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PHYTOCHEMICAL ANALYSIS AND CHROMATOGRAPHIC EVALUATION OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN. LEAVES

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Keywords:

Dillenia indica, Pharmacognosy, Physicochemical analysis, Phyto-chemical analysis, TLC and HPTLC

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ABSTRACT: The present study is about the pharmacognostic and phytochemistry study on *Dillenia indica* Linn. *Dillenia indica* is commonly known as chalta or chilta in Hindi. It is a medium sized tree, a native of South eastern Asia, India, Bangladesh, East Srilanka, South western China and Vietnam. The plant is evaluated for understanding the pharmacognostic and Phytochemical characteristics of the leaf. The physicochemical parameters were evaluated which revealed the moisture content as 10.71%. Water soluble and alcohol soluble extractives was found to be 11.97% and 42.32% respectively. Total ash content, water soluble ash content and acid insoluble ash content was found to be 17.48%, 14.03% and 10.56% respectively. Phytochemical analysis revealed the presence of Alkaloids, Glycosides, Steroids, Flavonoids, Tannins, Saponins and Phenolic compounds. TLC and HPTLC analysis revealed the presence of various phyto-constituents along with Flavonoids, Steroids and Amino acids. HPTLC analysis revealed the presence of Quercetine, Kaempferol, Cholesterol and Glutamic acid. Detection was done under 254 and 366nm.

INTRODUCTION: Since time immemorial plants are known to have a great potential for treatment and cure of various diseases. They possess a great range of phyto constituents that are helpful for various activities and have been documented in ancient Indian literatures. It is known that more than 80% of world population today is dependent on alternative medicines and traditional system of medicine following the Siddha Rasayana methods. The need for biological drugs from plant sources are increasing day by day for its comparably safer to synthetic drugs, less toxic, reliable and easy availability.

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Therefore the demand for promising drugs from plant sources is increasing continuously [1]. *Dillenia india* Linn. commonly known as Elephant Apple and locally known as chalta belonging to the family Dilleniaceae is an evergreen, large tree distributed in the South eastern regions of Asia, India, Bangladesh, Eastern Srilanka, South Western China and Vietnam. The fruits are edible and taste sour and astringent. It is traditionally used to prepare pickles and Jams. They are also used as flavouring agents.

Traditionally they are used as laxative and carminative. It also helps in relieving flatulence. Juice of the leaves, fruits and the bark is given orally for treatment of diarrhoea and Cancer. The review of various literature showed that the leaves, bark, fruits or the various part of the *D. indica* (Chalta) have extensive medicinal properties. It possesses various activities like Antimicrobial, Antioxidant, Analgesic, Anti-inflammatory,

Dysentery, Anti-diabetic and Anti-leukemic etc. Thus the *D. indica* has a wide range of activity which makes the fruit or the whole plant a gift for human being. Despite of various medicinal values expressed by the plant very little information is available about the phyto-chemical constituents till now. Therefore this prompted us to evaluate the plant for various phyto-chemical constituents as the source of good therapeutic value [2, 3, 4, 5].

MATERIALS AND METHODS:

Fresh leaves were collected from Ganeshpuri (Vasai), Maharastra during monsoon in the month of August. The material was identified and authenticated by Dr. A.K.S Rawat from National Botanical Research Institute, Lucknow. The leaves were collected and dried under shade at room temperature for 5 days. Later the leaves were grinded into powder coarsely and passed through mesh size no. 50. The powdered sample was stored in a closed container free from pollution and environmental contaminants [6].

Preparation of extract:

Preparation of *Dillenia indica* leaf extracts (DILE): 50 gm of powdered sample were evenly packed in Soxhlet's apparatus and the extraction was done with alcohol. The solvent was evaporated at low temperature. The residual extract was used for further investigation [6].

Pharmacognostic Evaluations:

Macroscopic and Organoleptic Studies:

Macroscopic investigation (evaluation of drugs by colour, odour, size, shape, taste and special features including touch and texture etc.) was done. The organoleptic investigations (condition, colour, odour, taste and texture) were performed [7, 8]. The results are depicted in **Fig. 1(A), Fig. 1(B)** and **Table 1.**

FIG. 1(A) **FIG. 1(B)**

FIG. 1(A) AND FIG. 1(B): LEAVES AND FRUITS OF *DILLENIA INDICA* LINN. SHOWING MORPHOLOGICAL CHRACTERISTICS

TABLE 1: ORGANOLEPTIC CHARACTERISTICS OF *DILLENIA INDICA* LINN. LEAVES

Sr. No.	Parameters	Leaves
1	Condition	Dried and powdered
2	Colour	Dark Green
3	Odour	Characteristic
4	Taste	Kashaya- Amla (Shukta) (Astringent- Sour)
5	Texture	Rough and fibrous

Microscopic Studies: Microscopic characteristics were studied for leaf and leaf powder. Microscopic study was done by taking transverse sections of leaf with help of sharp blade. The hand cut sections were subjected to double staining method using safranine and haematoxylin. Saffranine stains lignified tissues imparting deep red colour whereas Hematoxylin stains cellulose part imparting purplish violet colour. For this safranine solution was taken on watch glass. The hand cut sections

were placed in the solution for 10 min. Then the sections were transferred to 50% alcohol for 5 min. The sections were then washed with water and then again transferred to dilute hematoxylin in watch glass for 2min. The sections were again washed with distilled water. Then the sections were mounted on clean glass slide with help of glycerine water and covered by glass cover slip. Then slides were observed under light microscope [7]. The results are depicted in **Fig. 2 and Fig. 3.**

Powder Microscopy:
A small amount of powder was taken and few drops of 20% chloral hydrate solution were added to it in a watch glass. The sample was allowed to stand in watch glass for about 2 min. A few, drops of distilled water were added to the solution and was observed under light microscope. Similarly, the powder was also stained with dilute saffranine solution for identification of lignified cells. The powder microscopic characters were noted [7, 8]. The results are depicted in **Fig. 4(I) - Fig. 4(VIII)**

Determination of Physico-chemical characteristics: T
he moisture content, total ash content, water soluble ash content and acid insoluble ash content, water soluble extractives, alcohol soluble extractives were the parameters of determining the physicochemical characteristics. The physicochemical studies were done as per WHO guidelines [9, 10]. The results are depicted in **Table 2**.

Preliminary Phyto-chemical screening:
This involves analysis and screening of DILE for different phytochemical compounds. The preliminary screening gives a general idea regarding the presence of different compounds possessing therapeutic values. The Phytochemical screening was done as per who guidelines [7]. The results are shown in **Table 3(I), Table 3(II)** and **Table 3(III).**

Test for Carbohydrates:
Test for Reducing sugars (Fehling's Test):
1 ml. of Fehling's A and 1 ml. of Fehling's B solution were mixed thoroughly and boiled for one minute. 2 ml. of alcoholic extract was added to the boiled mixture. The mixture was heated in boiling water bath for 5-10 minutes. The mixture was observed for colour change from yellow to brick red ppt.

