PRELIMINARY PHYTOCHEMICAL SCREENING AND TO EVALUATE ANTI-OXIDANT PROPERTY ON ROOT EXTRACT OF DILLENIA INDICA (ELEPHANT APPLE)

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ABSTRACT

Objective: The objective of the present study was to carry out the presence of antioxidants activity on root extract of Dilleniaindica (Family-Dilleniaceae) which is believed to have the protective mechanisms in beneficial health effects.

Methods: Considering its medicinal importance, the plant was chosen for extraction with various solvents such as petroleum ether, chloroform and ethanol which was taken into considerations to determine the phytochemicals analyses present in it. The extracts of the roots were evaluated for antioxidant activity by using different in vitro models like Reducing Power method, TBA Method and DPPH method at different doses (20, 50, 100, 200, 400μg/ml). The IC50 values of each extract on different activity were carried out.

Results: The study shows that petroleum ether, chloroform and ethanolic extract of this plant showed potent antioxidant activity against the standard drug (Ascorbic acid). But chloroform extract of the roots shown most significant antioxidant activity as compared to petroleum ether and ethanol.

Conclusion: The root part of the plant shows active anti-oxidant activity that can be consumed by mankind.

Keywords: Phytochemical screening, Pharmacognostic evaluation, Anti-oxidant activity

INTRODUCTION

In the last few decades, traditional knowledge on primary healthcare has been widely acknowledged across the world. It is estimated that 60% of the world population and 80% of the population of developing countries rely on traditional medicine, mostly plant drugs, for the primary health care needs. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment [1, 2].

Use of plants as a source of medicine has been inherited and is an important component of the health care system in Egypt. Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is the most developed and widely practiced in India. Today this ancient system of medicine, believed to be more than 5000 y old, is based on two separate theories about the natural laws that govern good health and longevity, namely yin and yang, and the five elements (wu xing). Chinese medicine was systematized and written between 100 and 200 BC.

The Dillenia indica is comprised of about 100 species of evergreen and deciduous trees or shrubs of disjunct distribution in the seasonal tropics of Madagascar through South and South East Asia, Malaysia, North Australia. Mucilage from their fruits is used in drug formulations. Species from this have been widely used in medicinal folklore to treat cancers, wounds, jaundice, fever, cough, diabetes mellitus, and diarrhea as well as hair tonics. It also produces edible fruits and are cultivated as ornamental plants. Their extracts and pure compounds have been reported for their antimicrobial, anti-inflammatory, cytotoxic, antidiabetes, antioxidant, and antiprotozoal activities [3, 4].

MATERIALS AND METHODS

Collection of plant material

The roots of Dillenia indica were collected in the month of Feb, 2019 from Sivasagar, Assam.
• Cool in a desiccator’s and wt. (The loss in wt. is usually recorded as moisture.)

The results are shown in table 2.

**Determination of moisture content**

**Procedure**

• About 5 gm of air-dried crude drug was accurately weigh in a watch glass.
• The drug was kept in hot air woven at 105 °C and dry for a period until constant weigh obtained.
• The difference in weigh gives the moisture content of the drug.

The results are shown in table 3.

**Determination of foaming index**

**Procedure**

• Take 1g of coarse powdered drug in 500 ml conical flask.
• Add 100 ml boiling water and maintain moderate temperature for boiling for 30 min.
• Cool and filter into a volumetric flask and add water up to 100 ml.
• Take 10 test tubes with successive portions of 1,2 to 10 ml drug in each tubes.
• Adjust the volume with water up to 10 ml in each tubes and close the tubes with stoppers.
• Shake them for 15 seconds and allow to stand for 15 min. then measure the height.

The results are shown in table 4.

**Determination of swelling index**

**Procedure**

• Take 1g of the powder in a 25 ml stoppered cylinder.
• Adding water up to 25 ml.
• Shake occasionally for 23 h.
• Keep aside for 1h.

The volume of the mixture in ml is then read.

The results are shown in table 5.

**Determination of ash values of a crude drug**

• Use to determine the quality and purity of a crude drug and to establish the identity of it.
• Used to determine foreign inorganic matter present as an impurity.
• The results are shown in table 6.

**Determination of fluorescence powder drug analysis**

In the present study, dry root powder was used. The fluorescent analysis of the root powder of the plant *Dillenia indica* was carried out. The root of the plant after drying were then blended using a electric blender. This fine powder was analyzed for the fluorescent.

The results are shown in table 7 [5-6].

**Chemical studies**

**Determination of % yield:** It is done by following the formula

\[
\% \text{ of yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100
\]

The results are shown in table 8.

**In vitro anti-oxidant studies**

**Reducing power assay method**

**Procedure**

Taking 1 ml of methanolic extract (100-400 μg/ml), standard dilutions (20-400 μg/ml) and control sample (1 ml distilled water instead of sample solution) was mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml potassium ferricyanide (1%). Then the final mixture was properly mixed and incubated at 50 °C for 20 min. After incubation, the reaction mixture was rapidly cooled and mixed with 2.5 ml of 10% trichloroacetic acid. It was then centrifuged at 3000 rpm for 10 min. About 2.5 ml of the supernatant was then added, mixed well and allowed to stand for 10 min. The absorbance was measured at 700 nm [7, 8].

