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Original Article

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 anti-oxidant 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

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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 system of medicine is being practiced in countries like Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan. 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, ant diabetes, antioxidant, antidiarrheal, 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.

Authentication of plants material

The plant is authenticated by Department of Botany Guwahati University.

Drying and grinding of plant material

After authentication of the plant, the roots were shade dried and grinded. The material which is retained on the sieve was used for the extraction purpose.

Extraction of active constituents

The process of removing or extracting or separating of active constituents from the crude drugs by using suitable solvents is called extraction. The active constituents that have been extracted from crude drugs are known as extractives and the preparation so obtained are as extracts. About 400g of the powder of the roots of Dilleniaindica was weighed and extracted successively with the soxhlet apparatus. At first, it was extracted with petroleum ether and then according to their polarity lower to higher with ethanol and chloroform, respectively. The extract collected was filtered and evaporated using distillation.

Preliminary phytochemical screening

The chemical evaluation of different extracts was done by using a various standard procedure of different metabolites like alkaloids, tannins, saponins, glycosides, carbohydrates, flavonoids, proteins, ascorbic acid (vit c), phenolics. The results are shown in table 1.[6]

Pharmacognostic evaluation

Determination of loss on drying

Procedure

- Weight about 1.5g of the powdered drug into a weighed flat and thin porcelain dish.
- Dry in the oven at 100-degree C, until two consecutive weighings do not differ by more than 0.5 mg.

- 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 with2.5 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 taken, and 2.5 ml distilled water and 0.5 ml of ferric chloride (0.1%) were 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: Dertermination of phytochemical screening

Chemical test	Pet. ether	Chloroform	Ethanol
Alkaloid	-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 1**1.**

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	0.50g/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 (gm)	Constant wt. after drying (gm)	Loss on drying (gm)	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	+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	0.50g/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 H20	RED	HAUSER MEDIUM	LEMONATE
Powder+1N HCLin methanol	GEROGIA CLAY	OLIVE GREEN	AVOCADO
Powder+1N HCL in H20	YELLOW ORCHE	SOFT SAJE	DARK CHOCOLATE
Powder+1N HNO3 in methanol	GEROGIA CLAY	HAUSER LIGHT	AVOCADO
Powder+1N HCLin H2O	SOFT SAFE	LEMONATE	DARK CHOCOLATE
Powder+5% Iodine	COCOA	OLIVE GREEN	AVOCADO
Powder+50% 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

S. No.	Extracts	% Yield
1.	Petroleum ether	7.1
2.	Chloroform	2.4
3.	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 indicia* by RP method

S. No.	Extracts	20µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml
01	Ascorbic acid (Stand.)	34.32±0.001	48.66±0.002	56.23±0.001	66.62±0.05	74.88±0.01
02	Pet. Ether Extract	33.43±0.002	42.65±0.003	50.27±0.012	59.55±0.001	64.86±0.016
03	Chloroform Extract	19.23±0.020	23.36±0.018	26.22±0.022	34.86±0.012	48.26±0.010
04	Ethanol Extract	25.56±0.002	16.19±0.012	18.38±0.019	21.37±0.020	25.39±0.023

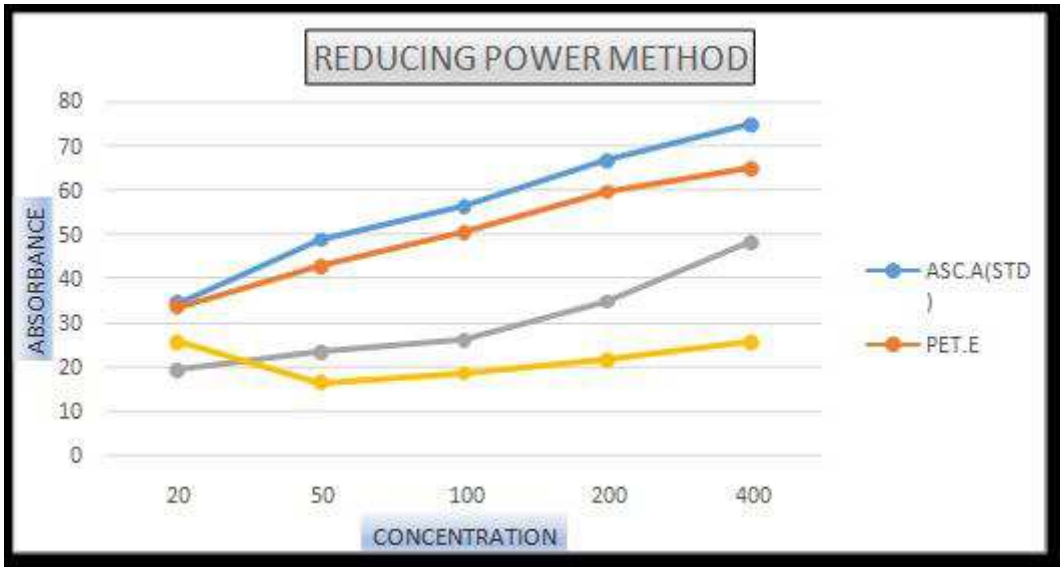

Fig. 1: Reducing power method of different extract of *Dillenia indica*

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.)	34.32±0.001	48.66±0.002	56.23±0.001	66.62±0.05	74.88±0.01
02	Pet. Ether Extract	15.55±0.021	26.09±0.017	33.023±0.023	37.151±0.013	44.024±0.040
03	Chloroform Extract	14.228±0.012	17.506±0.013	27.028±0.014	36.228±0.007	44.007±0.024
04	Ethanol Extract	18.470±0.016	24.330±0.016	34.123±0.024	50.220±0.024	64.125±0.026

