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PHYTOCHEMICAL INVESTIGATION AND CHROMATOGRAPHIC EVALUATION OF THE ETHANOLIC EXTRACT OF WHOLE PLANT EXTRACT OF *DENDROPHTHOE FALCATA* (L.F.) ETTINGSH

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ABSTRACT

The present study was aimed to investigate phytochemicals present in the whole plant extract of *Dendrophthoe falcata* and development of new solvent system for thin layer chromatography of ethanolic (whole plant) extract of medicinal plant *Dendrophthoe falcata*. The extract showed presence of Carbohydrates, Sterols, Glycosides, Flavonoids and Phenolic compounds. For TLC, new solvent system developed, best result was given by solvent system- Toluene: Ethyle acetate: Formic acid in the ratio of 2.5:1:1. HPTLC report of the extract in same solvent system- Toluene: Ethyle acetate: Formic acid in the ratio of 2.5:1:1 showed the presence of eleven active chemical constituents.

INTRODUCTION:

Dendrophthoe falcata is also known as "Vanda" in the Indian Ayurvedic System of Medicine. *Dendrophthoe falcata* (L. f.) ettingsh is a perennial climbing woody parasitic plant. It is indigenous to tropical regions especially in India, Srilanka, Thailand, China, Australia, Bangladesh, Malaysia and Myanmar. It is widely distributed throughout in India[1]. The accumulation of kaemferol, quercetin, myrecitin, and their glycosides are also present[2]. Several cardiac glycosides, flavonoids, and some pentacyclic triterpene present in methanolic leaves extract[3].

Ethnomedical claims: *Dendrophthoe falcata* (L.f.) Ettingsh is a popular hemi-parasitic plant and is used in folklore medicine for ailments including ulcers, asthma, impotence, paralysis, skin disorder, menstrual troubles, pulmonary tuberculosis and wounds. In addition to its medicinal value, the fruit of *D. falcata* testes sweet and is consumed as food. The entire plant is medicinally important and is used extensively in traditional medicine as an aphrodisiac, astringent, narcotic, and diuretic [4].

MATERIALS AND METHODS:

Plant material and its extract: The plant *Dendrophthoe falcata* was collected in the month of September from Ayurvedic college of Jhansi (U.P.) The plant material was taxonomically identified and authenticated by Dr. P. B. Singh, (Research Officer, Botany) Regional Research Institute (Ayurveda) Gwalior Road Jhansi. The shade dried plant material was grounded to coarse powder using grinder. The powdered drug (approx. 500 gm.) were then packed in the soxhlet apparatus and extracted with 95% Ethanol. After the extraction is complete, the extracted powder was discarded and the ethanolic extract so obtained was further processed. The excess solvent in the extract was removed by distillation and the concentrated extract so obtained was further dried under reduced pressure at a temperature not exceeding 40^0 C in rotary evaporator. The extract was then collected (extractive value 9 gm), kept in Petridish and stored in a dessicator at room temperature.

Preliminary phytochemical screening: It involves testing of DFEE for their content of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug [5]. The ethanolic extract was subjected to the preliminary phytochemical investigation [6] for detection of

Tests for carbohydrates:

Fehling's Test: 1 ml. Fehling's A solution and 1 ml. of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution (ethanolic extract) was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitate was observed.

Benedict's test: Equal volumes of Benedict's reagent and test solution (ethanolic extract) were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

Tests for proteins:

Xanthoproteic Test: To the small quantity of ethanolic extract 1ml. of conc. H_2SO_4

was added. This resulted in the formation of white precipitate which on boiling turned yellow. On addition of NH_4OH, yellow ppt. turned orange.

Biuret Test: Small quantity of ethanolic extract was dissolved in a few mL of water. To this test solution 4% NaOH solution and a few drops of 1% $CuSO_4$ solution was added. Appearance of violet colour showed presence of proteins.

Tests for glycosides:

Borntrager's Test: To the 3ml of ethanolic extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonical layer turned pink showing the presence of glycosides.

Legal's Test: To the concentrated ethanolic extract few drops of 10% NaOH were added, to make it alkaline. Then freshly prepared sodium nitroprusside was added to the solution. Presence of blue coloration indicated the presence of glycosides in the extract.

Keller-Killiani Test: To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides.

Test for steroids:

Salkowski Test: To 2 ml. of ethanolic extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Leibermann's Reaction: 3 ml. of ethanolic extract was mixed with 3 ml. of acetic anhydride. The test solution was then heated and cooled. A few drops of conc. H_2SO_4 were added to the test solution. Appearance of blue colour shows the presence of sterols.