The results are shown in table 9.

**Table 1: Determination of phytochemical screening**

| Chemical test          | Pet. ether | Chloroform | Ethanol |
|------------------------|------------|------------|---------|
| Alkaloids              | -ve        | +ve        | -ve     |
| Tannins                | -ve        | +ve        | +ve     |
| Saponins               | +ve        | +ve        | +ve     |
| Glycoside              | -ve        | -ve        | +ve     |
| Carbohydrates          | -ve        | -ve        | -ve     |
| Flavonoids             | -ve        | +ve        | -ve     |
| Proteins and amino acid| -ve        | +ve        | +ve     |
| Vitamin C(Ascorbic acid)| +ve      | +ve        | +ve     |
| Phenolics              | +ve        | +ve        | +ve     |

*(+ve) and (-ve) symbol indicates the presence and absence of respective plant constituents.*

**Thiobarbituric acid method**

**Procedure**

The test was conducted according to the method of Kikuzaki and Nakatani.

To 2.0 ml of the Sample solution, 1.0 ml of 20% aqueous trichloroacetic acid (TCA) and 2.0 ml of Aqueous thiobarbituric acid (TBA) solution were added. Then the final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 min. After cooling, it was then centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay. Both methods (FTC and TBA) described antioxidant activity by percent inhibition: [9-10]

The results are shown in table 10.

**1,1-Diphenyl-2-picryl hydrazil assay (DPPH assay)**

**Procedure**

The radical scavenging activity was determined by the use of DPPH free radical assay. Take 50 μL of various concentrations of
plant extracts in methanol were added to 5 ml of 100 μL Solution of DPPH in methanol. After 30 min incubation absorbance was read against blank taken as methanol at 517 nm and the % inhibition was calculated from the following equation below [11, 12].

The results are shown in table 11.

**RESULTS**

**Phytochemical screening**

Phytochemical screening was carried out for petroleum ether, chloroform and ethanolic extract of *Dillenia indica* for the presence of different phytoconstituents like flavonoid, phenolic, carbohydrate, glycoside and proteins.

Pharmacognostic evaluation

| Table 2: Determination of loss on drying |
|------------------------------------------|
| Wt. of porcelain | Initial wt. of the drug | Wt. of empty porcelain+drug before drying | Wt. of empty porcelain+drug after drying |
| 118.32 gm | 0.50 g/500 gm | 118.82 gm | 118.80 gm |

| Table 3: Determination of moisture content |
|-------------------------------------------|
| Wt. of the drug | Initial wt. of the drug+pet dish | Constant wt. after drying | Loss on drying | Moisture content |
| 0.50 gm | 11.82 gm | 118.80 gm | 0.02 gm | 4% |

| Table 4: Determination of foaming index |
|----------------------------------------|
| Concentration | 1 μg/ml | 2 μg/ml | 3 μg/ml | 4 μg/ml | 5 μg/ml | 6 μg/ml | 7 μg/ml | 8 μg/ml | 9 μg/ml | 10 μg/ml |
| Test Results | -ve | -ve | -ve | -ve | -ve | +ve | +ve | +ve |

| Table 5: Determination of swelling index |
|-----------------------------------------|
| Wt of coarse powder | Initial wt. of the powder | Wt. of empty porcelain+drug before drying | Wt. of empty porcelain+drug after drying |
| 1.2 gm | 0.50 g/500 gm | 118.82 gm | 118.80 gm |

| Table 6: Determination of ash values of a crude drug |
|-----------------------------------------------------|
| Wt. of drug (gm) | Wt. of crucible+drug (gm) | Wt. of total ash (gm) | % of total ash | Wt. of acid insoluble ash value (gm) | % of acid insoluble ash | Wt. of water soluble ash value (gm) | % of water soluble ash |
| Crucible 1 | 2 | 22.19 | 1.6 | 1.8 | 22.1 | 0.18 | - | - |
| Crucible 2 | 2 | 27.85 | 1.9 | 8.5 | - | - | 23.8 | 0.07 |

| Table 7: Determination of fluorescence powder drug analysis |
|-----------------------------------------------------------|
| Chemical test | Daylight | Short UV (254 nm) | Long UV (365 nm) |
| Powder+1N NAOH in methanol | MOON YELLOW | HAUSER LIGHT | LEMONETE |
| Powder+1N NAOH in H2O | RED | HAUSER MEDIUM | LEMONATE |
| Powder+1N HCl in methanol | GEROGIA CLAY | OLIVE GREEN | AVOCADO |
| Powder+1N HCl in H2O | YELLOW ORCHE | SOFT SAGE | DARK CHOCOLATE |
| Powder+1N HNO3 in methanol | GEROGIA CLAY | HAUSER LIGHT | AVOCADO |
| Powder+1N HCl in H2O | SOFT SAFE | LEMONATE | DARK CHOCOLATE |
| Powder+5% iodine | COCOA | OLIVE GREEN | AVOCADO |
| Powder+5% KOH | COUNTRY RED | HAUSER MEDIUM | HAUSER LIGHT |
| Powder+5% FeCl3 | DARK CHOCOLATE | GREEN | ARBOR GREEN |

