PRELIMINARY PHYTOCHEMICAL ANALYSIS AND PHARMACOGNOSTICAL STUDIES OF DIFFERENT PARTS OF *DILLENIA INDICA* LINN.

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Abstract

The present study was conducted to highlight the pharmacognostic and phytochemical analysis of different parts of *Dillenia indica* Linn, an important ethnomedicinal plant containing secondary metabolites. The plant has been blessed with numerous medicinal properties with edible constituents as well. The fruit is the main yield of the plant. The plant has a very vast range of traditional and medicinal properties with good therapeutic values. The fruits juice, stem, stem bark, root and leaves were recognised as a valuable material in day to day life by local and ethnic people. These produces were used as cough syrup, anti-diabetic, anti-cancerous, anti-diarrheal and aphrodisiac agent as they have rich amount of secondary metabolites as alkaloids, tannins, phenols, glycosides, saponins, steroids and terpenoids. Remarkable amount of carbohydrates are present in the plant sample. The physical parameters revealed the presence of total ash, water soluble ash, acid insoluble ash, moisture content etc. As the plant contains lots of medicinal values so there is a need to explore and standardize the plant so that more and more people should know about this plant and take advantage of this nature's gift.

Keywords*:* *Dillenia indica*, Pharmacognostic, Phytochemical, Ethnomedicinal, Therapeutic.

Introduction

Plants play an excellent role in revitalizing human's life for a very long time. Plants are used in many forms in indigenous system of medicines as Ayurveda, Siddha and Unani. The plant kingdom holds many species of plants containing substances of medicinal values which have to be discovered[1]. Among a vast kingdom of plants the plant *Dillenia* belongs to family Dilleniaceae having 60 species out of which 3 species are reported in India[2].

D. indica Linn. among other species is the most commonly used medicinal and edible species. *D. indica* Linn. has so many medicinal properties therefore it is used in traditional medicine since very long time. The medicinal properties of various parts of *D. indica* Linn. are described in Ayurveda, Yajurveda, and Charak Samhita. Various parts of plant are aphrodisiac decoction of different parts are used as universal antidote for poison, vata suppresent as an expectorant[3,4].

Material and Methods

Chemicals and reagents

All the chemicals and reagents used, are of analytical grade.

Collection of plant materials

Fresh plant materials (leaves, bark and fruits) of *D. indica* Linn are collected from botanical garden and university campus, D.D.U Gorakhpur University in different seasons and authentic identification was done by Herbarium, Department of Botany DDU Gorakhpur University Gorakhpur.

Sample processing

The fresh mature leaves, bark and fruits of *D. indica* Linn. were washed firstly with simple tap water and subsequently washed with distilled water. Now the fresh plant materials (leaves and bark) were air dried in shade at room temperature for 9-10 days and fresh fruits have been cut in small pieces and dried in oven at 65^0C for 6-7 days (as fruits contains great amount of moisture) in order to remove the moisture content. Dried plant materials were pulverized and powdered in a mixer grinder to fine powder form and store in air tight containers.

Solvent Extraction

Crude extracts of different plant materials of *D. indica* Linn. were prepared by continuous hot extraction method using Soxhelt apparatus. 200gms of plant materials were passed through sieve no. 40 and packed in Soxhelt apparatus and extractions were done using water, ethanol, methanol and acetone.

Pharmacognostic studies

Physicochemical evaluation

The different physicochemical parameters such as total ash content, water soluble ash, acid insoluble ash, extractive value (% yield), swelling index, foaming index and moisture content of different parts of *D. indica* Linn. were analysed as suggested by Indian Pharmacopeia and WHO guidelines[5-8].

Total ash content

About 2 grams each of powdered samples were weighed, taken and spread in a previously ignited and weighed silica crucible. The powdered samples were incinerated by increasing temperature to make it red hot and free from carbon. The silica crucibles were cooled, kept in desiccators and weighed. The procedure was repeated to find constant weight. They were done in triplicates.

