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ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *DILLENIA INDICA* L. FRUIT OF SONITPUR, ASSAM, INDIA

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ABSTRACT

Dillenia indica L. commonly known as elephant apple is distributed in the sub-Himalayan tract of India. The fruits of *D. indica* collected from Bhalukpong area of Sonitpur district of Assam (India) were dried, milled and extracted sequentially by Accelerated Solvent Extractor using four different solvents with increasing polarity (hexane < ethyl acetate < methanol < 50% aqueous methanol). Qualitative phytochemical analysis was carried out according to standard procedures. Total phenolic content (TPC) of all the extracts were determined by Folin-Ciocalteu's method using UV-Vis spectrophotometer. Free radical scavenging activity were determined by measuring the decrease in the visible absorbance of 2,2-diphenyl-1-picrylhydrazyl (DPPH) on addition of the plant extracts and the mean inhibitory concentration (IC_{50}) values were determined by plotting nonlinear regression curve. L-Ascorbic acid and gallic acid were used as standard in DPPH assay and TPC respectively. Phytochemical analysis confirms the presence of phenolic compounds in methanolic extract. The highest phenolic content (59.99±2.21 mg/g) was recorded in 50 % aqueous methanolic extract which also shows highest antioxidant activity with IC_{50} value of 56.66±1.55 µg/ml. The nutrient and antioxidant rich *Dillenia indica* L. fruit can be explored commercially to tap its health benefit properties by large scale processing in production of ready to serve juice, drink, squash etc.

INTRODUCTION: *Dillenia indica* L. (Dilleniaceae) commonly called as elephant apple (*Outenga* in Assamese) is a well known fruit in Assam. The plant is distributed in the sub-Himalayan tract from Uttarakhand eastwards to Assam and Bengal southwards to central and south India. It is grown in gardens for its attractive flowers and fleshy edible fruits [1]. *D. indica* is an ethno-medicinally important plant used for the treatment of severe diseases like cancer and diarrhea [2]. The fruit extract has shown significant anti-leukemic activity in human leukemic cell lines [3]. The fruit possesses tonic and laxative properties and is used for abdominal pains. The antioxidant effect of a plant is mainly due to phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes [4]. Phenolic compounds inhibit free radicals in the body which are responsible for oxidative damages.

The present study is undertaken to determine total phenolic content and mean inhibitory concentration (IC_{50}) value by DPPH scavenging activity of *D. indica* fruit extract in four different solvents and qualitative phytochemical analysis in methanolic extract of *D. indica* fruit.

MATERIALS AND METHODS:

Chemicals: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich; ascorbic acid, Folin-Ciocalteu's phenol reagent and sodium carbonate were obtained from Merck; and gallic acid was obtained from BDH. All other reagents used were of GR grade.

Preparation of Plant Extract: The mature fruits of five different trees were collected from Bhalukpong area of Sonitpur district of Assam (India) and cut into pieces to make shade dry. Dried sepals of the fruit were pulverized and extracted sequentially by Accelerated Solvent Extractor (ASE 150, Dionex, USA) using four different solvents with increasing polarity (hexane < ethyl acetate < methanol < 50% aqueous methanol). Two cellulose filters were placed into the bottom of the extraction cell. 30 g of finely powdered *D. indica* fruit were mixed with diatomaceous earth (nearly equals to 1/10 of the volume of the sample) and filled into the cell. The various conditions maintained for extraction under ASE are:

- Cell type: 100ml stainless steel
- Filter type: Cellulose
- Gas type: N_2
- Pressure: 1500 psi
- Temperature: 100^{o}C
- Static time: 5 minutes
- Rinse volume: 60 %
- Purging time: 90 seconds
- Static cycle: 3

The final volume was concentrated to dryness by rotary evaporator at 50°C under reduced pressure. The crude extract were weighed and stored at -4°C.

FIGURE 1: RIPE FRUIT OF *DILLENIA INDICA*

Preliminary Phytochemical Analysis: The methanolic extract of the plant were analyzed qualitatively for phytochemical content by standard methods given by Raaman [5].

