Nutritional Value and Antioxidant Activity of Fruit of *Dillenia indica* L. (Tha-byu) From Hinthada Township

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Abstract

Nowaday the potency of herbal drugs is significant and they have negligible side effects than the synthetic drug. Therefore there is increasing the demand by patients to use the natural products with medicinal activity. This research focused on some nutritive value and antioxidant activity of fruit of *Dillenia indica* L.(Tha Byu) from Hinthada Township. Preliminary phytochemical investigation have revealed the presence of carbohydrates, flavonoids, α-amino acids, phenolic compounds, saponins, glycosides, tannins, steroids and terpenoids, whereas alkaloids, starch, reducing sugars and cyanogenic glycosides were not detected in the fruit. The fruit sample was found to contain 12.16 % of moisture, 5.71 % of ash, 1.58 % of protein, 29.32 % of dietary fiber, 8 % of crude fat, 43.23 % of carbohydrate based on dried sample. According to EDXRF spectrum, the fruit also possess relatively highest amount of Potassium (2.219 %), Calcium (0.447 %) and minor components such as S, P, Fe, Zn, Cu and Rb. Moreover, the determination of the vitamin C content in the fruit were carried out by conventional method. Ascorbic acid content in mature *D.indica* fruit was found to be 19.81 mg in 100 g of fresh sample. The antioxidant activity of the fruit was also be studied by DPPH assay Method. Antioxidant activity was exhibited by IC_{50} (50 % Inhibition Concerntration) value. IC_{50} values of different extracts of the fruit were found to be 7.13µg/mL for methanol, 10.2 µg/mL for water, 12.78 µg/mL for pet-ether and 13.71 µg/mL for ethyl acetate.

Keywords: *Dillenia indica* L. Phytoconstituents, Nutritional value, EDXRF, Vitamin C, Antioxdiant Activity, IC_{50}

Introduction

In the plant kingdom, there are thousands of plants known and unknown, that yield medicine or drugs of great use to man. Myanmar is a country considered to be rich in medicinal plants genetic resources. *Dillenia indica* L., commonly known as elephant apple is an evergreen large shrub or small to medium-sized tree that grows Southeastern Asia. In Myanmar, it is abundant in Ayeyarwady Region, Rakhine state, Bago Division, Mon state, Tanintaryi Division and Kayin state. It was also found traditionally that in various part of Northeast India, the juices of leaves, bark and leaves were mixed and given orally for the treatment of cancer and diarrhea (Sunil *et al.,* 2011 and Yeshwante *et al.,* 2009). The leaves and bark are used as a laxative and astringent. The review of various literature showed that the leaves, bark, fruits or the various part of the *D. Indica* L. (Tha Byu) have extensive medicinal values. Juice of the fruit are utilized for preparing cough syrups and, blended with water and sugar, for reducing fever. Bark is used for arthritis. This plant also possesses various activities like Antimicrobial, Antioxidant Analgesic, Anti-inflammatory, Dysentery, Antidiabetic etc.The fruit juice is traditionally used for the treatment of various diseases and one of the major diseases is Diabetes Mellitus. It was also proved that this plant possesses some antidiabetic properties (Sunil kumar *et.al*.,2011).The sepals are traditionally used for stomach disorder (Talukdar *et al.*, 2012). As it possess higher medicinal values and easily available in Hinthada Township, the fruit of this plant was selected for this research work.The present finding validates the advantages of consumption of this fruit.

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Botanical Characteristics of *Dillenia indica* L.

Family	: Dilleniaceae
Scientific name	: *Dillenia indica* L.
English name	: Elephant Apple
Genus	: *Dillenia*
Species	: *indica*
Myanmar name	: Tha-Byu
Plant part used	: Fruit

Figure (1) Photographs of *Dillenia indica* L.

Materials and Methods

Preparation of Sample

The Fruits of the *Dillenia indica* L. (Tha-Byu) were collected from Chauk Ywar, Ingapu Township, Ayeyawady Region. After cleaning with water, the fruits were sliced into small pieces and air dried under shade at room temperature for two weeks. The dried sample was then ground into powder by the aid of grinding machine and stored in airtight containers to prevent moisture changes and other contamination.

Preliminary Phytochemical Investigation

The qualitative chemical tests for the presence of various phytoconstituents were carried out according to standard procedure. It involves testing of different extracts of the fruit for their contents of different classes of compound such as alkaloids, terpenoids, flavonoids, phenolic compounds, steroids, glycosides and amino acids.

