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Antioxidant activity of stem bark of Elephant Apple in different solvents

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ABSTRACT

Plants have been always played an important role in maintaining human health directly or indirectly. *Dillenia indica* Linn. is a plant known for its nutritive as well as medicinal properties. Bark of the plant has been blessed with various phytochemicals as alkaloids, tannins, phenols etc. which give the plant a defence mechanism to protect the plant. Presence of these phytochemicals proved that bark is also beneficial for human beings because of their therapeutic properties. In the present study the total phenolic content and antioxidant activities of the different extracts of bark of *Dillenia indica* Linn. have been examined and it is found that ascorbic acid as standard having the great antioxidant activity with lowest IC_{50}. Fifty per cent methanolic extract showed higher amount of total phenolic content 47.6± 0.92 mg/gm and lowest IC_{50} value 13.43 ± 1.25µg/ml having highest antioxidant activity. In contrast with its aqueous extract having highest IC_{50} value 239.1 ± 2.15 µg/ml with lowest antioxidant activity. The antioxidant activity has proved to be great source in the prevention of many life threatening diseases. The antioxidant activity of *Dillenia indica* Linn. should be further explored commercially to take its health benefits for serving the human society.

Keywords: Alkaloid, Antioxidant, *Dillenia indica,* Therapeutic, Life threatening.

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INTRODUCTION

Dillenia indica Linn. commonly known as Elephant Apple. It is a beautiful avenue tree often grown as an ornamental plant along road side. It is having beautiful and showy fragrant flowers and blessed with sour and tasty fruits. *Dillenia indica* Linn. has been nurtured by nature with various medicinal and therapeutic properties and generally used by tribal and folk people. Plants are always have been a prime responsible entity for human health and nutrition from the time immemorial as only they are available easily and freely and can be used as such in primitive era. Therefore, the plants are used as the important sources of medicines since the time when human civilization began. Some plants have the great value as medicines and therapeutic uses but they are not well known. The present work is based on an under explored genus *Dillenia indica* Linn. which belongs to family Dilleniaceae having 11 genera and 300 species. The genus *Dillenia* has almost 60 species out of which three species are found in India[1]

Human diet can be a great source of antioxidants as they contain various plants and vegetables rich in phenols, ascorbic acid etc., these phytochemicals are organic compounds having antioxidant properties [2,3]. Antioxidants play vital role by preventing oxidation in various contexts. Uses of antioxidants protect human health from various types of cancers, chronic health diseases, cardiovascular diseases etc[4]. Vegetables always play a vital role in serving as an antioxidant other than contributing nutritive role as proteins, carbohydrates and lipids. Antioxidants help in lowering the risk of many fatal and life threatening diseases like cancer and other chronic human ailments [5]. Fruits of *Dillenia indica* Linn. having sour taste having rich ascorbic acid content considered to be a great source of antioxidants. Bark of the plant like other parts having high phenolic contents should be used as rich source of antioxidant. The present work was performed to find the antioxidant activity of stem bark in various solvents.

MATERIAL AND METHODS

Collection of plant material

The bark of the plant has been collected from the botanical garden, D.D.U. Gorakhpur University, Gorakhpur. This plant parts was washed in distilled water. Now the bark was cut into small pieces and dried in shade for 10-12 days in order to remove the moisture. Now the shade dried bark was pulverised and converted into fine powdered form in a mixer grinder and stored in air tight containers.

Chemicals used

All the chemicals used in experiment are of analytical grade.

Solvent Extraction

The bark of *D. indica* L. was subjected to different solvents as absolute methanol, 50% methanol, absolute ethanol, 50% ethanol, acetone and water to get crude extract using Soxhlet apparatus. Two hundred fifty gram of plant material was passed through sieve no. 60 and packed in soxhlet apparatus and extraction procedures were carried out.