Test for Pentose sugars:
1 ml. of HCL for mixed with 1 ml. of alcoholic extract. The mixture was heated and few crystals of phloroglucinol were added. The mixture was observed for development of red colour.

Test for Hexose sugars (Tollen's Phloroglucinol test for galactose):
2.5 ml of conc. HCL was mixed with 4 ml 0.5% phloroglucinol. The mixture was heated and the solution was observed for appearance of colour change.

Cobalt-chloride Test:
3ml of alcoholic extract was mixed with 2ml cobalt-chloride solution and boiled. The mixture was allowed to cool and few drops of NaOH solution was added to it. The solution was observed for appearance of greenish blue or purplish colour solution.

Test for Non-reducing sugars:
Alcoholic extracts (Test solution) does not give response to Fehling's test.

Test for Non-Reducing Polysaccharides (Starch): Iodine Test: Few drops Iodine was mixed with 3 ml. of alcoholic extract. The solution was observed for appearance of blue colour and disappearance of blue colour on heating.

Tannic Acid Test: The alcoholic extract was mixed with 20% Tannic acid. The mixture was observed for ppt. formation.

Test for Proteins:
Biuret Test: Few drops of 4% NaOH was mixed with 3ml alcoholic extract and few drops of 1% $CuSO_4$ were added to the mixture. The mixture was observed for violet or pink colour appearance.

Xanthoprotein Test: 3ml. of alcoholic extract was mixed with 1ml. conc. H_2SO_4 and white ppt. is allowed to form. The ppt. is boiled with the solution and allowed to turn yellow. Few drops of NH_4OH were added to the solution for the ppt. to turn orange.

Test for proteins containing sulphur: 5 ml. alcoholic extract was mixed with 2 ml. 40% NaOH and 2 drops 10% lead acetate solution. The mixture was boiled and observed for black or brown colour solution formation.

Precipitation Test for proteins:
1 ml. Alcoholic extract was mixed with 5% $CuSO_4$ solution and observed for white colloidal ppt. formation.

1 ml. Alcoholic extract was mixed with 5% lead acetate solution and observed for white colloidal ppt. formation.

1 ml. Alcoholic extract was mixed with 5% ammonium sulphate solution and observed for white colloidal ppt. formation.

Tests for Amino Acids:
Ninhydrin Test (General Test): 3 ml. of alcoholic extract was mixed with 5% Ninhydrin solution and kept in boiling water bath. The mixture was observed for purple or bluish colour formation.

Test for Cysteine: Few drops of 40% NaOH and 10% lead acetate solution were added to 5 ml. of alcoholic extract. The mixture was boiled and observed for black or brown colour ppt. formation.

Test for Steroids:
Salkowski Reaction test:
To 2ml of alcoholic extract, 2 ml. chloroform and 2ml. conc. H_2SO_4 was added from the side of the test tube. The chloroform layer was observed for red colour formation and acid layer was observed for greenish yellow fluorescence.

Liebermann – Burchard reaction Test:
2ml. alcoholic extract was mixed with 1ml chloroform. To the mixture 1 ml. acetic anhydride and 2 drops of conc. H_2SO_4 from the side of the test tube. The solution was observed for grades of colour change initially red, then blue and finally green.

Test for Glycosides:
Tests for cardiac glycosides:
Legal's Test (Test for Cardenoloids): 1ml. pyridine and 1 ml. sodium nitroprusside was mixed with 1ml. alcoholic extract. The solution was observed for appearance of pink to red colour formation.

Keller-Killiani Test (Test for deoxysugars):
2ml. of alcoholic extract was mixed with few drops of glacial acetic acid, one drop 5% Fecl3 and conc. H_2SO_4 from side of the test tube. The solution was observed for reddish brown colour at junction of two liquid layers and the upper layer bluish green.

Test for Saponin Glycosides:
Foam Test:
2 ml. of extract was mixed with water and shaken vigorously. The solution was observed for persistent foam formation.

Test for Alkaloids:
Alcoholic extract was dried and mixed with dil. HCl and filtered. The following tests were performed with the filtrate.

Dragendorff's Test:
Few drops of Dragendorff's reagent were mixed with the filtrate. The solution was observed for orange brown ppt. formation.

Wagner's Test:
Few drops of Dragendorff's reagent were mixed with the filtrate. The solution was observed for orange brown ppt. formation.

Murexide test for purine alkaloids:
To 3 ml. filtrate 4 drops of conc. HNO_3 was added and evaporated to dryness. The mixture was cooled and 2 drops of NH_4OH was added. The mixture was observed for purple colour formation.

Tannic acid Test:
1 ml. Tannic acid was added to 1 ml. of filtrate extract and observed for buff coloured precipitate.

Mayer's Test:
1 ml. of filtrate was treated with few drops of Mayer's reagent and observed for ppt. formation.

Test for Flavonoids:
Shinoda Test:
A pinch of dried powder or extract was mixed with 5 ml. 95% alcohol and few drops of conc. HCl and 0.5 g of magnesium turnings. The solution was

observed for orange, pink or red to purple colour formation.

Sulphuric acid Test:
66% or 80% sulphuric acid when mixed with alcoholic extract it exhibits colour change.

Lead acetate solution Test:
To small quantity of residue, 1% lead acetate was added and observed for yellow colour ppt. formation.

Zinc + HCl Test:
The alcoholic extract was heated with zinc and HCl. The solution was observed for pink to red colour formation.

NaOH and Acid Test:
Addition of increasing amount of NaOH to the alcoholic extracts shows colouration which decolourises after addition of acid.

Tests for Tannins and Phenol compounds:
5% $Fecl_3$ Test: To 2 ml. of alcoholic extract 5% $Fecl_3$ was added. The solution was observed for deep blue-black colour formation.

1% Lead Acetate: To 2 ml. of alcoholic extract 1% Lead Acetate was added. The solution was observed for white ppt. formation.

1% Potassium Dichromate: To 2 ml. of alcoholic extract 1% Potassium Dichromate was added. The solution was observed for red ppt. formation.

Dil. Iodine Solution: To 2 ml. of alcoholic extract Dil. Iodine Solution was added. The solution was observed for transient red colour formation.

Dil. NH_4OH and 1% K_2FeCN_6: To 2 ml. of alcoholic extract Dil. NH_4OH and 1% K_2FeCN_6 was added. The solution was observed for red colour solution.

Dil. Potassium permanganate solution: To 2 ml. of alcoholic extract Dil. Potassium permanganate solution was added. The solution was observed for decolouration.