Determination of Nutritional Value

The nutritional values such as protein, fiber, fat and carbohydrates were also determined. The fat content was determined by the soxhlet extraction method.The protein and fiber content were also studied by acid alkali treatment. The quantitative analyses for the determination of total ash and moisture contents have been done according to methods described in the British Pharmacopoeia (1980), Myanmar medicine formulary (1989) and Ayuvedic formulation (1976).The moisture content and ash content of sample was determined by AOAC methods.The relative abundance of elements present in *D.indica* fruit was also studied by EDXRF spectrometer.

Determination of Vitamin C content

Ascorbic acid content (vitamin C) was determined by the conventional method given in "Laboratory Chemistry for the Health Sciences". 20 cm^3 of fruit sample solution was pipetted into a 250 cm^3 conical flask. About 150 cm^3 distilled water and 1cm^3 of 0.5 % starch indicator solution were added into that solution. It was titrated with 0.005 M iodine solution. The end point of the titration was identified as the first permanent trace of a dark blue-black color was occurred due to the starch-iodine complex.

The ascorbic acid content in sample was calculated from the volume of iodine consumed.

Determination of Antioxidant Activity

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of the fruit sample. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system. In this experiment, the antioxidant activity was studied on pet ether, ethyl acetate, methanol and water extracts of selected fruit sample by DPPH free radical scavenging assay.

Preparation of solutions

(a) 60μM DPPH solution

2.364 mg of DPPH was thoroughly dissolved in 95% ethanol (100 cm^3). This solution was freshly prepared in the brown coloured flask. Then it must be stored in the fridge for no longer than 24 hours.

(b) Test Sample Solution

Accurately weighed 2 mg of each test sample and 10 cm^3 of 95 % ethanol was thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. Desired concentrations (1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL) of each solution was prepared from this stock solution by dilution with appropriate amount of 95 % ethanol.

(c) Blank Solution

Blank solution was prepared by mixing the test sample solution (1.5 cm^3) with 95% ethanol (1.5 cm^3).

(d) Procedure

DPPH radical scavenging activity was determined by UV spectrophotometric method. The control solution was prepared by mixing 1.5 cm^3 of 60 µM DPPH solution and 1.5 cm^3 of 95 % ethanol using shaker. The sample solution was also prepared by mixing thoroughly 1.5 cm^3 of 60 µM DPPH solutions and 1.5 $cm^3$of test sample solution. The solution were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the equation. Then IC_{50} (50% inhibitory concentration) value were also calculated by linear regressive excel program.

$$\% \text{ Inhibition} = \frac{A_{DPPH} - (A_{\text{test sample}} - A_{Blank})}{A_{DPPH}} \times 100$$

% Inhibition = percent inhibition of sample

A_{DPPH} = absorbance of DPPH in EtOH solution

$A_{\text{test sample}}$ = absorbance of (sample + DPPH) solution

A_{Blank} = absorbance of (sample + EtOH) solution

Results and Discussion

Preliminary Phytochemicals Investigation

Before conducting other investigation, it is essential to carry out preliminary phytochemical investigation on fruit of *Dillenia indica* L. Test results revealed the presence of phenolic compounds, flavonoids, tannins, steroids, terpenoids, carbohydrates, saponins, α-amino acids and glycosides in the sample, whereas alkaloids, starch, reducing sugars and cyanogenic glycosides were not found in the fruit.

Nutritional Values

The fat content of the fruit of *Dillenia indica* L. was determined by the soxhlet extraction method and was found to be 8 %. In addition, the sample was also studied for fiber content by acid alkali treatment and protein content by AOAC method. The fiber, protein, fat and carbohydrates contents for *D. indica* fruit were found to be 29.32 %, 1.58 %, 8 % and 43.23 % respectively.

The total ash in the fruit sample is the inorganic residue remaining after the organic matter has been burnt away. It was obtained by using Muffle furnace and found to be 5.71 %. The moisture content of the sample was determined by oven dried method and found to be 12.16 %. All the results obtained were represented in Table 1. Moreover, the elemental compositions in the dried fruit sample were studied by EDXRF spectrometry. The relative abundance of the elements were 2.219 % for K, 0.447 % for Ca, 0.337 % for S, 0.105 % for P, 0.006 % for Fe, 0.002 % for Zn, 0.002 % for Cu and 0.001 % for Rb. It can be seen that potassium present in greater amount than the others.Potassium is crucial to cardiovascular and nerve functions in our body. But it can be lost in diuretic therapy for edema or hypertension. Therefore, drinking the fruit juice of *Dillenia indica* prevents the lost of potassium from the body. All the results were reported in Table (2).