Water soluble ash

The ash obtained from each sample after total ash treatments were boiled for 10 minutes with 20ml of distilled water. The insoluble ash was collected on ashless filter paper and washed at least two times with hot water. The insoluble ash was transferred to pre-ignited silica crucibles and again ignited for 20minutes and weighed. The process was repeated to find a constant weight. Now the weight of insoluble matter was subtracted from weight of total ash and the difference of weight was determined the water soluble ash**.**

Acid insoluble ash

The ash obtained from each sample after total ash treatment was boiled with 20ml of 2N HCl for 10minutes. Now the insoluble ash was collected on ash less filter paper and washed for atleast two times with hot water. The insoluble ash was transferred into preignited silica crucible, again ignited and weighed. The process was repeated to get a constant weight for each sample. The percentage of acid insoluble ash was calculated with reference to the pre air dried plant samples.

Moisture content

About 2gm of each drug (powdered samples of different plant parts) were taken and kept in oven at 105^{o}C to get a constant weight. Amount of moisture content in the sample was calculated by subtracting the oven dried weight of sample from air dried sample.

Extractive value determination

For determination of extractive value of dry samples of different plant parts, 5gm of dried sample was placed in glass stoppered conical flask and macerated with 100ml of solvents (Ethanol, methanol, acetone and aqueous) shaking gently and allowed to stand for 18 hours. Now filtrates were transferred to flat bottom dish and solvents were evaporated on a water bath, followed by drying at 105^{o}C for 6hours, cooled in desiccator and weighed immediately. Now the contents of extractable matters were calculated in percentage (%).

Swelling index

One gram of the powdered was sample taken in a measuring cylinder. The initial volume of the measuring cylinder with powdered sample was noted. Now the volume of the cylinder was made upto 100ml with 0.1 N HCl. The cylinder was shaken gently and set undisturbed for 24 hours. The volume covered by sample was noted after 24 hours and calculated by the formula-

Swelling index (%) = Ws- Wc/WcX100

Where Ws = Height of swollen sample after 24 hours

Wc= Initial height of powdered sample in graduated cylinder.

Foaming index

About one gram of the powdered sample was taken in 500ml conical flask. Now 100 ml of boiling water was added to flask and mildly boiled at 80^{0}C-90^{0}C for about 30minutes. Then the mixture was cooled and filtered into a volumetric flask and again water was added to make the volume up to 100ml (v). Now 10 stoppered test tubes were taken and successive portions of 1,2,3ml upto 10ml samples were taken in test tubes separately and final volume was adjusted to 10ml by adding water and test tubes were closed with stoppers. All the test tubes were shaken for 15seconds and allowed to stand for 15 minutes and the height of the formed foam was measured. The height of he formed foam in each test tubes is less than 1cm, then the foaming index is less than 100 and calculated as-

Foaming index = 1000/ V

Where V= Volume (ml) of sample used for making dilutions in the test tubes where foam of 1cm or more foam was formed.

Fluorescence analysis

The colour of dry plant samples *D. indica* Linn. were examined under daylight and U.V light. Now the dry plant samples were treated with different chemicals and allowed to stand at room temperature for 5 minutes. Now the mixtures were filtered using Whatman No. 1 filter paper and colour changing behaviour of the plant powders under daylight and U.V light were observed[9,10].

Organoleptic analysis

The organoleptic parameters of dry plant samples and their extracts of *D. indica* were examined by analysing colour, texture, odour and taste[11].

Phytochemical analysis

Preliminary phytochemical evaluations were done for all the extracts using the standard methods[6,12,13].

Test for Alkaloids

Dragendorff's Test: Few drops of extract were taken in a test tube and few drops of dragendorff reagent (Potassium Bismuth Iodide Solution) was added to it. Formation of reddish brown precipitate confirms the presence of alkaloids.

Mayer's Test: To the extract few drops of Mayer reagent (Potassium Mercuric Iodide solution) was added and presence of alkaloids was confirmed by cream coloured precipitate.

Test for Tannins and Phenols

Lead subacetate Test: Few ml of extract was mixed with few drops of lead sub-acetate solution. A cream coloured gelatinous precipitate indicates the presence of tannin.

Ferric Chloride Test: 1 ml of the extract was diluted with distilled water and mixed with few drops of ferric chloride solution (1%). A greenish or bluish black colour indicates the presence of tannins and phenols.

Test for Glycosides

Legal's Test: Few drops of extract was mixed with sodium nitroprusside containing sodium hydroxide and pyridine. The formation of blood red colour indicates the presence of glycosides.

