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Antioxidant activity of the extracts from *Dillenia indica* fruits

Md.H. Abdille [b], R.P. Singh [a], G.K. Jayaprakasha [a], B.S. Jena [a,*]

[a] *Human Resource Development, Central Food Technological Research Institute, Mysore 570 020, India*
[b] *Department of Applied Botany and Biotechnology, University of Mysore, Mysore, India*

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Abstract

There has been growing interest in the beneficial health effects of consuming fruits and vegetables. Mainly, the presence of phenolic antioxidants is believed to have the protective mechanisms. In the present study the fruit of *Dillenia indica* was extracted with ethyl acetate, methanol and water. The total phenolic content of the extracts was determined by Folin–Ciocalteu method and antioxidant activity of the extracts was assayed through some in vitro models such as antioxidant capacity by phosphomolybdenum method, β-carotene-linoleate model system, and radical scavenging activity using α,α-diphenyl-β-picrylhydrazyl (DPPH) method. The total phenolic contents of the fruit extracts as tannic acid equivalents were found to be highest in methanol extract (34.1%) followed by ethyl acetate extract (9.3%) and water extract (1.4%). Antioxidant capacity of the extracts as equivalent to ascorbic acid (μmole/g of the extract) was in the order of methanol extract > ethyl acetate extract > water extract. In comparison with butylated hydroxyanisole (BHA), at 100 ppm of concentration, the antioxidant and free radical scavenging activities of the extracts assayed through β-carotene-linoleate model system, and DPPH method were also found to be highest with methanol extract followed by ethyl acetate and water extracts. The results indicated that the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract and the fruit of *D. indica* is rich in phenolics may provide a good source of antioxidant.
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Keywords: *Dillenia indica*; Total phenolics; Antioxidant activity

1. Introduction

Dillenia a small genus of trees found in Indo-Malaysian region extending to Tropical Australia. *D. indica* occurs in the moist and evergreen forests of sub-Himalayan tract, from Kumaon and Garhwal eastwards to Assam, West Bengal and Orissa. It is a evergreen tree, 30–50 ft. in height, which bears large and hard fruit of 3–5 in. in diameter, consisting of five closely fitted imbricate sepals enclosing numerous seeds embedded in a gelatinous pulp. The tree flowers during May–August and fruits ripen during September–February. The ripe fruits are gathered while the sepals thickened, which are sour in taste and widely used in flavouring of curries and preparation of jam and jelly. The acid juice is sweetened with sugar and used as cooling drink (The Wealth of India, 1952). The fruits are rich in nutrients (Gopalan, Ramasastri, & Balasubramaniam, 1971) and the fruit could be processed to commercial products such as clear beverage and ready-to-serve beverage and squash (Saikia & Saikia, 2002).

Epidemiological studies have shown that increased consumption of fruits and vegetables has been associated with protection against various diseases including certain forms of cancer, diabetes, Alzheimer's disease and cardiovascular disease (Ames, Shigenaga, & Hagen, 1993; Steinmetz & Potter, 1996; Willet, 2002). The protective effects of fruits and vegetables are attributed to the occurrence of antioxidants, particularly antioxidant

* Corresponding author. Tel.: +91 821 514310; fax: +91 821 517233.
E-mail address: bsjena@yahoo.com (B.S. Jena).

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vitamins such as ascorbic acid, α-tocopherol and β-carotene (Kalt & Kushad, 2000; Prior & Cao, 2000). The putative beneficial effects of a high intake of fruits and vegetables on the risk of diseases may not result exclusively from the action of well-characterized antioxidant vitamins rather, they may result from the action of lesser-known compounds or from a concerted action of a combination of different antioxidants present in these foods (Cao, Booth, Sadowski, & Prior, 1998). In addition to α-tocopherol, ascorbic acid and carotenoids, plant tissues synthesize a wide variety of phenolic compounds (Loliger, 1991). Several studies have revealed that the majority of the antioxidant activity may be from compounds such as flavonoids, isoflavones, flavones, anthocyanin, catechin and other phenolics (Kahkonen, Hopia, & Vuorela, 1999).