Determination of Total Phenolic Content (TPC)

TPC of different extracts of stem bark were determined by the Folin- Ciocalteu method as followed by Wu. *et. al.* 2007[6]. Gallic acid was used as standard and a standard curve was plotted by taking six different concentrations (20, 40, 60, 80,100,120 µg/ml) of standard. Stock solution of Gallic acid standard was prepared by mixing 10 mg gallic acid in 100 ml methanol. Different volumes were taken in separate test tubes and mixed with 0.1ml of Folin- Ciocalteu reagent. After 6 min. again the reaction mixtures were added with 0.5ml of 20% sodium carbonate.

Two gram of the sample was defatted with 100 ml of diethyl ether using Soxhlet apparatus for 2-3 hrs. Now the mixture was dried in oven and dissolved in absolute methanol. Now 0.1 ml of extract was mixed with 2.5 ml of deionised water followed by 0.1ml Folin- Ciocalteu reagent. The mixture was stirred and allowed to stand for 6 min. Then the mixture with 0.5 ml of Na_2CO_3 was added and allowed to stand for 30 min at room temperature. The reaction mixture developed colour whose absorbance was measured at 760 nm using UV-VIS Spectrophotometer. Same process has been applied for different solvent extracts. The result obtained were expressed in mg/gm Gallic acid Equivalent and TPC was calculated by this formula-

$$TPC = C \times \frac{V}{M}$$

Where,

c = concentration of gallic acid as established from calibration curve (mg/ml)

v = volume of plant extract (ml)

m = weight of plant sample (gm)

Determination of Free Radical Scavenging Activity

Free radical scavenging activity was determined by method of Lee *et. al.,* 1996 [7] using 1,1, di-phenyl-2-picryl hydrazyl (DPPH) assay. For DPPH assay 0.4mM DPPH was prepared in 100% methanol by adding 15.7mg DPPH in 100ml methanol. Different Stock solutions were prepared by dissolving 10mg of dried plant sample in 10ml of different solvents separately (absolute methanol, 50% methanol, absolute ethanol, 50% ethanol, acetone and water) to get a concentration of 1mg/ml. Control solution was prepared by methanol and DPPH solution only. Now the varying concentrations (20, 40, 60, 80, 100 and 120µg/ml) were taken from stock solution and mixed with methanol to make the volume 1ml and added with 3ml of DPPH solution. The reaction mixtures were shaken and covered with black paper and allowed to stand in dark at room temperature for 30 mins. The absorbance was measured after 30 mins by using UV/ VIS spectrophotometer at 517 nm. The percentage inhibition was calculated by the formula-

$$\% \, Inhibition = \frac{\text{Ao} - \text{A1}}{Ao} \times 100$$

Where Ao is Control, A1 is Sample

Statistical analysis

All experiments were done in triplicates and the results were expressed as mean± Standard Deviation (SD).

RESULT AND DISCUSSION

The phytochemical analysis of bark of *D. indica* Linn. showed the presence of different phytochemicals as alkaloids, glycosides, Saponins, steroids, tannins, phenols, proteins, carbohydrates etc.[8]. Phytochemical analysis showed that all extracts of bark showed the presence of phenol. Quantitative estimation revealed that 50% methanolic extract showed highest phenolic content i.e., 47.6± 0.92 mg/gm GAE(Table-1). The antioxidant assay of bark of *D. indica* Linn. revealed that it possesses higher amount of antioxidant activity. The test revealed that aqueous extract of bark showed lowest antioxidant activity with IC_{50} 239.1±2.15µg/ml. Highest antioxidant activity is shown by ascorbic acid with IC_{50} 6.04± 2.89µg/ml. Among different extracts used 50% methanolic extract showed higher antioxidant activity with lowest IC_{50} 13.43±1.25 µg/ml. The study reveals that bark of *D. indica* Linn. is rich in antioxidant property. This proves that the bark of the plant can be used as medicine for various ailments. The antioxidant activity of *D. indica* Linn. should be further explored commercially to take its health benefits for serving the human society either alone or in synergistic form with others.

Figure 1: Bark of *D. indica* Linn.

Table 1: IC_{50} values and Total Phenolic Content (TPC) of *D. indica* Linn. Stem Bark Extracts in different Solvents.