Borntrager's Test: The extract was treated with few drops of benzene and dilute ammonia solution. The presence of glycosides was confirmed by reddish pink coloured solution.

Test for Saponins

Foam Test: The extract was mixed with 2ml of distilled water and vigorously shaken together. Formation of foam which persist or remain for atleast 10minutes indicates the presence of saponins.

Test for Proteins

Biuret Test: One drop of 40% NaOH was added to the extract followed by addition of two drops of 1% copper sulphate solution. Formation of violet colour indicates the presence of protein.

Xanthoproteic Test: To the extract few drops of concentrated nitric acid was added. Formation of yellow coloured solution confirmed the presence of protein.

Test for Carbohydrates

Tannic Acid Test: To the extract few drops of 20% tannic acid was added. Formation of white precipitate indicates the presence of carbohydrates.

Benedict's test: To the extract few ml of Benedict's solution (alkaline solution of cupric citrate complex) was added and boiled for some time. The formation of reddish brown precipitate indicates the presence of carbohydrates.

Fehling's Test: Few drops of extract was added to Fehling's solution (Fehling A and B solution) and boiled in water bath. Formation of brick red precipitate showed the presence of carbohydrates.

Test for Steroids and Terpenoids

Liebermann Burchard's Test: Few drops of extract was added with equal amount of anhydrous acetic acid and chloroform and cooled at 0^{o}C. Now a drop of sulphuric acid was added from side wall of test tube. At the junction of two layers a brown ring formed, out of which upper layer turns green in colour showing the presence of steroids and formation of dark red colour at lower layer indicates the presence of terpenoids

Salkowski Test: Few drops of extract were added to few drops of equal amount of sulphuric acid and chloroform. Upper layer showed yellow colour which indicated the presence of terpenoids and red colour of lower layer confirmed the presence of steroids.

Test for Flavonoids

Alkaline Reagent Test: Few drops of 1% NaOH solution was added to extract which showed the formation of yellow colour which disappeared on addition of concentrated HCl, confirms the presence of flavonoids.

Aluminium Chloride Test: To extract 1ml of 1% aluminium chloride solution was added and shaken vigorously. Formation of light yellow precipitate indicated the presence flavonoids.

Test for Coumarin

Fluorescence Test: To the extract few drops of sodium hydroxide was added and this solution is boiled in water bath for 10minutes. The solution now was exposed to UV light. A greenish yellow colour indicated the presence of Coumarin.

Result and Discussion-The result showed that the extractive value of bark(20.4±1.4) and fruit(26.1±2.72) were higher in methanol extract whereas the extractive value of leaf was higher in ethanolic extract (17.8±2.02). The extractive values of all the three parts were minimum in aqueous extract i.e.7.18±2.33 for leaf, 5.2±1.10 for bark and 6.73±1.44 for fruits (Table 1). The physicochemical parameters revealed the total ash content was 8.42± 0.48 for leaf, 11.52±0.30 for bark and 7.16±0.51 for fruits. Acid insoluble ash of leaf is 3.02±0.83, 4.66±0.29 for bark and 3.24±0.61 for fruit. Water soluble ash for leaf 6.62± 1.19, 4.26± 0.30 for bark and 3.43±0.45 for fruits. The moisture content of crude drug sample for leaf was found 7.33±0.42, 18.11±0.87 for bark and 16.96±1.02 for fruit whereas swelling index was 18.56±0.46, 12.11±.0.43 and 16.1±0.217 for leaf, bark and fruit respectively. Foaming index were not more than 100 for all samples (Table 2). Result showed that the phytochemical screening indicates the presence of secondary metabolites as alkaloids, flavonoids, tannins, phenols, steroids and terpenoids as well as primary metabolites as carbohydrates and proteins (Table 3). The results of different organoleptic parameters and fluorescence analysis for different parts of plant in different chemical were given in Table 4 to 9 respectively. Secondary metabolites as alkaloids, flavonoids, tannins, glycosides, steroids etc. have proved to be a good therapeutic source in today's pharma world. These secondary metabolites are variously used in medicine production, recreational drugs and flavouring. As the plant contain

these phytochemicals in enough amount which will proved to be a good source of nutraceuticals. Along with these, the organoleptic characters, moisture content, ash content, fluorescent analysis are also used as helping tools in exploring the medicinal values of plant.