NH_4OH and 10% $AgNO_3$: One drop of NH_4OH and excess of 10% $AgNO_3$ solution was added to 2ml of alcoholic extract and heated for 20 min. in boiling water bath. Initially white coloured ppt. was observed which shows dark silver mirror formation on wall of the test tube.

Test for Triterpenes: To 1 ml. of alcoholic extract 1 ml. of chloroform and 1 ml. of conc. H_2SO_4 were added. The solution was observed for appearance of red colour indicating presence of Triterpenes.

Analysis and Determination of Phyto-Chemical Constituents Using TLC and HPTLC:
Thin Layer Chromatography:
The alcoholic extract of *Dillenia indica* Linn. (DILE) was subjected to thin layer chromatographic analysis to find the presence of chemical constituents in support to the phytochemical analysis. To analyse the phytochemical constituents qualitatively, 10µl of 0.1% DILE was applied as a single band spot in a row along one side of precoated TLC plate, at a distance about 1cm from the edge, by using capillary tubes. The choice of solvent system depends upon two factors: (a) Nature of substance to be separated, (b) material on which separation is to be carried. To make a choice of suitable solvent system, firstly elutropic series of different solvents was tried by running on the TLC plate 8.

The TLC plate containing the sample spot was placed at an angle of 450 in the development chamber covering the bottom of the plate by the solvent up to nearly 1mm. The ascending technique was used. The solvent front was marked and the plate was finally allowed to dry. The coloured substances were visual on the chromatogram. Colourless components were detected by using various visualizing agent. The qualitative evaluation of the plate was done by determining the migrating behaviour of the separated substances given in the form of RF value [6, 11, 12].

TLC Analysis: The presence of different phyto-chemical constituents was analysed with Thin Layer Chromatography using two different solvent system Chloroform: Acetone: Di-ethylamine (10:8:2) and Toluene: Chloroform: Ethanol (28.5: 57: 14.53) as the mobile phases. Natural Product and Anisaldehyde sulphuric acid reagent were used for derivatization. The results are depicted in **Fig.**

5, Fig. 6, Fig. 7, Fig. 8, Fig. 9 and **Fig. 10.** The RF values are depicted in **Table 4(I)** and **4(II).**

HPTLC Analysis: It can be said that HPTLC is a powerful tool among the modern age analysis tools. HPTLC produces visible chromatograms. Complex information about the entire sample is available at a glance. Multiple samples can be observed simultaneously, So that reference and test samples can be compared for identification. Here the reference standards used are Quercetine, Kaemferol, Cholesterol and Glutamic Acid [6, 12]. The optimized chromatographic conditions for flavonoids (Quercetine and Kaemferol), Steroids (Cholesterol) and Amino acid (Glutamic acid) are shown in Table 5(I), 6(I) and 7(I) respectively. Whereas the chromatographic images for flavonoids are shown in Fig. 11(A) and 12(A). Their respective densitogram is shown in Fig. 11(B) and 12(B) with their Rf values depicted in Table 5(II) and 5(III). The chromatographic images for steroids are shown in Fig. 13(A) and 14(A). Their respective densitogram is shown in Fig. 13(B) and 14(B) with their Rf values depicted in Table 6(II) and 6(III). The chromatographic images for amino acids are shown in Fig. 15(A) and 16(A). Their respective densitogram is shown in Fig. 15(B) and 16(B) with their Rf values depicted in Table 7(II) and 7(III).

RESULTS AND DISCUSSION:

Pharmacognostic study:

Macroscopic (Morphological) Characteristics:

The leaves of *Dillenia indica* Linn. are simple, alternate, broad, large, long, elliptical and petiolated with acute apex. The leaves show serrate margin and reticulate venation. Older leaves are dark green in colour whereas young leaves are yellowish green in colour. The leaves are 25cms long and 15cms broad. The leaves have characteristic odour and astringent - sour in taste.

Microscopic Characteristics:

Microscopic evaluation revealed the presence of Collenchyma cells, Upper epidermis, Palisade cells, Parenchyma cells, Spongy parenchyma, Vascular bundles with Phloem and Xylem and Anomocytic Stomata.

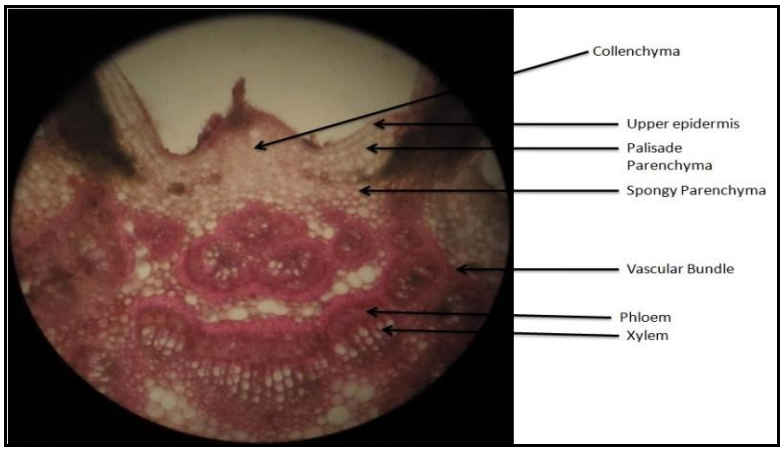

FIG. 2: T.S. OF LEAF OF *DILLENIA INDICA* LINN. THROUGH MIDRIB (10x X 40x)

FIG. 3: ANOMOCYTIC STOMATA (10x X 40x)

Powder Microscopy: The powder microscopy of the leaves revealed the presence of aciculars, trichome, crystals, stone cells, stomata and sclereid.

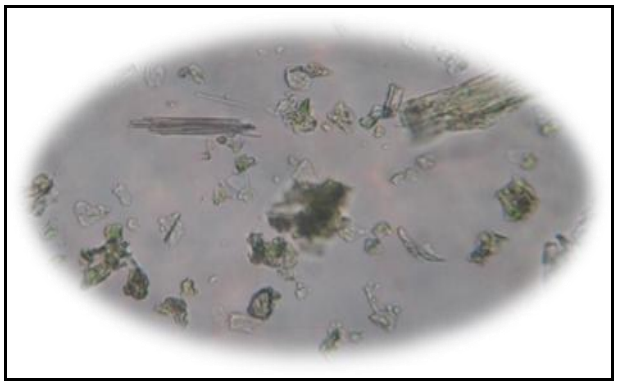

FIG. 4(I): BUNDLE OF ACICULAR (10xX10x)

