

STANDARDIZATION OF STEM-BARK OF DENDROPHTHOE FALCATE LINN

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ABSTRACT

Dendrophthoe Falcate Linn belonging to family Loranthaceae is an evergreen parasitic plant grown on different host plant throughout India. Present investigation deals with Macroscopic, Microscopic, Physico-chemical investigation, Optimization of extraction conditions, Heavy metal analysis and Microbiological assay of stem-bark of *Dendrophthoe Falcate Linn*. Screening of physico-chemical revealed the determination of foreign organic matter, ethanol extractable matter, water extractable matter, total ash, acid insoluble ash, water soluble ash, loss on drying, and moisture content in *Dendrophthoe Falcate Linn*. The percentages of foreign organic matter, ethanol extractable matter, water extractable matter, total ash, acid insoluble ash, water soluble ash, loss on drying, moisture content were of 0.032%-0.040%, 21.95%-23.42%, 29.66%-30.13%, 3.17%-3.23%, 0.85%-0.95%, 2.28%-2.34%, 8.16%-8.86%, 6.23%-6.66% respectively. In the present work different solvents, from non-polar to polar, were used to optimize the extractive values of *Dendrophthoe falcate Linn*.

The results obtained from this study can be used to prepare a monograph for the proper identification of the *Dendrophthoe Falcate Linn*. plant.

Keywords: *Dendrophthoe Falcate Linn*, Parasitic plant, Physico-chemical parameters, Optimization of extraction condition, Heavy metal analysis, Microbiological assay.

INTRODUCTION

Dendrophthoe falcate Linn. is genus of evergreen, shrubby, parasite that is distributed in the tropical and sub-tropical regions of the old world.

Dendrophthoe falcate Linn. (Loranthaceae) is an important plant in Indian system of medicine. It is a parasitic shrub that grows on a variety of host plants namely mango, jack and other trees. The plant has been used as an aphrodisiac, astrigent, narcotic and diuretic¹.

Dendrophthoe falcate Linn has been used traditionally in the treatment of pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesical calculi and vitiated conditions of kapha and pitta¹.

In Nepal, the leaf along with *Urtica doica* is made into a paste and used to treat bone fractures.

Members of genus *Dendrophthoe* are reported to have anti-oxidant, anti-microbial, anticancer, antidiabetic², anti-lithiatic and antihypertensive³ properties.

Chemically the plant has been found to be rich in phenols and flavonoids; catechins, gallic acid, ellagic acid, chebulinic acid, quercetin⁴, kaempferol, rutin and quercetrin⁵.

Therefore the study was undertaken to investigate Macroscopic, Microscopic, Physico-chemical parameters, Optimization of extraction conditions, Heavy metal analysis and Antimicrobial study of stem-bark of *Dendrophthoe Falcate Linn.* parasite on *Mangifera indica* (Anacardiaceae).

MATERIALS AND METHODS

Collection of plant material and authentication

The stem-bark of *Dendrophthoe falcate* (Loranthaceae) a parasite on *Mangifera indica*, (Anacardiaceae) was collected in November and December from Maharashtra (India) and were positively identified and confirmed by the botanist Mrs. Anjali, Department of Botany, K.J Somaiya College of Science and Commerce. A voucher specimen has been deposited in the herbarium of the botany department of K.J Somaiya College of Science and Commerce. The fresh plant material and young stem were used for the study of macroscopic and microscopic characters, whereas the dried uniform stem-bark powder was used for the Physico-chemical parameters,

Optimization of extraction conditions, Heavy metal analysis and Antimicrobial study investigation.

Pharmacognostic studies

Macroscopic studies

The morphological studies were done by naked eyes. The shape, apex, base, margin, and size of the *Dendrophthoe falcate* and its part were determined.

Microscopic studies

Microscopic studies were done by preparing thin hand section of young stem of *Dendrophthoe Falcate Linn.* The section was stained with safranin and mounted with glycerin.

Physico-chemical investigation

Powder of stem-bark of *Dendrophthoe falcate Linn* were subjected to physicochemical study for determination of foreign organic matter, extractive value, ash value, loss on drying and moisture content using the method described by Indian Herbal Pharmacopeia and United State Pharmacopeia. The results are presented in Table 1.

Optimization of extraction conditions for various solvents

1) Optimization of amount of solvent and time of extraction

In this experiment, the amount of *Dendrophthoe falcate* stem bark powder taken was kept constant throughout the experiment. In different sets of stoppered conical flasks, accurately weighed *Dendrophthoe falcate* powder was taken. In these different sets of stoppered conical flasks different amounts of different solvents were added and kept for 1.0, 2.0, 3.0, 4.0 hours. Then these solvents were filtered through Whatmann filter paper (No. 41) in pre-weighed dry beakers separately and solvents were evaporated on a water-bath to dryness. The dried residues were then weighed separately and the percentage extractions were calculated. The percentage extraction values the volume of solvent was optimized.

2) Optimization of number of extractions

For optimization of the number of extractions, the optimized amount of the selected solvent was added to the sample in different sets of stoppered conical flasks and these flasks were kept aside for the optimized time. Then the contents of the stoppered conical flasks were filtered separately through Whatmann filter paper (no. 41) in pre-weighed dry beakers. The residues were again taken in a flask

and extracted again using the optimized solvent and time. The above procedure was repeated and the percentage extraction values were calculated. From these values the numbers of extractions were optimized.

