Dilleniaceae which is known to possess a wide range of therapeutic activities. The plant *D. bracteata,* commonly known as Racemed fish-bone tree, is locally known chiruthaeku in Telugu, bettadakanagalu in Kannada, and colikkay in Tamil. It is widely distributed throughout South India. Trees of *D. bracteata* grow up to 20 m high, bark grayish-brown, young shoots are tomentose and leaf scars clap the branches half the circumference. Leaves are simple, alternate, and spiral; stipules lateral, deciduous; flowers are bisexual and yellow in color. Pseudocarp is orange and subglobose. Fruits are ovoid, dark reddish-brown to black, arillate, and glabrous.[9] Plants of Dilleniaceae family play an important role in traditional medicine, and some of them are used for arthritis, diabetes,[10,11] dysentery, hepatitis, blennorrhagia,[12] and to treat gastrointestinal disorders, inflammation,[13-16] hemorrhoids, wounds,[17] and leishmanial ulcers.[18] Studies have shown that extracts from of them as well as their isolated compounds possess diverse biological activities, including antihemorrhagic,[19] anti-inflammatory, antioxidant, antimicrobial,[20,21] antitumoral,[22] antiulcer, immunological,[23] and cancer chemoprevention.[24] Therefore, the antioxidant potential of *D. bracteata* was also evaluated in the present study.

MATERIALS AND METHODS

Fresh leaves of *D. bracteata* were collected from Tirumala hills, Andhra Pradesh, in the month of February. The taxonomical identification and authentication of the plant were done by Dr. K. Madhava Chetty, Assistant Professor, S. V. University, Tirupati, Andhra Pradesh. The leaves were collected and dried under shade at room temperature for 5 days. Later the leaves were ground into coarse powder and passed through mesh size no. 50. The powdered sample was stored in a closed container free from pollution and environmental contaminants.[25]

Preparation of Extract

About 50 g of powdered sample was evenly packed in Soxhlet apparatus, and the extraction was done with 90% ethanol (based on extractive value). The solvent was evaporated at low temperature. The residual ethanolic extract of *D. bracteata* (EEDB) was used for phytochemical screening.[25]

Preliminary Phytochemical Screening

This involves the analysis and screening of EEDB for different phytochemical compounds. The preliminary screening gives a general idea regarding the presence of different compounds possessing therapeutic values. The phytochemical screening was done as per the WHO guidelines.[8,26-28] The results are shown in Table 1.

Estimation of Total Phenolic Content

Total phenolic content was estimated using the Folin-Ciocalteu method.[29] Samples (100 µL) were mixed thoroughly with 2 ml of 2% sodium carbonate. After 2 min, 100 µL of Folin–Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min, and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as a gram of gallic acid equivalents (GAE) per 100 g of dry weight of the plant samples.

Estimation of Flavonoids

The flavonoids content was determined according to Eom *et al*.[30] An aliquot of 0.5 ml of sample (1 mg/mL) was mixed with 0.1 ml of 10% aluminum chloride and 0.1 ml of potassium acetate (1M). In this mixture, 4.3 ml of 80% methanol was added to make 5 mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

In vitro Antioxidant Activity

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of an antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH-H. The free radical scavenging activity of all the extracts was evaluated by DPPH according to the previously reported method.[31] Briefly, an 0.1 mm solution of DPPH in methanol was prepared, and 1 mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (125, 250, 500, and 1000 µg/mL). The absorbance was measured at 517 nm using an ultraviolet–visible (UV-VIS) spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as a reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated using the following formula.