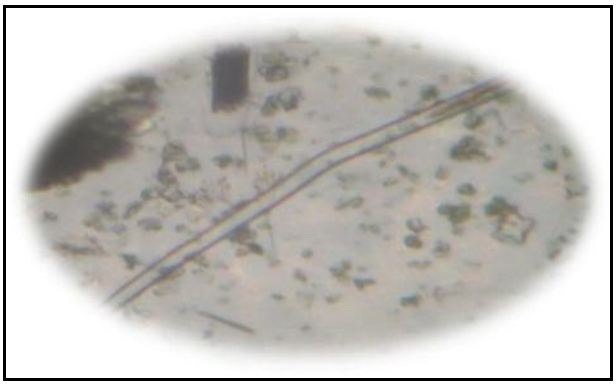

FIG. 4(II): TRICHOME (10xX10x)

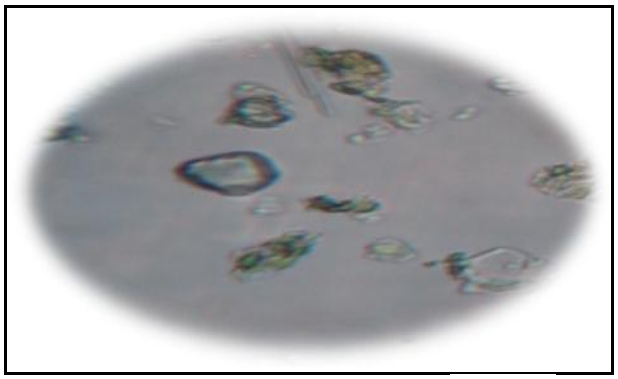

FIG. 4(III): PRISMATIC CRYSTAL (10xX10x)

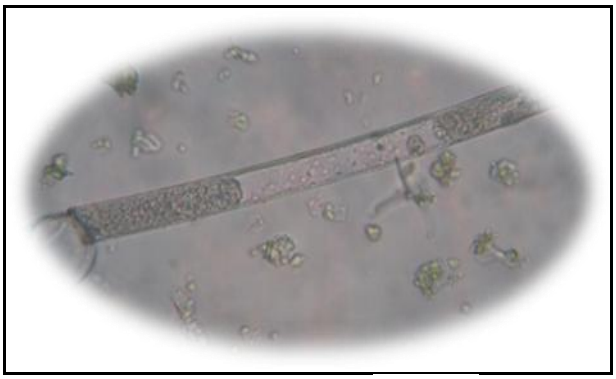

FIG. 4(IV): PHLOEM (10xX10x)

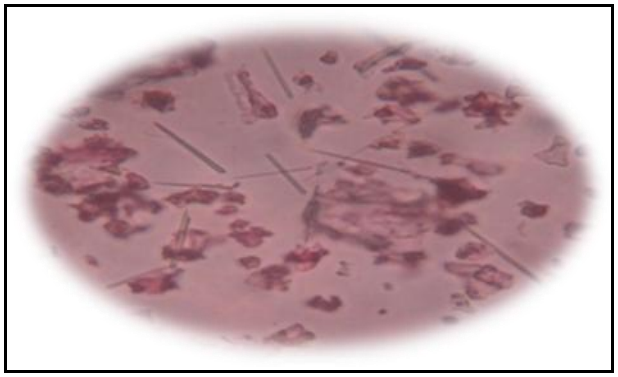

FIG. 4(V): SINGLE ACICULARS (10xX10x)

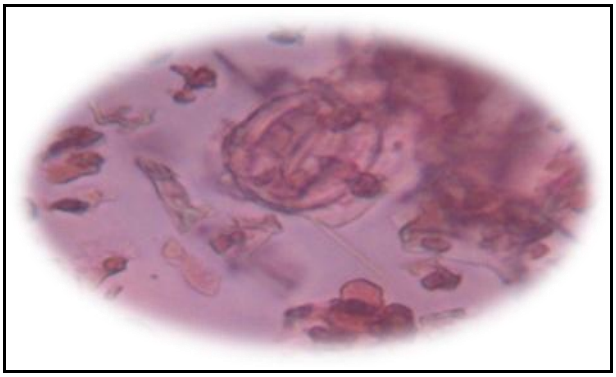

FIG. 4(VI): STOMATA (10xX40x)

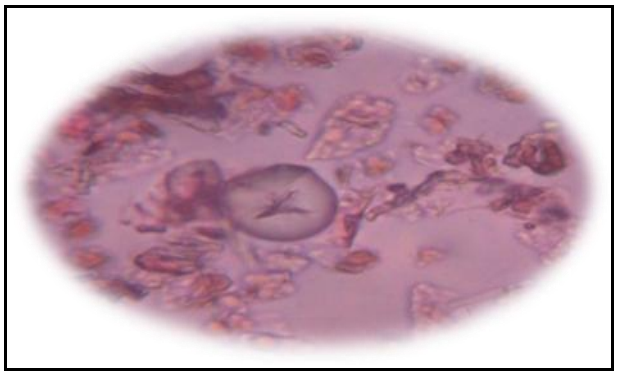

FIG. 4(VII): STARCH GRANULE (10xX40x)

FIG. 4(VIII): SCLEREID (10xX10x)

FIG. 4: POWDER MICROSCOPY OF *DILLENIA INDICA* LINN. LEAVES UNDER DIFFERENT MAGNIFICATIONS

TABLE 2: PHYSICOCHEMICAL ANALYSIS OF LEAVES OF *DILLENIA INDICA* LINN. RESULTS EXPRESSED AS, MEAN±SD, N=9

Analysis Parameters	(% w/w)
Moisture content	10.71±0.25
Water soluble extractives	11.97±0.41
Alcohol soluble extractives	42.32±0.52
Total Ash content	17.48±0.43
Acid insoluble ash content	10.56±0.18
Water soluble ash content	14.03±0.18

TABLE 3(I): PHYTOCHEMICAL SCREENING OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN.

Chemical Constituents	Test	Inference
Carbohydrates	Fehling's Test	-ve
Pentose sugars	Pentose sugars	+ve
Hexose sugars	Tollen's Test	+ve
	Cobalt chloride Test	+ve
	Non-reducing Sugars	+ve

Key: "+ve"- Present; "-ve"- Absent

TABLE 3(II): PHYTOCHEMICAL SCREENING OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN.

Chemical Constituents	Test	Inference
Non-Reducing Polysaccharides	Iodine Test	-ve
	Tannic acid test	+ve
Proteins	Biuret Test	-ve
	Xanthoprotein Test	-ve
Proteins containing Sulphur (Precipitation Tests)	5% $CuSo_4$ Test	-ve
	5% Lead acetate	+ve
	5% Ammonium sulphate	-ve
Amino acids	Ninhydrin Test	-ve
	Cysteine Test	-ve
Steroids	Salkowski Reaction test	+ve
	Liebermann- Burchard Test	+ve
Cardiac Glycosides	Legal's Test	+ve
	Keller-Killiani Test	-ve
Saponin Glycosides	Foam test	+ve
Alkaloids	Dragendorff's Test	+ve
	Wagner's Test	-ve
	Murexide Test	-ve
	Tannic Acid Test	+ve
	Mayer's Test	+ve

KEY: "+ve"- Present; "-ve"- Absent

TABLE 3(III): PHYTOCHEMICAL SCREENING OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN.