Oxidative stress has been linked to various diseases (Halliwell, 1994) while food industry long has been concerned with issues such as rancidity and oxidative spoilage of foodstuffs (Shahidi & Wanasundara, 1992). The enzymatic oxidation as well as autooxidation of lipids during storage and processing is the major reaction responsible for the deterioration in food quality affecting the colour, flavour, texture and nutritive value of the foods. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation step leading to the termination of the reaction and a delay in the oxidation process. However, the commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens (Imaida et al., 1983; Madhavi & Salunkhe, 1996). Therefore, there has been a considerable interest by the industry and a growing trend in consumer preferences for natural antioxidants over synthetic compounds and elimination of synthetic antioxidants in food applications has given more impetus to explore natural source of antioxidants. Thus, antioxidants are of interest to both food scientists and health professionals and there has been a convergence of interest among researchers in these fields as the role of antioxidants in the diet and their impact on human health has come under attention.

In the present study, the antioxidant activity of the extracts of *D. indica* fruit was assayed through various in vitro models. This is the first report on antioxidant activity of *D. indica* fruit.

2. Materials and methods

2.1. Materials

β-Carotene, linoleic acid, α,α-diphenyl-β-picrylhadrazyl (DPPH) and butylated hydroxyanisole (BHA) were obtained from Sigma Chemical Co., (St. Louis, MO, USA). All solvents/chemicals used were of analytical grade and obtained from Merck, Mumbai, India. Visible spectra measurements were done using Spectronic 20 visible spectrophotometer (Spectronic Instruments Inc., NY, USA). The fruits of *D. indica* were obtained from Balasore, Orissa.

2.2. Extraction

The fruits of *D. indica* were cut into pieces, sun dried and powdered in a grinder to 40-mesh size powder. 25 g of fruit powder was extracted with 150 ml of ethyl acetate by mixing, using a magnetic stirrer at 30 °C for 2 h. The extract was filtered through Whatman No. 41 filter paper to obtain particle free extract. The residue was re-extracted twice and filtered. The extracts were pooled and concentrated and dried under vacuum. The same procedure was followed for the other solvents such as methanol and water for antioxidant fractions (Jena, Jayaprakasha, Singh, and Sakariah, 2002) and the dried extracts were used to explore their antioxidant activity.

2.3. Determination of total phenolics

The concentration of phenolic compounds in the extracts was determined as described by Jayaprakasha, Singh, and Sakariah (2001) and results were expressed as tannic acids equivalents. The extracts were dissolved in a mixture of methanol and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of tenfold diluted Folin–Ciocalteu reagents and 0.8 mL of 7.5% sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using Spectronic 20 visible spectrophotometer. The estimation of phenolic compounds in the fractions was carried out in triplicate and the results were averaged.

2.4. Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of ethyl acetate, methanol and water extracts of fruit of *D. indica* was evaluated by the method of Prieto, Pineda, and Aguilar (1999). An aliquot of 0.1 ml of sample solution (100 μg/ml) was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in Spectronic 20 visible spectrophotometer. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the

sample. For samples of unknown composition, water-soluble antioxidant capacity was expressed as equivalents of ascorbic acid (μmole/g of extract).

2.5. Antioxidant assay using β-carotene-linoleate model system

The antioxidant activity of fruit extracts of *D. indica* was evaluated using β-carotene-linoleate model system as described by Jayaprakasha et al. (2001). 0.2 mg of β-carotene in 0.2 ml of chloroform, 20 mg of linoleic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed. Chloroform was removed at 40 °C under vacuum and the resulting mixture was diluted with 10 ml of water and was mixed well. To this emulsion, 40 mL of oxygenated water was added. Four milliliter aliquots of the emulsion were pipetted into different test tubes containing 0.2 ml of extracts (50 and 100 ppm) and BHA (50 and 100 ppm) in ethanol. BHA was used for comparative purposes. A control containing 0.2 ml of ethanol and 4 ml of the above emulsion was prepared. The tubes were placed at 50 °C in a water bath and the absorbance at 470 nm was taken at zero time ($t = 0$). Measurement of absorbance was continued till the color of β-carotene disappeared in the control tubes ($t = 120$ min) at an interval of 15 min. A mixture prepared as above without β-carotene served as blank. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula, $\mathrm{AA} = 100[1 - (A_o - A_t)/(A_o^0 - A_t^0)]$, where A_o and A_o^0 are the absorbance values measured at zero time of the incubation for test sample and control, respectively. A_t and A_t^0 are the absorbance measured in the test sample and control, respectively, after incubation for 120 min. The results were expressed in % basis of preventing bleaching of β-carotene.