Tests for alkaloids:

The ethanolic extract was evaporated in a test tube. To the residue dilute HCl was added, shaken well and filtered. With the filtrate following tests were performed:

Hager's Test: To the 2-3 ml of filtrate Hager's reagent was added. Yellow precipitate was formed showing the presence of alkaloids.

Mayer's Test: To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

Tests for flavonoids:

Shinoda Test: To the ethanolic extract, added 5 ml of 95% ethanol and few drops of conc. HCl. To this solution 0.5 g of magnesium turnings were added. Observance of pink coloration indicated the presence of flavonoids.

With Lead Acetate: To the small quantity of ethanolic extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoid.

With Sodium Hydroxide: On addition of an increasing amount of sodium hydroxide, the ethanolic extract showed yellow coloration, this decolorized after addition of acid.

Tests for Tannins and Phenolic compounds:

$FeCl_3$ Solution Test: On addition of 5% $FeCl_3$ solution to the ethanolic extract, deep blue black colour appeared.

Lead Acetate Test: On addition of lead acetate solution to the ethanolic extract white precipitate appeared.

Dil. HNO_3 Test: On addition of dilute HNO3 solution to the ethanolic extract, reddish colour appeared.

Test for saponins:

Foam Test: Drug extract was shaken vigorously with water. No persistent foam was formed

Test for triterpenes: To the ethanolic extract chloroform and conc. H_2SO_4 was added. Appearance of red colour indicated the presence of triterpenes[7].

Thin Layer Chromatography:

The Ethanolic extract of the *Dendrophthoe falcata* was subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test. The details of procedure are as following:

Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Prepared chromo plates were then divided of the liquid associated with thin layer by drying the thin layer plates, for 30 minutes in air and then in an oven at 110^0C for another 30 minutes. For qualitative work, 0.1% of DFEE applied as a single spot in a row along one side of chromo plate, about 2 cm from the edge, by using capillary tubes. The range of sample volume applied was controlled, spreading not more than 0.5 cm. The choice of solvent 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 45^0 in the development chamber covering the bottom of the plate by the solvent up to nearly 1 mm. 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 visualizing agent, iodine vapours. The qualitative evaluation of the plate was done by determining the migrating behaviour of the separated substances given in the form of R_F value [9].

High performance thin layer chromatography:

After quantitative analysis of DFEE by TLC, DFEE was further analyzed by HPTLC for better resolution. The separated spots were visualized under UV 366 nm.

RESULT AND DISCUSSION:

The leaves were extracted using solvent 95% ethanol in the soxhlet apparatus. The semi-solid extract so obtained was aromatic and greenish-black in colour. The extractive value was found 9 gm.

Table1: Characteristics of DFEE

Characteristics	Ethanolic Extract
Extractive Value	9 gm
Physical Appearance	Semisolid mass
Color	Greenish
Odour	Aromatic
Taste	Bitter

Preliminary phytochemical screening:

DFEE was tested for its content of different classes of compounds. Various qualitative chemical tests for preliminary phytochemical screening of the extract for different types of chemical constituents were applied. Phytochemical tests on the extract gave positive reactions for carbohydrates, glycosides, steroids, tannins & phenolic compounds, flavonoids and triterpenes.

Table2. Data for phytochemical screening of DFEE

Chemical Constituents	Test	Inference
Carbohydrates	Molisch test	+
	Benedicts Test	+
Glycosides	Borntrager's Test	+
	Legal's Test	+
	Keller- Killiani Test	+
Steroids	Salkowski Test	+
	Liebermann's Reaction	+
Tannins & Phenolic Compounds	$FeCl_3$ Sol. Test	+
	Lead Acetate Test	+
	Dil. HNO_3 Test	+
Flavanoids	Shinoda Test	+
	Lead acetate Test	+
	Sodium Hydroxide Test	+
Saponins	Foam Test	+
Triterpenes	Chloroform + Conc. H_2SO_4	+

Chromatography:

The separation and purification of phyto-constituents of the extract was mainly carried out using a combination of the chromatographic techniques. The choice of technique depends largely upon the solubility properties and volatilities of compound to be separated.

Thin Layer Chromatography:

It is a method of choice for separating all lipid soluble components, i.e., the lipids, steroids, carotenoids, simple quinines and chlorophyll. The special advantage includes versatility and speed. TLC of DFEE resulted in identification of ten spots with the R_f Value 0.08, 0.12, 0.16, 0.24, 0.32, 0.48, 0.56, 0.76, 0.88, 0.96.The solvent system used for the TLC is shown in table 3.