Chemical Constituents	Test	Inference
Flavonoids	Schinoda Test	-ve
	Sulphuric Acid Test	+ve
	Lead acetate solution Test	+ve
	Zinc + HCl Test	-ve
	NaOH and Acid Test	+ve
Tannins and Phenol Compounds	5% Fecl3 Test	+ve
	1% Lead Acetate	+ve
	1% Potassium Dichromate	+ve
	Dil. Iodine Solution	-ve
	Dil. NH_4OH and 1% K_2FeCN_6	+ve
	Dil. Potassium permanganate Solution.	-ve
	NH4OH and 10% $AgNO_3$	-ve
Triterpenes	Chloroform and H_2SO_4 Test	-ve

KEY: "+ve"- Present; "-ve"- Absent

TLC Finger Print Profile of Alcoholic Extract of *Dillenia Indica* Linn.

The TLC finger print profile showed the presence of different phytoconstituents. Two different solvent systems were used to analyse the presence of different phytochemical constituents.

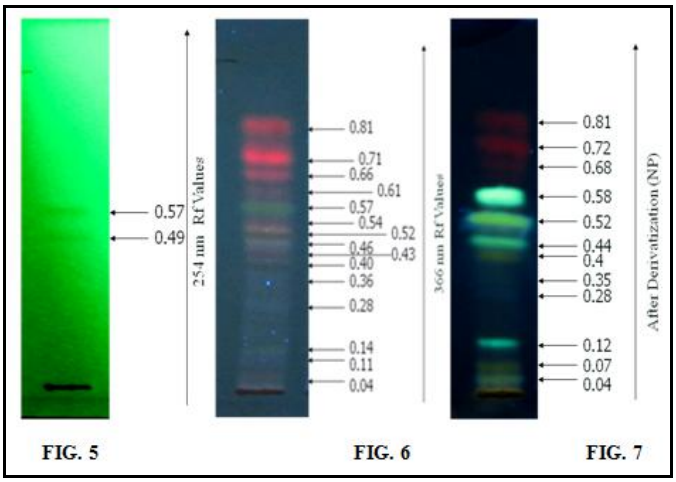

FIG. 5, FIG. 6 AND FIG. 7: TLC CHROMATOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN SHOWING SEPARATION OF 2 DIFFERENT BANDS UNDER 254nm, SEPERATION OF 15 DIFFERENT BANDS UNDER 366nm AND SEPERATION OF 12 DIFFERENT BANDS UNDER 366nm AFTER DERIVATIZATION WITH NATURAL PRODUCT INDICATES THE PRESENCE OF DIFFERENT PHYTO-CHEMICAL CONSTITUENTS.

SOLVENT SYSTEM: CHLOROFORM: ACETONE: DI-ETHYLAMINE (10: 8: 2, v/v/v)

TABLE 4(I): TLC OF DILE INDICATING THE RF (RETENTION FACTOR) VALUES OF DIFFERENT BANDS IN DIFFERENT WAVELENGTH BEFORE AND AFTER DERIVATIZATION

Sr.No.	Rf Factor in 254 nm	Rf Factor in 366 nm	Rf value after derivatization with Natural Product (NP) in 366 nm
1	0.49	0.04	0.04
2	0.57	0.11	0.07
3	-	0.14	0.12
4	-	0.28	0.28
5	-	0.36	0.35
6	-	0.40	0.40
7	-	0.43	0.44
8	-	0.46	0.52
9	-	0.52	0.58
10	-	0.54	0.68
11	-	0.57	0.72
12	-	0.61	0.81
13	-	0.66	-
14	-	0.71	-
15	-	0.81	-

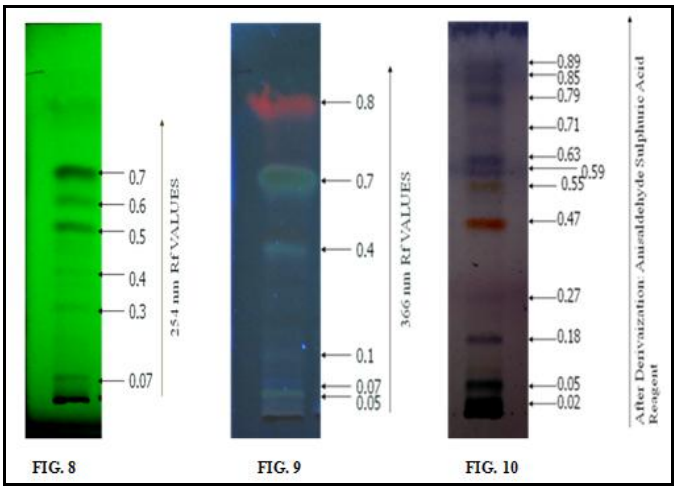

FIG. 8 FIG. 9 FIG. 10

FIG. 8, FIG. 9 AND FIG. 10: TLC CHROMATOGRAM OF ALCOHOLIC EXTRACT OF DILLENIA INDICA LINN SHOWING SEPARATION OF 6 DIFFERENT BANDS UNDER 254nm, SEPERATION OF 6 DIFFERENT BANDS UNDER 366nm AND SEPERATION OF 12 DIFFERENT BANDS UNDER DAY LIGHT AFTER DERIVATIZATION WITH ANISALDEHYDE SULPHURIC ACID INDICATES THE PRESENCE OF DIFFERENT PHYTO-CHEMICAL CONSTITUENTS.

SOLVENT SYSTEM: TOLUENE: CHLOROFORM: ETHANOL (5.68: 11.36: 2.89)

TABLE 4(II): TLC OF DILE INDICATING THE RF VALUES OF DIFFERENT BANDS IN DIFFERENT WAVELENGTH

Sr.No	Rf Factor in 254nm	Rf Factor in 366 nm	Rf Factor after derivatization with Anisaldehyde Sulphuric acid
1	0.07	0.05	0.02
2	0.3	0.07	0.05
3	0.4	0.1	0.18
4	0.5	0.4	0.27
5	0.6	0.7	0.47
6	0.7	0.8	0.55
7	-	-	0.59
8	-	-	0.63
9	-	-	0.71
10	-	-	0.79
11	-	-	0.85
12			0.89

HPTLC Profile:

HPTLC profile showed the presence of Quercetin, Kaemferol, Cholesterol and Glutamic Acid.