**DETERMINATION**

| Table 8: The % of the yield of different extracts of *Dillenia indica* roots |
|-------------------------------|-----------------|-----------------|
| Extracts | % Yield |
| Petroleum ether | 7.1 |
| Chloroform | 2.4 |
| Ethanol | 4.7 |

*In vitro* anti-oxidant studies

Reducing the power method
Table 9: *In vitro* antioxidant activity of ascorbic acid (Stand.), pet ether, chloroform and ethanol extract of *Dillenia indica* by RP method

| S. No | Extracts               | 20μg/ml | 50 μg/ml | 100 μg/ml | 200 μg/ml | 400 μg/ml |
|-------|------------------------|---------|----------|-----------|-----------|-----------|
| 01    | Ascorbic acid (Stand.) | 34.3±0.001 | 48.6±0.002 | 56.2±0.001 | 66.6±0.05  | 74.4±0.01  |
| 02    | Pet Ether Extract      | 33.4±0.002 | 42.6±0.003 | 50.2±0.012 | 59.5±0.001 | 64.8±0.016 |
| 03    | Chloroform Extract     | 19.2±0.020 | 23.3±0.018 | 26.2±0.022 | 34.8±0.012 | 48.2±0.010 |
| 04    | Ethanol Extract        | 25.5±0.002 | 16.1±0.012 | 18.3±0.019 | 21.3±0.020 | 25.3±0.023 |

Fig. 1: Reducing power method of different extract of *Dillenia indica*

**Thiobarbituric acid method**

Table 10: *In vitro* antioxidant activity of ascorbic acid (Stand.), pet ether, chloroform and ethanol extract of *Dillenia indica* by TBA method

| S. No | Extracts               | 20μg/ml | 50 μg/ml | 100 μg/ml | 200 μg/ml | 400 μg/ml |
|-------|------------------------|---------|----------|-----------|-----------|-----------|
| 01    | Ascorbic acid (Stand.) | 34.3±0.001 | 48.6±0.002 | 56.2±0.001 | 66.6±0.05  | 74.4±0.01  |
| 02    | Pet Ether Extract      | 15.5±0.021 | 26.0±0.017 | 33.0±0.023 | 37.1±0.013 | 44.0±0.040 |
| 03    | Chloroform Extract     | 14.2±0.012 | 17.5±0.013 | 27.0±0.014 | 36.2±0.007 | 44.0±0.024 |
| 04    | Ethanol Extract        | 18.4±0.016 | 24.3±0.016 | 34.1±0.024 | 50.2±0.024 | 64.1±0.026 |

Fig. 2: TBA method of different extract of *Dillenia indica*
DPPH assay method

Table 11: *In vitro* antioxidant activity of ascorbic acid (Stand.), Pet ether, chloroform and ethanol extract of *Dillenia indica* by DPPH ASSAY method

| S. No. | Extracts               | 20 μg/ml | 50 μg/ml | 100 μg/ml | 200 μg/ml | 400 μg/ml |
|--------|------------------------|----------|----------|-----------|-----------|-----------|
| 01     | Ascorbic acid (Stand.) | 34.3±0.001| 48.6±0.002| 56.2±0.001| 66.6±0.05 | 74.8±0.01 |
| 02     | Pet. Ether Extract     | 11.2±0.02 | 16.0±0.006| 18.0±0.020| 22.3±0.025| 29.3±0.001|
| 03     | Chloroform Extract     | 33.3±0.005| 46.0±0.006| 50.0±0.016| 61.5±0.002| 78.3±0.002|
| 04     | Ethanol Extract        | 25.3±0.001| 30.4±0.003| 36.3±0.003| 46.3±0.025| 52.0±0.030|

DISCUSSION

Phytochemical screening
The phytochemical screening of powdered roots of *Dillenia indica* indicates the presence of active constituents. Solvent selection was made for root and extraction was performed. From this study, it may be concluded that petroleum ether, chloroform and ethanolic extracts of this root of plant (*Dillenia indica*) have various phytoconstituents which was shown in (table 1).

Pharmacognostic evaluation
The pharmacognostic evaluation of the roots of this plant *Dillenia indica* gives us a brief idea about the various potential pharmacognostic activities in the plant.

Anti-oxidant activity
The antioxidant activity of this roots of *Dillenia indica* by using different models gives us a positive response that the roots of the plant shows active anti-oxidant properties which was compared with reference standard Ascorbic acid.

CONCLUSION

The current study shows that petroleum ether, chloroform and ethanolic extract of *Dillenia indica* have significant anti-oxidant property. *Dillenia indica* is widely available and also cultivated in different region in the world. The scientific research suggests a huge biological potential of this plant. A detailed study on the pharmacognostical, phytochemical and antioxidant properties of the root have been discussed and also provided details evidence for use of this root in different diseases. These results also justify the use of roots in traditional medicines.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally

CONFLICT OF INTERESTS
Declare none

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