Extract type	IC_{50} (µg/ml) mean ±SD	TPC(mg/gm GAE) mean±SD
L-Ascorbic acid	6.04 ± 0.32	_
Acetone	217.38 ± 3.25	1.59 ± 1.15
Aqueous	239.10 ± 1.36	<1
Absolute ethanol	16.04 ± 0.74	38.21 ± 1.02
50% ethanol	31.75 ± 2.10	29.6 ± 1.21
Absolute methanol	14.06 ± 1.40	42.9 ± 0.15
50% methanol	13.43 ± 1.25	47.6 ± 0.92

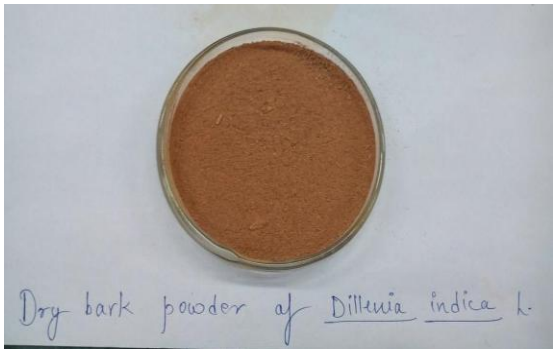

Figure 2: Pulverized bark powder of *D.indica* Linn.

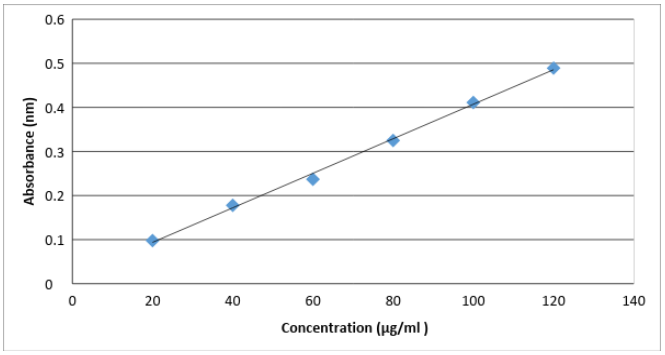

Figure 3: Standard curve of Gallic acid

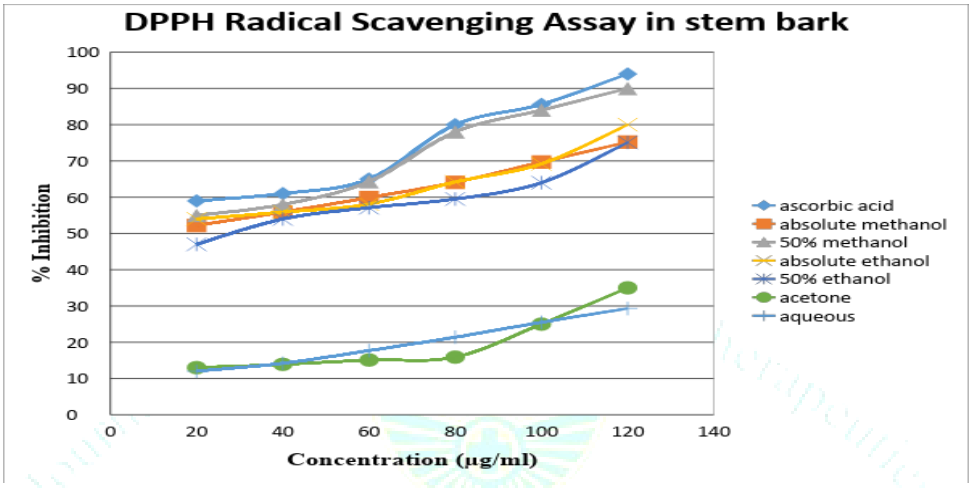

Figure 4: DPPH Radical Scavenging Activity in different extracts of stem bark

CONCLUSION

The present research work enables to find out the antioxidant activity by DPPH radical scavenging assay along with phenolic content. In this work it work it is found that 50% methanolic stem bark extract shows the highest phenolic content as well as highest antioxidant activity. Hence the plant can be recommended as an excellent food supplement which can be utilized as a food source. Being a natural source D. *indica* Linn. Can serve human society which will be an effective and low-cost medicinal food for all.

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