DPPH scavenging effect (% inhibition) = {(A0–A1)/A0) *100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Ferric reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions was measured using the method described by Benzie and Strain.[32] The FRAP reagent

was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) tripyridyltriazine (TPTZ) solution, and 20.0 mM ferric chloride ($FeCl_3$).$6H_2O$ solution in a ratio of 10:1:1 in volume. Samples at different concentrations (100, 200, 300, 400, and 500 µg/ml) were then added to 3 ml of FRAP reagent, and the reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of ferrous sulfate ($FeSO_4$) were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of the sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al*.[33] Stock solutions of ethylenediaminetetraacetic acid (EDTA) (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), hydrogen peroxide (H_2O_2) (10 mM), and deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$, 0.1 mL H_2O_2, 0.36 mL of deoxyribose, 1.0 mL of the extract of different concentration (125, 250, 500, and 1000 µg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.9), and 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h. 1.0 mL portion of the incubated mixture was mixed with 1.0 mL of 10% trichloroacetic acid and 1.0 mL of 0.5% thiobarbituric acid (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as percentage inhibition of deoxyribose degradation is calculated using the following equation.

Hydroxyl radical scavenging activity= {(A0–A1)/A0) *100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al*.[3] The superoxide anion radicals were generated in 3.0 ml of Tris-HCl buffer (16 mM and pH 8.0), containing 0.5 mL of nitroblue tetrazolium (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 mL extract of different concentration (125, 250, 500, and 1000 µg/ml), and 0.5 mL Tris-HCl buffer (16 mM and PH 8.0). The reaction was started by adding 0.5 mL phenazine methosulfate solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid.

The percentage of inhibition was calculated using the following equation:

Superoxide radical scavenging activity= {(A0–A1)/A0) *100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of all of the extract samples and reference. All the tests were performed in triplicates, and the results were averaged.

Nitric oxide (NO) radical scavenging

Griess reagent is containing 1% sulfanilamide, 2% phosphoric acid, and 0.1% naphthyl-ethylenediamine dihydrochloride. NO was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generate NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of the Griess reagent. Scavenger of NO competes with oxygen leading to reduced production of NO. The absorbance of chromophore created during diazotization of nitrite with sulfanilamide following coupling with naphthyl-ethylenediamine dihydrochloride was read at 540 nm and compared to the absorbance of the standard. The decrease in absorbance of Griess reagent at its absorption maximum of 540 nm is proportional to the concentration of free radical scavenger added to the Griess reagent solution.[34] Ascorbic acid was used as a standard solution. NO radical scavenging activity was calculated using the following equation:

$$\text{NO Scavenged } (\%) = \frac{(\text{A cont.} - \text{A test})}{(\text{A cont.})} \times 100$$

Where, Acont. = Absorbance of control reaction; A test = Absorbance of test reaction

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The phytochemical study [Table 1] shows that the ethanol extract of *D. bracteata* leaves contains carbohydrates, proteins, gums and mucilages, glycosides, phenolic compounds, flavonoids, triterpenoids, and saponins.

Total Phenol Content and Total Flavonoid Content in EEDB

The total phenolic and flavonoid contents of the ethanol extract of *D. bracteata* leaves were found to be 168.67 mg GAE per gram of dry extract and 61.67 mg quercetin equivalents per gram of dry extract, respectively.

In vitro Antioxidant Activity

DPPH radical scavenging activity

The reactivity of EEDB was analyzed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the

Table 1: Phytochemical screening of EEDB

S. No.	Test	Phytochemicals present in EEDB
1	Alkaloids	–
2	Glycosides	+
3	Carbohydrates	+
4	Steroids	–
5	Triterpenoids	+
6	Fixed oils and fats	–
7	Tannins	–
8	Proteins and amino acids	+
9	Gums and mucilages	+
10	Flavonoids	+
11	Saponins	+
12	Phenols and Phenolic compounds	+

Key: +: Present, –: Absent. EEDB: Ethanolic extract of *Dillenia bracteata*

resulting discoloration are stoichiometrically related to the number of electrons gained.[35] The DPPH radical scavenging (%) activity is shown in Figure 1, EEDB exerted an inhibition of 74% and that of ascorbic acid was 83% at 500 µg/ml, and the IC_{50} of the extract was 241.3 µg/ml, while that of ascorbic acid was 219.38 µg/ml.