Determination of Total Phenolic Content (TPC): TPC of different extracts were determined by the Folin-Ciocalteu method as followed by Tsering *et al.,* [6] with reduced volumes. Standard curve was plotted by using gallic acid at five different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml). Stock solution of standard was prepared by mixing 10mg gallic acid in 100ml of water. Different volumes of stock solution (100µl-500µl) were mixed with 2ml water and 0.3ml Folin-Ciocalteu's phenol reagent in test tubes.

After 5 minutes 0.8ml 20% sodium carbonate was added and the final volume was made to 5ml. In separate test tubes 200µl (1mg/ml) of each extracts were mixed individually with the same reagents as described above and the final volume was made to 5ml. After 30 minutes the absorbance was measured at 765 nm using UV-Vis spectrophotometer (SPECORD 250, Analytik Jena, AG, Germany). Results were expressed as mg/g Gallic Acid Equivalent and TPC was calculated using the formula:

$$TPC = c \times \frac{V}{m}$$

where, c is the concentration of gallic acid established from the calibration curve (mg/ml); V is the volume of plant extract (ml); and m is the weight of pure plant extract (g).

Determination of Free Radical Scavenging Activity: Free radical scavenging activity was determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay given by Brand-Williams *et al.,*[7] with some modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (25, 50, 100, 175, 250, 375, 500 and 1000 µg/ml methanol). Mixture of 1ml methanol and 1ml DPPH solution was used as control and L-Ascorbic acid as standard. The decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer (SPECORD 250, Analytik Jena, AG, Germany) and the inhibition % was calculated using the following formula:

Inhibition %=

$$\frac{[A_{(Control)} - A_{(Test\ Sample)}]}{A_{(Control)}} \times 100$$

Statistical Analysis: All reactions were carried out in triplicate and results were expressed as mean±SD. The free radical scavenging activity was expressed as IC_{50} in µg/ml determined by non linear regression curve using Graph Pad Prism-5 software and total phenolic content (TPC) was expressed in mg/g [Gallic Acid Equivalent (GAE)/dry weight (dw)].

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening: The result of various phytochemical tests is shown in **Table 1**. The presence of phenolic compounds in methanolic fruit extract of *D. indica* is confirmed by ferric chloride test. The screening also showed the presence of carbohydrates in bulk and fixed oils in low amount. Test for other compounds like alkaloids, saponins, gums and mucilage, proteins, amino acids and phytosterols showed negative results.

TABLE 1: PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS OF METHANOLIC FRUIT EXTRACT OF *D. INDICA* FRUIT

Phytochemicals/ tests	Inferences
Alkaloids	
i. Mayer's test	-
ii. Wagner's test	-
iii. Hager's test	-
Carbohydrates	
i. Molish's test	+++
ii. Fehling's test	++
iii. Barfoed's test	++
iv. Benedict's test	++
Saponins test	-
Gums and Mucilages test	-
Proteins/Amino acids	
i. Biuret test	-
ii. Ninhydrin test	-
Phytosterols	
i. Libermann-Burchard's test	-
Fixed oils	
i. Spot test	+
Phenolic compounds	
i. Ferric chloride test	+
ii. Gelatin test	-
iii. Lead acetate test	-

(-) absent; (+) present

Extract yield: Yield of extract is given in **Table 2** which is recorded highest for methanolic extract followed by 50 % aqueous methanol > ethyl acetate > hexane extract.

TABLE 2: EXTRACT YIELD, IC_{50} AND TPC OF *D. INDICA* FRUIT EXTRACT

Extract type	Yield of extract (g %, w/w)	IC_{50} (µg/ml) (Mean±SD)	TPC (mg/g) (Mean±SD)
L-Ascorbic acid	-	4.98±0.24	-
Hexane extract	0.77	428.07±28.54	< 1
Ethyl acetate extract	1.54	85.10±4.64	50.20±1.92
Methanol extract	10.07	62.17±0.62	49.80±0.40
50% Aqueous methanol extract	8.80	56.66±1.55	59.99±2.21

Total phenolic content (TPC): Phenols are very important plant constituents and there is a highly positive relationship between total phenolic content and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups [8]**.** It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidants [9].