Determination of Vitamin C Content

The ascorbic acid content in freshly blended *D. indica* fruit juice was determined by the redox titration using iodine solution. In this experiment, a known volume of fruit juice was titrated with iodine solution using starch as an indicator.

Due to this reaction, the iodine formed is immediately reduced to iodide as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidized to dehydroascorbic acid, the excess iodine is free to react with the starch indicator, forming starch-iodine complex. The end point of the titrations were identified as the first permanent trace of a dark blue-black color due to the starch-iodine complex.

Vitamin C (Ascorbic acid) is an antioxidant that need for human nutrition. One way to determine the amount of Vitamin C in food is redox titration. The redox reaction is better

than acid-base titration since there are additional acids in juice, but few of them interfere with the oxidation of ascorbic acid by iodine.

This titration procedure is appropriate for testing the vitamin C in vitamin C tablets, juices and fresh, frozen, or packaged fruits and vegetables. The result of calculated ascorbic acid content in fruit sample was reported in Table (3).

Free Radical Scavenging Activity

Free Radical Scavenging Activity **(**antioxidant activity) was studied on the petroleum ether, ethyl acetate, methanol and water, extracts of *D.indica* fruit by DPPH free radical scavenging assay method. DPPH (1,1-diphenyl -2-picryl-hydrazyl) method is the most widely reported method for screening of antioxidant activity on many plant drugs. This method is based on the reduction of coloured free radical DPPH in ethanolic solution by different concentration of the samples.The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}) value. IC_{50} is the concentration of sample or antioxidant required to inhibit the initial absorbance of DPPH free radical by 50 % and a lower IC_{50} value would reflect greater antioxidant activity of the sample.

In this study, five different concentrations (1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL) of each crude extract were prepared in ethanol solvent. Ascorbic acid was used as standard and ethanol without crude extract was employed as control. Determination of absorbance was carried out at wave length 517 nm using UV visible spectrophotometer. Each experiment was done triplicate.

The percent oxidative inhibition values of crude extracts measured at different concentrations and the results were summarized in Table 4. From these experimental results, it was found that as the concentrations increased, the absorbance values decreased i.e, increase in radical scavenging activity of crude extracts usually expressed in term of percent inhibition. From the average values of percent inhibition, IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program.

The IC_{50} values were found to be 7.13 µg/mL for methanol, 10.2 µg/mL for water, 12.78 µg/mL for petroleum ether and 13.71 µg/mL for ethyl acetate. Among these extracts, methanol extract was found to be more effective than the others as it possesses lower the IC_{50}. It was observed that all the extracts possess the significant amount of antioxidant activity.

Table (1) Some Nutritional Values of *D. indica* L. Fruit.

No.	Parameters	% Contents (w/w)
1	Protein	1.58
2	Ash	5.71
3	Fats	8.00
4	Moisture	12.16
5	Crude Fiber	29.32
6	Carbohydrate	43.23

Table (2) Relative Abundance of Some Elements in *D. indica* L. Fruit by EDXRF Spectrometry.

No	Elements	Relative Abundance (%)
1	Potassium (K)	2.219
2	Calcium (Ca)	0.447
3	Sulphur (S)	0.377
4	Phosphorous (P)	0.105
5	Iron (Fe)	0.006
6	Zinc (Zn)	0.002
7	Copper (Cu)	0.002
8	Rubidium (Rb)	0.001

Table (3) Vitamin C Content in Fruit of *Dillenia indica* L.

Name of sample	Observed value (mg/100g)
Fruit	19.811
Vitamin C	98.616

Table (4) Oxidative Inhibition Percentage and IC_{50} Values of Different Extracts of Fruit of *D. indica* L. and Standard Ascorbic Acid.