TABLE 5(I): OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR DETECTION OF FLAVONOIDS

Parameters	Description
Stationary Phase	Merck Silica gel 60 F_{254} TLC pre-coated plates
Plate size	11.0 cm x 10.0 cm
Mode of separation	Normal phase
Mobile phase	Cyclohexane: Ethyl acetate: Formic acid (6: 4.5: 0.2, v/v/v)
Development chamber	Camag twin trough chamber
Chamber saturation	20 min
Sample applicator	CAMAG Linomat 5
Syringe	Hamilton, 100.0 µL
Band width	7.0 mm
Distance from the edges of the plate	12.5mm
Rate of sample application	100 nL/sec
Spotting volume	10 µL
Development distance	85.0 mm
Densitometric scanner	CAMAG Scanner 4 equipped with winCATS Planar Chromatography manager software version 1.4.7
Photodocumentation	CAMAG Reprostar 3

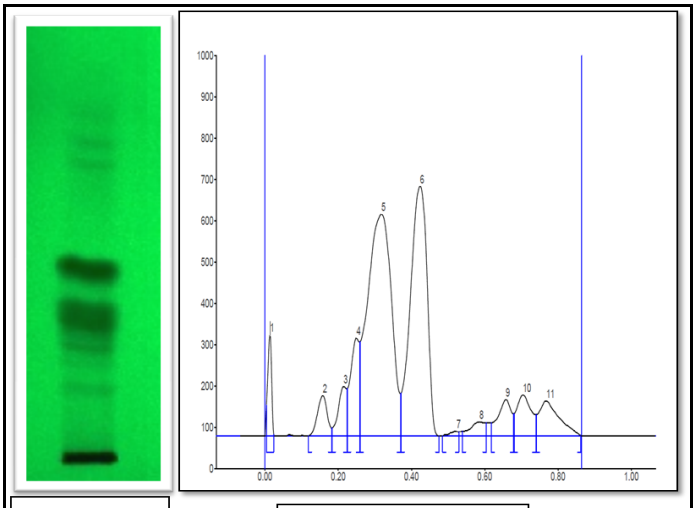

FIG. 11(A) DILE FIG. 11(B) DILE

FIG. 11(A) AND FIG. 11(B): HPTLC CHROMATOGRAM AND DENSITOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN. FOR FLAVONOIDS SHOWING SEPARATION OF 11 DIFFERENT BANDS UNDER 254 nm

TABLE 5(II): HPTLC PEAKS OF *DILLENIA INDICA* LINN. FOR FLAVONOIDS

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	74.4	0.01	245.2	11.35	0.02	4.3	2220.7	2.84
2	0.12	0.1	0.16	97.9	4.53	0.18	19.7	2277.0	2.91
3	0.18	19.9	0.21	120.0	5.55	0.22	114.6	2358.2	3.01
4	0.23	114.9	0.25	236.9	10.96	0.26	227.6	4934.0	6.30
5	0.26	228.1	0.32	537.3	24.86	0.37	102.7	30704.7	39.22
6	0.37	103.5	0.42	605.0	27.99	0.48	0.0	24024.0	30.69
7	0.49	0.0	0.52	11.4	0.53	0.53	10.5	241.3	0.31
8	0.54	10.9	0.59	34.1	1.58	0.61	31.8	1226.2	1.57
9	0.62	31.7	0.66	88.7	4.10	0.68	53.6	2796.6	3.57
10	0.68	54.0	0.71	99.8	4.62	0.74	51.5	3431.1	4.38
11	0.74	51.6	0.77	85.0	3.93	0.86	1.2	4067.0	5.20

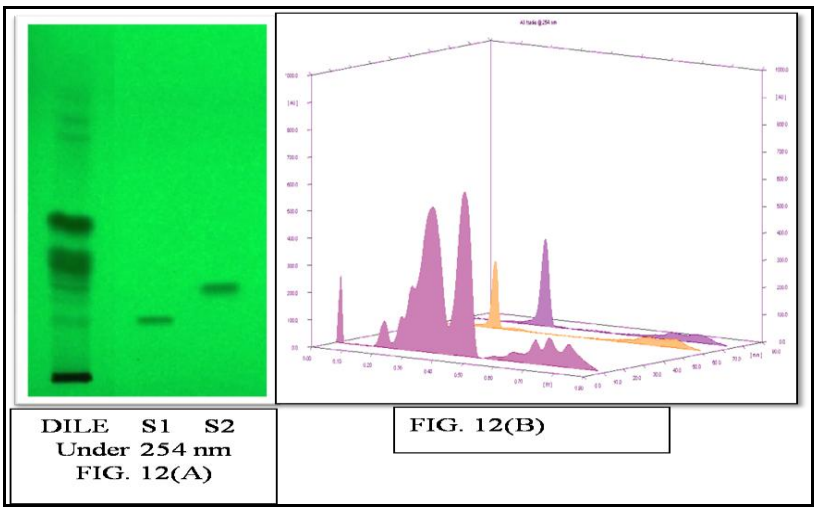

FIG. 12(A): DILE: HPTLC CHROMATOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN FOR FLAVONOIDS UNDER 254 nm; S1: STANDARD QUERCETIN; S2: STANDARD KAEMFEROL

FIG. 12(B): HPTLC DENSITOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN OVERLAPPED WITH STANDARDS QUERCETIN AND KAEMFEROL UNDER 254 nm

TABLE 5(III): RF VALUES OF STANDARD FLAVONOIDS QUERCETIN AND KAEMFEROL COINCIDING WITH TWO SPOTS OBSERVED IN DILE.

HPTLC FIG.	Name Of Phytochemical Constituent	Compound	Rf Value Obtained (Standards)	Rf Value Obtained (*Dillenia indica* Linn.)
11 A,B	Flavonoid	Quercetin	0.18	0.16
12 A,B		Kaemferol	0.27	0.25

TABLE 6(I): OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR DETECTION OF STEROIDS

Parameters	Description
Stationary Phase	Merck Silica gel 60 F_{254} TLC pre-coated plates
Plate size	11.0 cm x 10.0 cm
Mode of separation	Normal phase
Mobile phase	Toluene: methanol (8: 1, v/v)
Development chamber	Camag twin trough chamber
Chamber saturation	20 min
Sample applicator	CAMAG Linomat 5
Syringe	Hamilton, 100.0 µL
Band width	7.0 mm
Distance from the edges of the plate	12.5mm
Rate of sample application	100 nL/sec
Spotting volume	10 µL
Development distance	85.0 mm
Densitometric scanner	CAMAG Scanner 4 equipped with winCATS Planar Chromatography manager software version 1.4.7
Photodocumentation	CAMAG Reprostar 3
Derivatizing agent:	10% Methanolic sulphuric acid