Conclusion- In the present investigation, a set of pharmacognostic and phytochemical standardization parameters were done on various plant parts as per WHO guidelines and Indian pharmacopoeia in *D. indica* Linn. which shows the presence of ash, water soluble ash, acid insoluble ash, swelling index, foaming index and organoleptic values. Phytochemical screening shows that alkaloids, terpenoids, glycosides, tannins etc. are present in the plant samples. These studies reveal the medicinal importance of different plant parts. The importance of various parts of plant have already described in Ayurveda which is need to explore today to serve human's life.

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Reference-

1. Dubey NK, Rajesh Kumar and Pramila Tripathi (2004). Global promotion of herbal medicine: India's opportunity. Current Science, 86(1): 37-41
2. Dickison WC (1979) A note on the wood anatomy of *Dillenia* (Dilleniaceae), IAWA Bulletin. 2-3.
3. CRC Press, World Dictionary of Medicinal and Poisonous Plant (4): 851, 2012.
4. Edible Medicinal and Non-medicinal Plants: Fruits Volume 2, 410-415: 2012.
5. Anonymous Indian Pharmacopoeia, Vol II 3rdEd. Controller of publications, Govt. of India, New Delhi, 1985.
6. Evans WC (2002): Trease and Evans Pharmacognosy. WB Saunders Ltd 3rd Edn Lond. 32-33
7. Kagithoju S, Godishaa V, Pranuaparthi et.al; (2013). Pharmacognostic and Phytchemical Investigatios in *Strychnos potatorum* Linn F. Journal of Pharmacognosy and Phytochemistry. 2(4): 46-51.
8. Quality Control methods for medicinal plant materials by WHO Guidelines: 34 (A.I.T.B.S. Publishers and Distributers. Delhi 2002).
9. Chase CR and Pratt RJ (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J. American Pharm. Asso. 38:324-331.
10. Kokoshi CJ, Kokoshi RJ and Sharma PJ (1958). Fluorescence of powdered vegetable drug under UV radiations. Journal of American Pharmaceutical Assocation.47: 715-
11. Trease GE and Evans WC (1983). Pharmaognosy (12th ed. 46-53). East bourne: Balliere Tindall.
12. Harborne JB (1973). Phytochemical methods: A guide to modern techniques of plant analysis. 2nd Edition. Chapman and Hall London. New York. USA: 48-189.
13. Sofowara A. (1993) Medicinal plants and traditional medicine in Africa, 2nd Edn. Spectrum Books Ltd.: 289-300.

Table – 1 Extractive values of different parts of *D. indica* Linn.

Parts used	Extractive values in different solvents			
	Methanol	Ethanol	Acetone	Aqueous
Leaf	14.2 ± 0.64	17.8 ± 2.02	9.9 ± 0.284	7.18 ± 2.33

Fruit	26.1 ± 2.72	21.8 ± 2.23	15.9 ± 1.10	6.73 ± 1.44
Bark	20.4 ± 1.4	17.3 ± 1.04	14.0 ± 4.45	5.2 ± 1.10