Extracts	Inhibition percent in different concentrations (%)					IC_{50} (µg/mL)
	1.25	2.5	5	10	20	
MeOH	30.54	36.47	44.2	57.47	68.04	7.13
H_2O	23.5	30.21	37.73	49.77	61	10.2
PE	28.21	36.28	38.05	46.68	58.63	12.78
EA	21.21	25.95	33.06	45.23	57.23	13.71
Ascorbic acid	28.53	31.25	45.38	57.07	69.84	6.98

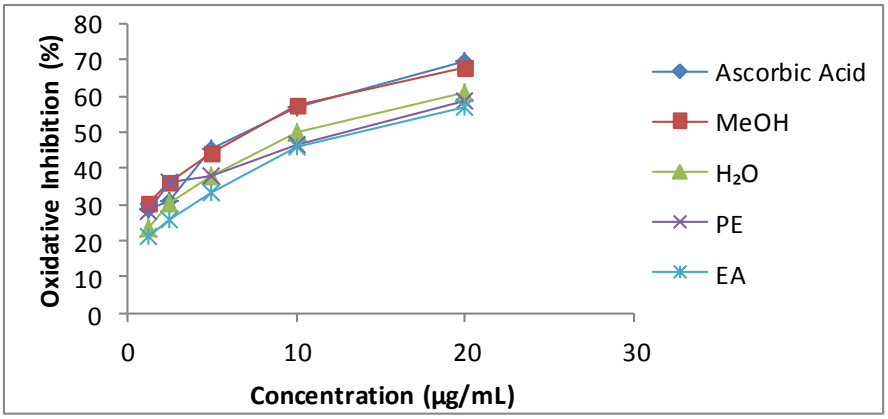

Figure (2) Plot of oxidative inhibition percent vs concentration (µg/mL) of jPE EtOAc, MeOH and H_2O extracts of *Dillenia indica* L. fruit compare with standard ascorbic acid.

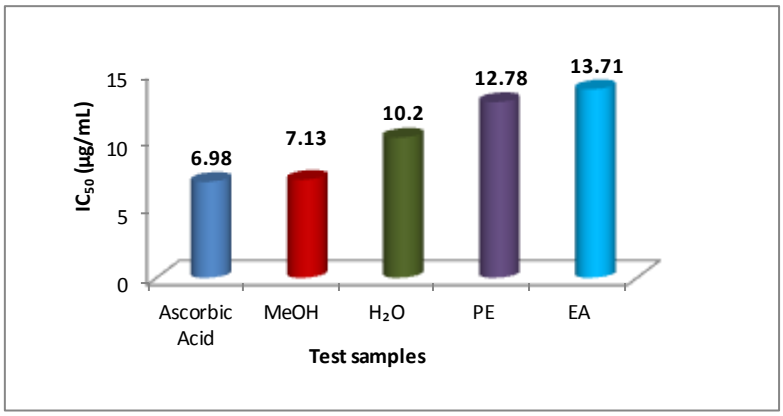

Figure (3) A bar graph of IC_{50} values of, PE, EtOAc, MeOH and H_2O extracts of *Dillenia indica* L. fruit compare with standard ascorbic acid.

Conclusion

The present work evaluated some nutritional values and free radical scavenging activity of fruit of *Dillenia indica* L. (Tha-byu). Preliminary phytochemical investigation reviled the presence of phenolic compounds, flavonoids, steroids, terpenoids, tannins, saponins, α-amino acids, carbohydrates and glycosides, whereas alkaloids, cyanogenic glycosides, reducing sugars and starch were not detected in the fruit. Study on nutritional value indicated protein (1.58%), Fat (8%), fiber (29.32%), carbohydrate (43.23%), moisture (12.16%) and ash (5.71%). Elemental composition by EDXRF provided 2.219% of potassium, 0.447% of calcium and trace amount of elements S, P, Fe, Zn, Cu, Rb. Moreover, vitamin C content of 19.81mg/100g of the sample was obtained by redox titration. Free radical scavenging activity (Antioxidant activity) of the *D.indica* fruit was represented in terms of IC_{50} value. The lower the IC_{50} value, the greater is the antioxidant activity. Among the different soluble portions of the fruit, methanol extract showed the lowest IC_{50} value (7.13 µg/mL) followed by water (10.2 µg/mL), petroleum ether (12.78 µg/mL) and ethyl acetate (13.71 µg/mL). On the basis of the results that have been observed in the present study, nutrients and antioxidant rich *D.indica* fruit can be explored commercially for the production of fruit juice, drink and squash. Moreover, it could be applied as the local health remedy to the local indigenous communities of our country. Therefore, the fruit of the *D.indica* Linn. is one of the valuable fruit in Myanmar.

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