2.6. Radical scavenging activity using DPPH method

Radical scavenging activity of the extracts was determined essentially as described by Blois (1958). Different concentrations (25, 50 and 100 μl equivalent to 25, 50 and 100 ppm, respectively) of *D. indica* fruit extracts and BHA (25, 50 and 100 ppm) were taken in different test tubes. The volume was adjusted to 100 μl by adding MeOH. 5.0 ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract and MeOH was used for the baseline correction. The changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula, % radical scavenging activity = (Control OD − sample OD/Control OD) × 100.

3. Results and discussion

The yields of ethyl acetate, methanol and water extracts of dried fruits of *D. indica* were 4.9%, 21.5% and 4.3%, respectively. The total phenolic contents of the fruit extracts of *D. indica* as determined by Folin–Ciocalteu method, are reported as tannic acid equivalents (Table 1). Among the three extracts, methanol extract was containing highest (34.1%) amount of phenolic compounds followed by ethyl acetate extract (9.3%) and water extract (1.4%). In our recent studies, it has been reported that the yield of extractable compounds was highest in methanol extract from the peel and seeds of pomegranate in comparison with the solvents such as ethyl acetate and water (Negi, Jayaprakasha, & Jena, 2002; Singh, Chidambara Murthy, & Jayaprakasha, 2002). Furthermore, the extraction of phenolic compounds from the fruit is commonly achieved with methanol or aqueous methanol (Amiot, Fleuriet, & Macheix, 1986; Antolovich, Prenzler, Robards, & Ryan, 2000). The levels of total phenolics determined in this way are not absolute measurements of the amounts of phenolic compounds, but are in fact based on their chemical reducing capacity relative to tannic acid. It has been observed that the phenol antioxidant index, a combined measure of the quality and quantity of antioxidants in vegetables (Vinson, Hao, & Zubic, 1998). In the present study the responses of the extracts in this assay may arise from the variety and/or quantity of phenolics found in three different fruit extracts of *D. indica.*

Fruit and vegetables are the main sources of antioxidant vitamins (vitamin E, vitamin C, precursor of vitamin A i.e., β-carotene), which act as free radical scavengers, making these foods essential to human health (Elliot, 1999). However, more than 80% of the total antioxidant capacity in fruits and vegetables comes from the ingredients other than antioxidant vitamins, indicating the presence of other potentially important antioxidants in these foods (Miller & Rice-Evans, 1997) and phenolic compounds are the dominant antioxidants that exhibit scavenging efficiency on free radicals and reactive oxygen species are numerous and widely distributed in the plant kingdom (Namiki, 1990; Prior & Cao, 2000). In the present study, the relative antioxidant ability of the *D. indica* fruit extracts was investigated through some in vitro models such as

Table 1
Phenolic content (as tannic acid equivalent) extracts of *D. indica* fruit

Extract	Phenolics (% w/w)
Water extract	1.41 ± 0.079
Ethyl acetate	9.37 ± 0.108
Methanol	34.14 ± 0.130

Here and in Tables 2 and 3, the values are means ± SD of three replicates.

antioxidant capacity by phosphomolybdenum method, β-carotene-linoleate model system, and radical scavenging activity using, α-diphenyl-β-picrylhydrazyl (DPPH) method.

The antioxidant capacity of the extracts was measured spectrophotometrically through phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of fruit extracts of *D. indica* was found to decrease in the order, methanol extract > ethyl acetate extract > water extract (Table 2).

The antioxidant activity through β-carotene-linoleate model system of *D. indica* fruit extracts at 50 and 100 μg/mL concentrations were compared with butylated hydroxyanisole is presented in (Table 3). The addition of *D. indica* fruit extracts and butylated hydroxyanisole at 50 μg/mL concentrations prevented the bleaching of β-carotene to different degrees. β-Carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidised and broken down in part, subsequently the system looses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically. In our present study, the extracts from *D. indica* fruits were found to hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system. Methanol extract, ethyl acetate extract and water extracts showed 80.2%, 55.5% and 45.5% antioxidant activity, respectively, at 100 μg/ml concentration.