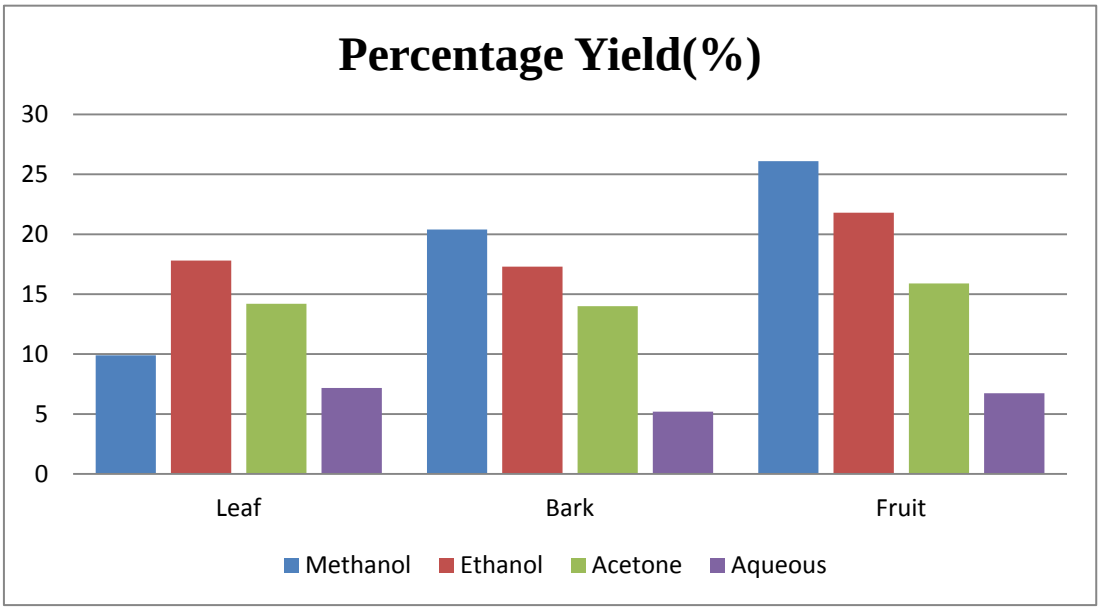

Fig-1 Percentage yield of leaf, bark and fruit of *D. indica* Linn. in different solvents.

Table–2 Physicochemical parameters of different parts of *D. indica* Linn.

Parts used	**Physicochemical parameters values (% w/w)**					
	Total ash	Water soluble ash	Acid Insoluble ash	Moisture content	Swelling index	Foaming index
Leaf	8.42 ± 0.485	6.62 ± 1.195	3.02 ± 0.830	7.33 ± 0.420	18.56 ± 0.464	Less than100
Fruit	7.16 ± 0.513	3.43 ± 0.450	3.24 ± 0.616	16.96 ± 1.025	16.1 ± 0.217	Less than100
Bark	11.52 ± 0.302	4.26 ± 0.302	4.66 ± 0.294	18.11 ± 0.877	12.11 ± 0.431	Less than100

Table – 3 Preliminary phytochemical analysis of different parts of *D. indica* Linn.in different solvents

Phytochemical Groups	Test Performed	Leaf				Bark				Fruit			
		EtE	MeE	AE	AqE	EtE	MeE	AE	AqE	EtE	MeE	AE	AqE
Alkaloids	Dragendorff Test	-	+	-	-	+	-	+	-	-	+	+	-
	Mayer's Test												
Carbohydrates	Benedict's Test	+	+	+	+	+	+	-	-	+	+	+	+
	Tannic Acid Test												
	Fehling's Test												
Glycosides	Legal's Test	+	-	-	-	+	+	-	-	+	+	+	-
	Borntrager's Test												
Flavonoids	Ammonium Test	+	+	+	+	-	-	+	-	+	+	+	+
	Aluminium Chloride Test												
Proteins	Biuret Test	-	-	-	-	+	-	+	-	+	+	+	-
	Xanthoproteic test												
Saponins	Foam test	+	+	+	-	+	+	+	+	+	+	+	+
Steroids	LibermannBurchard Test	-	-	+	-	+	+	+	+	+	+	-	-
	Salkowski Test												
Tannin	$FeCl_3$ Test	+	+	+	+	+	+	+	+	+	+	+	+
	Lead subacetate Test												
Terpenoid	Salkowski Test	+	+	+	-	+	+	-	-	+	+	+	+
	LibermannBurchard's Test												
Coumarin	Fluorescence Test	-	+	-	-	-	-	+	-	+	+	+	+
Phenol	$FeCl_3$ Test	+	+	+	+	+	+	+	+	+	+	+	+

Abbreviations: EtE- Ethanolic Extract, MeE: Methanolic Extract, AE: Acetone Extract, AqE: Aqueuos Extract.

Table – 4 Organoleptic characters of Leaves of *D. indica*Linn. in different solvents

Solvents Used	Colour	Consistency	Odor	Taste
Methanol	Dark Green	Semisolid	Characteristic	Bitter

Ethanol	Dark Green	Semisolid	Characteristic	Bitter
Acetone	Dark Green	Solid	Characteristic	Mild Sweet
Aqueous	Reddish Brown	Semisolid	Very Mild	Mild Bitter

Table – 5 Organoleptic characters of fruits of *D.indica* Linn in different solvents.