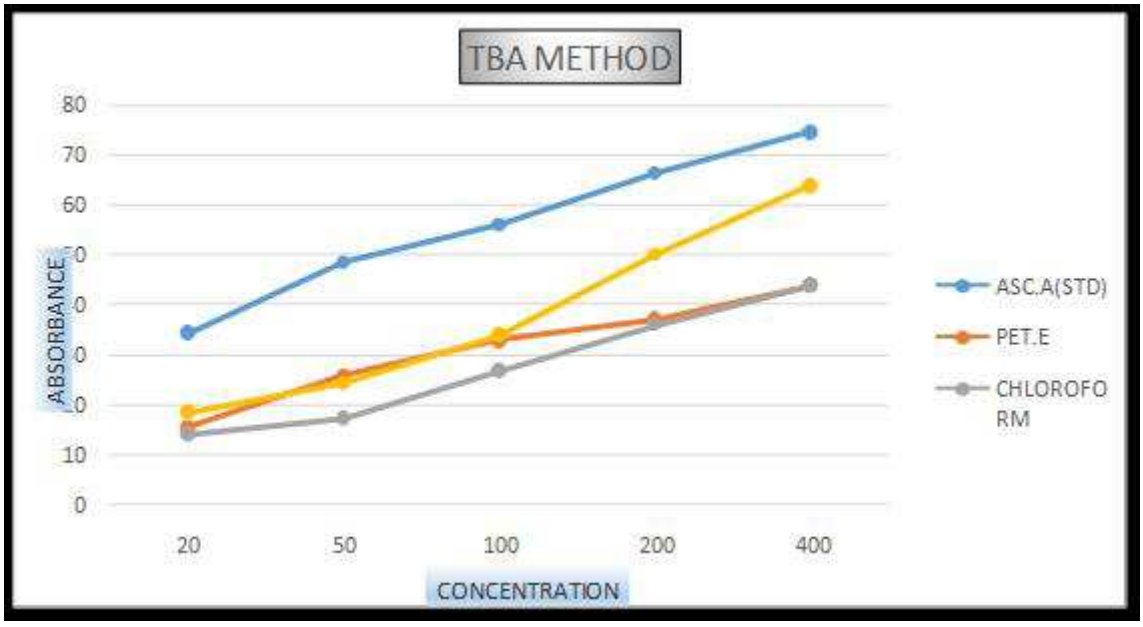

Fig. 2: TBA method of different extract of *Dilleniaindica*

DPPH assay method

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

S. No.	Extracts	20µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml
01	Ascorbic acid (Stand.)	34.32±0.001	48.66±0.002	56.23±0.001	66.62±0.05	74.88±0.01
02	Pet. Ether Extract	11.23±0.02	16.06±0.008	18.04±0.020	22.36±0.025	29.32±0.001
03	Chloroform Extract	33.36±0.005	46.09±0.006	50.61±0.016	61.56±0.002	78.38±0.002
04	Ethanol Extract	25.36±0.001	30.44±0.003	36.33±0.003	46.32±0.025	52.01±0.030

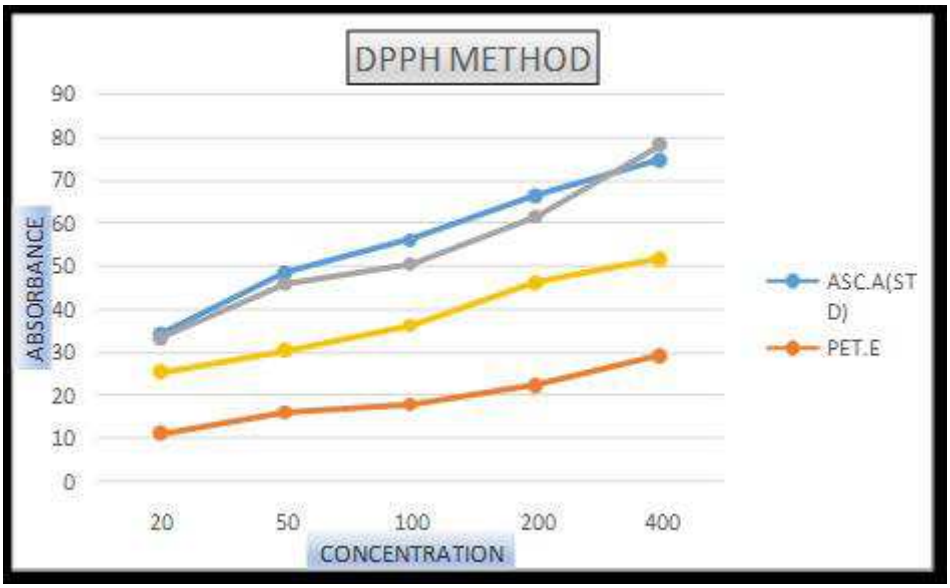

Fig. 3: DPPH scavenging activity of different extract of *Dilleniaindica*

Table 12: IC_{50} value from reducing power method, tba method and DPPD scavenging activity of *Dilleniaindica*

S. No.	Activity	Extract	IC50
01	Reducing Power	Ascorbic acid(stnd)	2.38
		Pet. Ether	2.98
		Chloroform	5.81
		Ethanol	62.1
02	TBA	Ascorbic acid(stnd)	2.38
		Pet. Ether	5.76
		Chloroform	5.83
		Ethanol	4.10
03	DPPH	Ascorbic acid(stnd)	2.38
		Pet. Ether	10.23
		Chloroform	2.62
		Ethanol	4.72

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 author have contributed equally

CONFLICT OF INTERESTS

Declare none

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