FRAP assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric TPTZ complex and producing a colored ferrous TPTZ (Fe2+-TPTZ).[32] In general, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom.[36] FRAP assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction.[37] In the present study, the trend for ferric ion reducing activities of EEDB and ascorbic acid is shown in Figure 2. The absorbance of EEDB clearly increased, due to the formation of the Fe2+-TPTZ complex with increasing concentration. Hence, they should be able to donate electrons to free radicals stable in the actual biological and food system.

Hydroxyl radical scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once, inside the cell, H_2O_2 can probably react with Fe2+, and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects.[38] Hydrogen peroxide scavenging activity of the extract is presented in Figure 3; the extract exerted a concentration-dependent scavenging. At a concentration of 500 µg/ml, the hydroxyl radical scavenging activity of the EEDB was 67% while at the same concentration; the ascorbic acid reached 74%. The IC_{50} value of the extract was 201.6 µg/ml, whereas the standard exerted an IC_{50} of 130.63 µg/ml.[39]

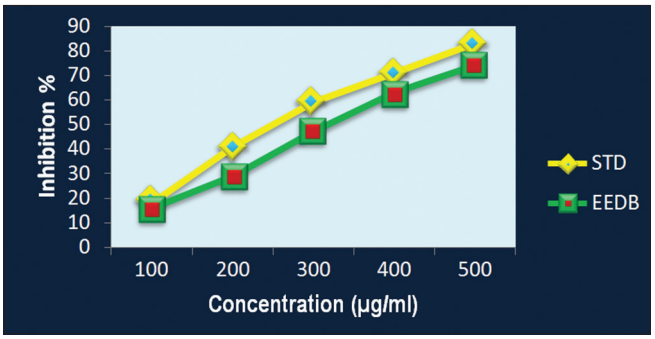

Figure 1: 1, 1-diphenyl-2-picryl-hydrazyl radical scavenging activity of the ethanolic extract of *Dillenia bracteata*

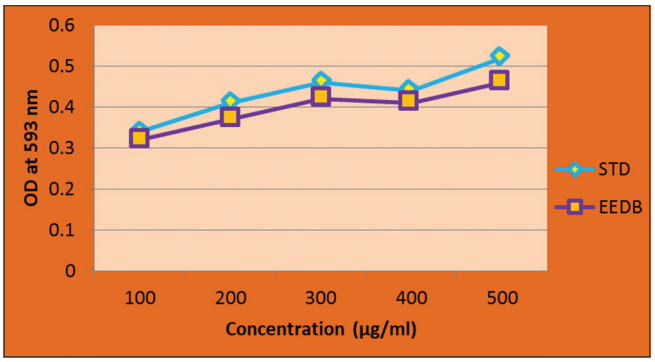

Figure 2: Ferric reducing antioxidant power assay of the ethanolic extract of *Dillenia bracteata*

Superoxide radical scavenging activity

Although superoxide anion is a weak oxidant, it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress.[40] Numerous biological reactions generate superoxide anions which are highly toxic species. At a concentration of 1000 µg/ml, the superoxide radical scavenging activity of the EEDB was 87% while at the same concentration; ascorbic acid reached 89%, which was significantly comparable

[Figure 4]. The IC_{50} values were found to be 70.6 µg/mL and 40.8 µg/mL, respectively, for EEDB and ascorbic acid. The results clearly indicate that EEDB has a noticeable effect as scavenging superoxide radical.

NO radical scavenging assay

NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation, and regulation of cell-mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation, and antimicrobial and antitumor activities.[41] Suppression of released NO may be partially attributed to direct NO scavenging, as the EEDB decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. The scavenging of NO by the extract was increased in concentration-dependent manner. Figure 5 illustrates a significant decrease in the NO radical due to the scavenging ability of extract and ascorbic acid. At a concentration of 500 µg/ml, the NO radical scavenging activity of the EEDB reached 69.21% while at the same concentration; the ascorbic acid reached 84.01%. The IC_{50} values were found to be 242.7 µg/ml and 228.9 µg/ml for EEDB and ascorbic acid, respectively.