Table 2
Antioxidant capacity of extracts of *D. indica* fruit by phosphomolybdenum method

Extract	Antioxidant capacity as equivalent to ascorbic (μmoles/g of extract)
Water	594.6 ± 25.9
Ethyl acetate	1067.0 ± 11.9
Methanol	1904.8 ± 12.1

Table 3
Antioxidant activity of extracts from D. *indica* fruit and BHA by β-carotene-linoleate model system (% inhibition of bleaching of β-carotene)

Extarct/BHA	50 ppm	100 ppm
BHA	93.3 ± 0.02	97.5 ± 0.03
Water	29.66 ± 1.01	45.5 ± 0.09
Ethyl acetate	45.6 ± 0.08	55.5 ± 1.10
Methanol	64.2 ± 0.40	80.2 ± 0.75

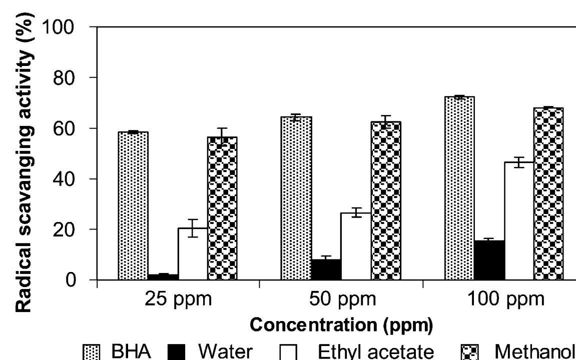

Fig. 1. Radical scavanging actvity of *D. indica* fruit extracts by DPPH method.

The free radical scavenging activity of the fruit extracts of *D. indica* were tested through DPPH method and the results are presented in the (Fig. 1). The role of antioxidants is their interaction with oxidative free radicals. The essence of DPPH method is that the antioxidants react with the stable free radical i.e., α,α-diphenyl-β-picrylhydrazyl (deep violet colour) and convert it to α,α-diphenyl-β-picrylhydrazine with discolouration. The degree of discolouration indicates the scavenging potentials of the sample antioxidant. In the present study the extracts of *D. indica* fruit were able to decolourise DPPH and the free radical scavenging potentials of the extracts of were found to be in the order of methanol extract > ethyl acetate extract > water extract. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.), and aromatic amines (*p*-phenylene diamine, *p*-aminophenol etc.), reduce and decolourise α,α-diphenyl-β-picrylhydrazyl by their hydrogen donating ability (Blois, 1958). It appears that the extracts from the fruits of *D. indica* possess hydrogen donating capabilities to act as antioxidant.

In our present study, the decreasing order of antioxidant activity among the *D. indica* fruit extracts assayed through all the three methods was found to be methanol extract > ethyl acetate extract > water extract. This order is similar to the phenolic contents of the extracts that showed the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract. In the present study it is found that the methanol extract of *D. indica* fruit contains substantial amount of phenolics and it is the extent of phenolics present in this extract is responsible for its marked antioxidant activity as assayed through various in vitro models. Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables (Deighton, Brennan, Finn, & Davies, 2000; Tsushida, Suzuki, & Kurogi,

1994; Vinson et al., 1998; Velioglu, Mazza, Gao, & Oomah, 1998). Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also needs their characterization (Heinonen, Lehtonen, & Hopla, 1998). For instance, it has been reported that phenolic compounds with *ortho*- and *para*-dihydroxylation or a hydroxy and a methoxy group are more effective than simple phenolics (Frankel, Waterhouse, & Teissedre, 1995; Shahidi & Naczk, 1995). However, synergistic or additive actions of the phenolics present in the extracts cannot be ruled out. This is the first report that envisages the antioxidant activities of *D.indica* fruit extracts. Hence the fruits of *D. indica* could be a good source of antioxidant phenolics. Further studies are warranted for the isolation and identification of individual phenolic compounds and also in vivo studies are needed for better understanding their mechanism of action as antioxidant.

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