In the present study, TPC was determined by plotting standard gallic acid curve with regression equation y=0.0737+15.8978*x; R^2= 0.9895**.** TPC of various extracts are presented in **Table 2**. Moderate amount of TPC was found in methanol, 50 % aqueous methanol and ethyl acetate extract while hexane extract has a very low phenolic content.

IC_{50} (50% Inhibitory concentration): IC_{50} values of different extracts of *D. indica* fruits along with L-ascorbic acid are shown in **Table 2**. IC_{50} is the concentration of samples or antioxidant required to inhibit the initial absorbance of DPPH free radical by 50% [10] and a lower IC_{50} value would reflect greater antioxidant activity of the sample [11]. IC_{50} value of 50% aqueous methanol, methanol and ethyl acetate extracts ranges from 56.66±1.55 µg/ml to 85.10±4.64 µg/ml showing moderate antioxidant capacity while hexane extract with high value of IC_{50} has least antioxidant property. IC_{50} of L-Ascorbic acid was recorded lowest followed by methanol extract < 50 % aqueous methanol extract < ethyl acetate extract. **Figure 2** shows inhibition curves of different extracts of *D.indica* fruit with increasing concentrations. The three extracts *viz.*, 50 % aqueous methanol, methanol and ethyl acetate other than hexane extract shows almost same inhibition curve.

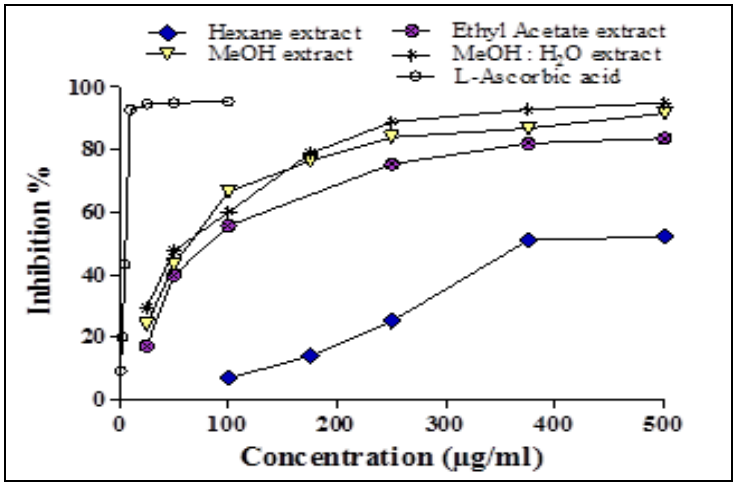

FIGURE 2: DPPH SCAVENGING ACTIVITY OF *D. INDICA* FRUIT EXTRACT ALONG WITH L-ASCORBIC ACID

CONCLUSION: The fruit of *D.indica* has potent antioxidant activity. The extent of the antioxidant activity of *D. indica* fruit extract is in accordance with its phenolic content. The hexane extract of *D. indica* fruit has a very low phenolic content and antioxidant activity. The aqueous methanol (50%), methanol and ethyl acetate extracts has moderate phenolic content as well as antioxidant property.

The phenolic content of *D. indica* fruit extract is responsible for its free radical scavenging activities which are polar compounds in nature. *D. indica* is widely available in the plains of the Brahmaputra valley in Assam.

The unripe fruits are used to make curries because of its sour taste and ripe fruits are used for making pickles. The present finding partially validates the traditional knowledge about the goodness of consumption of this fruit. Further study is going on to isolate the bioactive phytochemicals. The nutrient and antioxidant rich *Dillenia indica* L. fruit can be explored commercially to tap its health benefit properties by large scale processing in production of ready to serve juice, drink, squash etc.

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