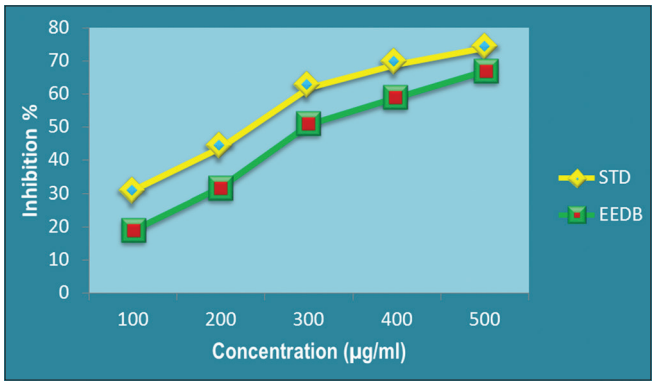

Figure 3: Hydroxyl radical scavenging activity of the ethanolic extract of *Dillenia bracteata*

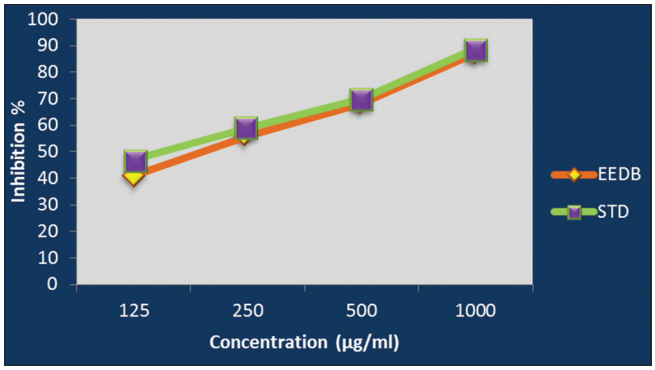

Figure 4: Superoxide radical scavenging activity of the ethanolic extract of *Dillenia bracteata*

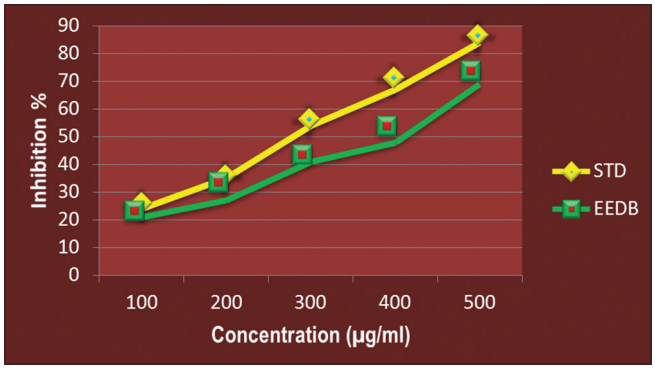

Figure 5: Nitric oxide radical scavenging activity of the ethanolic extract of *Dillenia bracteata*

CONCLUSION

The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing a number of diseases through free radical scavenging activity.[42] Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activity is very important due to the deleterious role of free radicals in biological systems. Many secondary metabolites which include flavonoids, triterpenoids, and phenolic compounds serve as sources of antioxidants and do scavenging activity.[43] In this study, it is evident that the extract of the study species, *D. bracteata* leaf possess effective antioxidant activity. The ethanol extract of *D. bracteata* leaf exhibited potent *in vitro* antioxidant activity in DPPH radical scavenging assay, FRAP assay, hydroxyl radical scavenging assay, superoxide free radical scavenging assay, and NO radical scavenging assay, in comparison to the known antioxidant such as ascorbic acid.

The antioxidant effect of *D. bracteata* leaf may be due to the phenolics and flavonoids present in it. The high antioxidant activity exhibited by the extract of *D. bracteata* leaf provided justification for the therapeutic use of this plant in folkloric medicine due to the phytochemical constituents. The present study suggests that this extract could be of great importance for the treatment of radical-related diseases and age-associated diseases.

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