Table3: TLC of DFEE performed in the Best Solvent System

Solvent System	No. of Spots	Visualizing Agent
Toluene:Ethyl acetate: Formic Acid (2.5:1:1)	10	Iodine Vapor

Separation of ten spots showed the separation of ten chemical constituents. Thus the screening of DFEE for different chemical constituents was further verified by TLC. Fig. 1

Fig. 1

High performance thin layer chromatography:

It is a major advancement of TLC with a better resolution in a shorter time, differ from TLC only in particle and pore size of the sorbents. It is most applied to obtain "finger-print" patterns of herbal formulation, perform qualitative determination and quantitative analysis of active ingredients of herbal extract and synthetic drugs, standardize herbal extracts & formulation detection of adulteration, perform stability studies of herbal extracts and estimate synthetic/natural drugs in formulation. In present study HPTLC of DFEE showed eleven spots in UV, further resolving the separation of DFEE done by TLC. (Table 4)

The peaks obtained in HPTLC of DFEE are shown in Fig. 2.

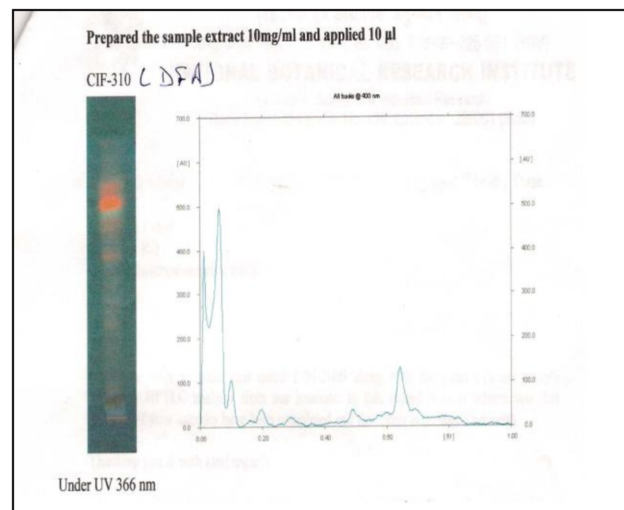

Fig. 2

Table 4: HPTLC peaks of DFEE

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area%	Assigned Substance
1	1	0.00 Rf	12.1 AU	0.01 Rf	399.4 AU	30.20%	0.03 Rf	227.5 AU	5644.8 AU	18.53%	unknown *
1	2	0.03 Rf	227.6 AU	0.06 Rf	496.4 AU	37.54%	0.08 Rf	43.3 AU	14024.7 AU	46.04%	unknown *
1	3	0.08 Rf	43.6 AU	0.10 Rf	105.4 AU	7.97%	0.12 Rf	4.9 AU	2029.2 AU	6.66%	unknown *
1	4	0.14 Rf	1.1 AU	0.16 Rf	14.8 AU	1.12%	0.17 Rf	11.0 AU	290.8 AU	0.95%	unknown *
1	5	0.18 Rf	9.0 AU	0.20 Rf	38.8 AU	2.94%	0.22 Rf	6.0 AU	909.3 AU	2.99%	unknown *
1	6	0.27 Rf	4.1 AU	0.29 Rf	20.6 AU	1.56%	0.32 Rf	7.2 AU	613.3 AU	2.01%	unknown *
1	7	0.47 Rf	9.9 AU	0.49 Rf	37.4 AU	2.83%	0.53 Rf	11.2 AU	1153.4 AU	3.79%	unknown *
1	8	0.55 Rf	11.1 AU	0.57 Rf	23.4 AU	1.77%	0.58 Rf	21.7 AU	502.9 AU	1.65%	unknown *
1	9	0.62 Rf	25.7 AU	0.64 Rf	130.9 AU	9.90%	0.68 Rf	32.7 AU	3940.8 AU	12.94%	unknown *
1	10	0.68 Rf	33.3 AU	0.69 Rf	37.3 AU	2.82%	0.72 Rf	16.7 AU	1061.4 AU	3.48%	unknown *
1	11	0.82 Rf	14.8 AU	0.83 Rf	18.0 AU	1.36%	0.85 Rf	2.1 AU	289.9 AU	0.95%	unknown *

Sample Preparation- 10mg/ml
Application-Linomat 5 Applicator (Camag)
Volume applied-10 µl
Solvent System-Toluene:Ethyl acetate:Formic acid (2.5:1:1)
TLC plate Development-Presaturated Camag Twin Trough Chamber

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