Solvent used	Colour	Consistency	Odor	Taste
Methanol	Dark Orange	Solid	Characteristics	Bitter
Ethanol	Orange	Semisolid	Characteristics	Bitter
Acetone	Cream	Semisolid	Mild fruity	Sweet
Aqueous	Cream	Solid	Strong fruity	Sweet

Table – 6 Organoleptic characters of bark of *D. indica* Linn.in different solvents.

Solvent used	Colour	Consistency	Odor	Taste
Methanol	Dark Red	Solid	Characteristics	Bitter
Ethanol	Dark Red	Solid	Characteristics	Bitter
Acetone	Red	Solid	Pungent	Bitter
Aqueous	Brick Red	Semisolid	Pungent	Bitter

Table – 7 Fluorescence analysis of leaf powder of D. *indica* Linn in different solvents

Treatments	Daylight	UV light

Leaf powder	Dark Green	Brown
Powder + Distilled Water	Dark Green	Dark Brown
Powder + Aq. NaOH	Leave Green	Brown
Powder + Met. NaOH	Green	Wine Red
Powder + Chloroform	Dark Green	Dark Pink
Powder + Ethanol	Dark Green	Fluorescent Red
Powder + Methanol	Dark Green	Dark Pink
Powder + 50% HCl	Green	Brown
Powder + Cone. HCl	Green	Dark Brown
Powder +50% H_2SO_4	Dark Green	Brown
Powder +Cone. H_2SO_4	Sap Green	Parrot Green
Powder +50% HNO_3	Green	Purple
Powder +Cone. HNO_3	Brown	Red
Powder + Benzene	Dark Green	Dark Pink
Powder + Acetic Acid	Brown	Pink
Powder +Ammonia solution	Sap Green	Dark Green
Powder + $FeCl_3$ solution	Black	Purple

Table – 8 Fluorescence analysis of bark powder of D. *indica* Linn in different solvents

Treatments	Daylight	UV light
Bark powder	Reddish Brown	Red
Powder + Distilled Water	Red	Violet
Powder + Aq. NaOH	Dark Brown	Black
Powder + Met. NaOH	Brown	Grey
Powder + Chloroform	Orange	Brown
Powder + Ethanol	Red	Brown
Powder + Methanol	Reddish Brown	Brown
Powder + 50% HCl	Brick Red	Dark Red
Powder + Cone. HCl	Brown	Brown
Powder +50% H_2SO_4	Reddish Brown	Black
Powder +Cone. H_2SO_4	Blackish Brown	Green
Powder +50% HNO_3	Red	Violet
Powder +Cone. HNO_3	Brownish Brown	Chocolaty
Powder + Benzene	Brick Red	Brown
Powder + Acetic Acid	Orange	Brown
Powder +Ammonia solution	Brown	Soil Brown
Powder + $FeCl_3$ solution	Black	Black
Powder + Picric acid	Orange	Reddish Brown

Table – 9 Fluorescence analysis of fruit powder of D. *indica* Linn in different solvents

Treatment	Daylight	U.V. light

Fruit Powder	Yellowish Brown	Dark Brown
Powder + Distilled Water	Brown	Brown
Powder + Aq. NaOH	Brown	Brown
Powder + Met. NaOH	Dark Brown	Brown
Powder + Chloroform	Orange	Orange
Powder + Ethanol	Brown	Creamish White
Powder + Methanol	Muddy Brown	Red
Powder + 50% HCl	Light Brown	Brown
Powder + Cone. HCl	Dark Brown	Green
Powder +50% H_2SO_4	Black	Sap Green
Powder +Cone. H_2SO_4	Greenish Black	Peacock Green
Powder +50% HNO_3	Pale Yellow	White
Powder +Cone. HNO_3	Dark Brown	Black
Powder + Benzene	Dark Brown	Red
Powder + Glacial acetic acid	Brown	White
Powder +Ammonia solution	Brown	Dirty Brown
Powder + $FeCl_3$solution	Black	Black
Powder + Picric acid	Mustard Yellow	Reddish Brown