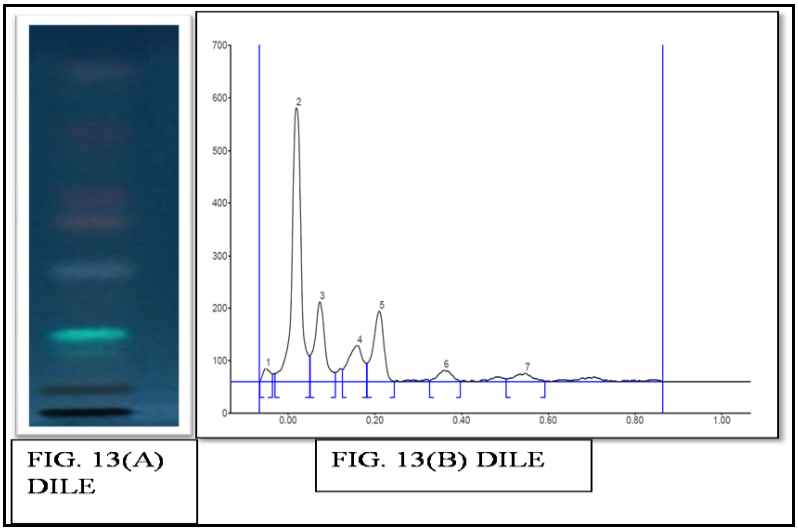

FIG. 13(A) AND FIG. 13(B): HPTLC CHROMATOGRAM AND DENSITOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN. FOR STEROIDS SHOWING SEPARATION OF 7 DIFFERENT BANDS AND PEAKS UNDER 366 nm RESPECTIVELY

TABLE 6(II): HPTLC PEAKS OF *DILLENIA INDICA* LINN. FOR STEROIDS

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.07	1.4	-0.05	25.3	2.68	-0.04	15.2	396.8	2.03
2	-0.03	15.3	0.02	522.1	55.34	0.05	49.8	9713.1	49.74
3	0.05	50.3	0.07	152.7	16.19	0.11	18.1	3212.6	16.45
4	0.13	23.4	0.16	69.3	7.34	0.18	35.3	2066.3	10.58
5	0.18	35.5	0.21	134.5	14.26	0.25	1.0	2888.6	14.79
6	0.33	2.7	0.36	22.6	2.39	0.40	1.1	634.8	3.25
7	0.50	5.8	0.55	17.0	1.80	0.59	1.3	617.3	3.16

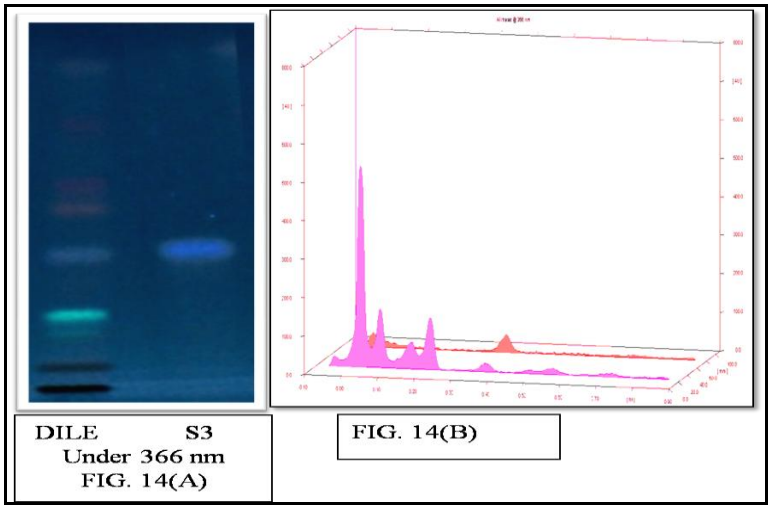

FIG. 14(A): DILE: HPTLC CHROMATOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN FOR STEROIDS UNDER 366 nm; S3: STANDARD CHOLESTEROL.

FIG. 14(B): HPTLC DENSITOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN OVERLAPPED WITH STANDARD CHOLESTEROL UNDER 366 nm.

TABLE 6(III): RF VALUES OF STANDARD CHOLESTEROL COINCIDING WITH A SPOT OBSERVED IN DILE.

HPTLC Image No.	Name Of Phytochemical Constituent	Compound	Rf Value Obtained (Standards)	Rf Value Obtained (*Dillenia indica* Linn.)
13 A, B **14 A, B**	Steroid	Cholesterol	0.35	0.36

TABLE 7(I): OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR DETECTION OF AMINO ACIDS

Parameters	Description
Stationary Phase	Merck Silica gel 60 F_{254} TLC pre-coated plates
Plate size	20.0 cm x 10.0 cm
Mode of separation	Normal phase
Mobile phase	n-Butanol: acetic acid: water (6.5: 3.5: 2, v/v/v)
Development chamber	Camag twin trough chamber
Chamber saturation	30 min
Sample applicator	CAMAG Linomat 5
Syringe	Hamilton, 100.0 µL
Band width	6.0 mm
Distance from the edges of the plate	13.0mm
Rate of sample application	100 nL/sec
Spotting volume	10 µL
Development distance	85.0 mm
Densitometric scanner	CAMAG Scanner 4 equipped with winCATS Planar Chromatography manager software version 1.4.7
Photodocumentation	CAMAG Reprostar 3
Derivatizing agent	Ninhydrin reagent

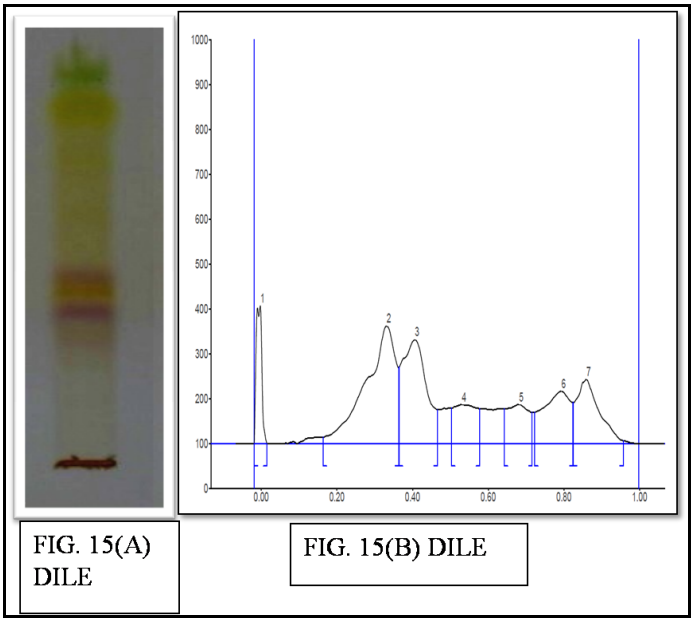

FIG. 15(A) AND FIG. 15(B): HPTLC CHROMATOGRAM AND DENSITOGRAM OF ALCOHOLIC EXTRACT OF *DILLENIA INDICA* LINN. FOR AMINO ACIDS SHOWING SEPARATION OF 7 DIFFERENT BANDS AND PEAKS UNDER VISIBLE LIGHT (550 nm) RESPECTIVELY.

TABLE 7(II): HPTLC PEAKS OF *DILLENIA INDICA* LINN. FOR AMINO ACIDS

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	0.0	-0.00	307.8	24.81	0.01	0.7	4088.3	6.91
2	0.16	15.0	0.33	263.3	21.22	0.36	170.6	18064.3	30.53
3	0.37	171.2	0.41	232.6	18.75	0.47	76.6	12864.2	21.74
4	0.50	80.2	0.53	88.0	7.10	0.58	79.2	4785.6	8.09
5	0.64	78.0	0.68	87.7	7.07	0.72	70.7	4514.7	7.63
6	0.72	70.6	0.79	117.3	9.45	0.83	92.1	7337.8	12.40
7	0.83	92.1	0.86	144.0	11.61	0.96	7.5	7523.6	12.71

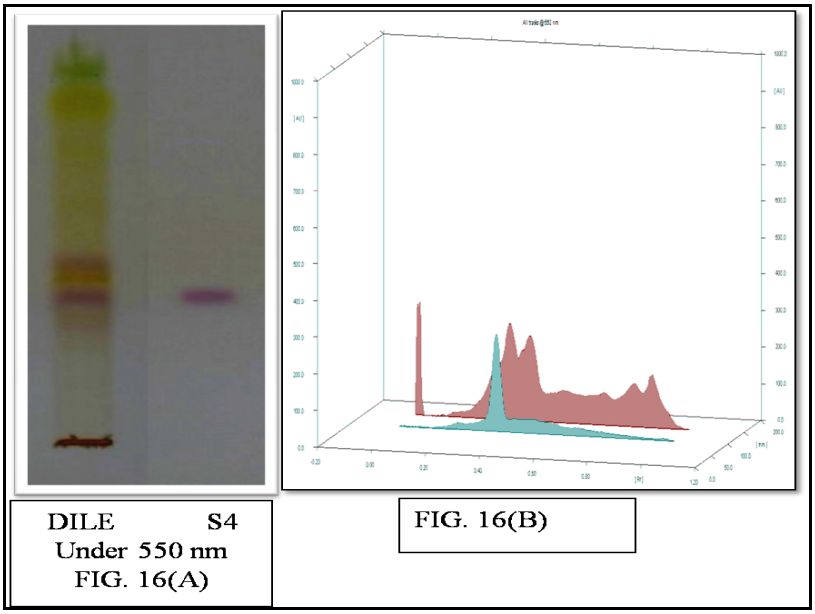

FIG. 16(A): DILE: HPTLC CHROMATOGRAM OF ALCOHOLIC EXTRACT OF DILLENIA INDICA LINN. FOR STEROIDS UNDER 550 nm; S4: STANDARD GLUTAMIC ACID.

FIG. 16(B): HPTLC DENSITOGRAM OF ALCOHOLIC EXTRACT OF DILLENIA INDICA LINN. OVERLAPPED WITH STANDARD GLUTAMIC ACID UNDER 550nm (VISIBLE LIGHT).

TABLE 7(III): RF VALUES OF STANDARD CHOLESTEROL COINCIDING WITH A SPOT OBSERVED IN DILE.

HPTLC Image No.	Name Of Phytochemical Constituent	Compound	Rf Value Obtained (Standard)	Rf Value Obtained (*Dillenia indica* Linn.)
15 A, B 16 A, B	Amino Acids	Glutami Acid	0.34	0.33

DISCUSSION: The macroscopic study (**Table 1**) of leaf indicated that its colour, odour, taste and texture may be an important characteristic feature for identifying the plant. The anatomy of the leaf was studied by taking transverse section. Transverse section (**Fig. 2**) of the leaf showed epidermis with palisade parenchymatous cells followed by Spongy parenchymatous cells. Collenchyma cells were seen in the midrib region below the epidermis and vascular bundles were also present. Anomocytic stomata were seen scattered (**Fig. 3**). Powder studies (**Fig.4**) indicated the presence of Aciculars, Trichomes, Prismatic crystals, Phloem, Stomata, Starch grains and Sclereid. Physicochemical parameters are important parameters in detecting adulteration and are adopted to confirm the purity and quality of drug. The moisture content of the drug plays an important role as too high moisture content may encourage the bacterial, fungal or yeast growth. The moisture content, extractive values and ash values, of leaves were determined. The results are depicted in **Table 2.** Ash values are particularly important parameter as it shows the presence and absence of foreign matters like metallic salts or silica.

The Phytochemical study (Table 3(I), 3(II) and 3(III)) shows Alcoholic extracts of *Dillenia indica* L. contains Carbohydrates, Starch, Proteins containing sulphur, Steroids, Cardiac Glycosides, Saponin Glycosides, Alkaloids, Flavonoids, Tannins and Phenol compounds.

T.L.C. Profile (Fig. 5, 6, 7, 8, 9 and 10) of alcoholic extract using Chloroform: Acetone: Di-ethylamine (10: 8: 2, v/v/v), showed fifteen spots under 366 nm whereas Toluene: Chloroform: Ethanol (5.68: 11.36: 2.89, v/v/v) showed twelve spots on derivatization under visible light.

HPTLC Profile of alcoholic extract using Cyclohexane: ethyl acetate: formic acid (6: 4.5: 0.2, v/v/v) showed presence of Quercetin and Kaemferol (11 A, B and 12 A, B) with 0.16 and 0.25 Rf values respectively. Toluene: methanol (8: 1, v/v) solvent system showed the presence of Cholesterol (13 A, B and 14 A, B) at 0.36 Rf value. n-Butanol: acetic acid: water (6.5: 3.5: 2, v/v/v) showed the presence of Glutamic acid (15 A, B and 16 A, B) with 0.33 Rf value.

Pharmacognostic standardization including physico-chemical evaluation in **Table 2** is meant for identification, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs. Since the plant *Dillenia indica* Linn. is useful in traditional medicine for the treatment of various ailments it is important to standardize it for use as a drug.

CONCLUSION:

Since time immemorial plants have been playing an important role in treatment of various ailments. Herbal plants are considered to be source of varied phytoconstituents exhibiting various pharmacological properties. Therefore it becomes a necessity to study the phytochemical constituents and pharmacognostic characteristic before its use in the field of research and pharmaceutical formulation. From the present study, it can be concluded that most of the biologically active phytochemicals were present in the methanolic extract of *Dillenia indica* Linn. leaves. In other words, the results confirmed the presence of therapeutically potent compound in leaf extract of *Dillenia indica*. The chromatogram of thin layer chromatography showed the presence of various unknown compounds whereas HPTLC densitogram showed the presence of flavonoids (quercetin and kaemferol), sterols (cholesterol) and amino acids (glutamic acid).

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