Heavy metal analysis

The concentration of seven heavy metals viz., Ni, Cu, Zn, Pb, Fe, Cd and Cr in *Dendrophthoe Falcate Linn.* using AAS technique has been studied. For this 500mg of the plant powder of *Dendrophthoe Falcate Linn.* was accurately weighed and transferred in to a conical flask. 13cm³ of concentrated HNO₃ was added to the flask and heated on a sand bath till all brown fumes ceased. Ones the brown fumes were ceased the solution was kept for cooling. After cooling solution was transferred to the 25cm³ of standard volumetric flask and diluted up to the mark with distilled water followed by the filtration with Whatmann filter paper 41 and a filtrate is used as a sample solution whereas accurately measured 13cm³ of concentrated HNO₃ was heated on a sand bath till all brown fumes cease, ones the brown fumes are cease the solution is kept for cooling. After cooling solution is transferred to the 25cm³ of standard volumetric flask and diluted up to the mark with distilled water followed by the filtration with Whatmann filter paper 41 and a filtrate is used as a blank.

Microbiological activity

In present investigation an attempt was made to test the antimicrobial activity of *Dendrophthoe Falcate Linn.* using Disc Diffusion method^{6,7,8}.

The test organisms used for the antimicrobial study for *Dendrophthoe Falcate Linn.* were Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi. These organisms were maintained on the nutrient agar and sabouraud's agar slants.

The ability of the test substances to inhibit bacterial growth is indicated by the appearance of a zone of inhibition around the disc containing the test solution. After specified incubation period, the agar plates were examined for growth. First the positive control without *Dendrophthoe Falcate Linn.* extract was checked to ensure that each test strain was capable of providing adequate growth. The negative control was checked for the absence of growth thereby indicating the sterility of the medium. The remaining plates were examined for the presence or absence of growth. In reading the end points a faint haze of growth of a single colony was evident for anti microbial activity. A dense film of growth or more than one colony was considered as evidence that the plant extract failed to inhibit the growth. The zone of inhibition was measured by using vernier calipers.

RESULTS AND DISCUSSION

The plant of *Dendrophthoe falcate Linn* were observed to be large, bushy, evergreen, branched hemiparasite with smooth dark grey bark, young parts glabrous or nearly so. Leaves are thick, coriaceous, and highly variable in size and shape and opposite, most often ovate-oblong, 7.5 to 20cm long and 2 to 10cm wide, apex and base usually obtuse, margins often minutely white; petioles 0 to 13mm long. Flower borne in short, spreading axillary racemes; pedicels short; bract 1.6mm long; broadly ovate, concave; calyx 4mm, more or less tomentose, tube cylindrical, limb capular, truncate or shortly toothed.(Fig. 1)



Fig. 1: Twig of plant



Fig. 1(a): Flowers



Fig. 1(b): Leaves

A T.S of stem of *Dendrophthoe Falcate Linn* is circular in outline. The outer cork consist of a few layers of brown, irregular parenchymatous cells, the inner cork is made up of few layers of radially arranged in regular rows of lignified parenchymatous cells. Cortex consisting of many layers of tangentially elongated and rounded cells interspersed with a well developed belts of sclereids in group of 2 to 4; many cells of cortex, specially those of outer few layers contain tannins ranging in colour from yellow, orange to dark brown; groups of pericycle fibers appear outside phloem; phloem

seen in several thin patches around the well developed xylem. Xylem is traversed regularly by 1 to 4 seriat radially elongated lignified medulary ray cells and consists of well developed vessels, xylem fibers, tracheids and xylem parenchyma. Modularly ray cells are interrupted by small groups of sclereids, pith occupies the central part of stem, consists of thin walled, rounded or polygonal lignified parenchymatous cells, small groups of sclereids also seen in this region, prismatic crystals present in association with sclereids and medulary ray cells. Fig.2.

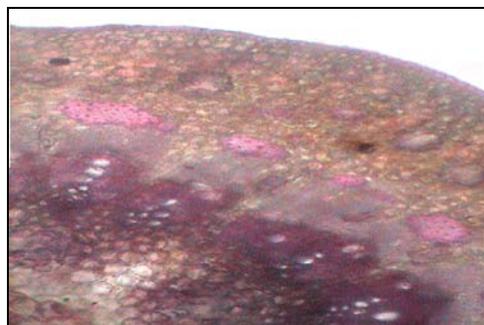


Fig. 2: T.S of Young Stem of *Dendrophthoe Falcate Linn*

Physico-chemical investigation of stem-bark of *Dendrophthoe Falcate Linn* shows the following results (Table 1)

Table 1: Results of physicochemical parameters of stem-bark of *Dendrophthoe Falcate*

Sr.No	Parameters	% Content
1	Foreign organic matter	0.036
2	Ethanol soluble extractive	22.86
3	Water soluble extractive	29.90
4	Total ash	3.19
5	Acid-insoluble ash	0.90
6	Water soluble ash	2.31
7	Loss on drying	8.42
8	Moisture content	6.43

The amount of solvent added and time for extraction and corresponding percentage extraction are given in Table 2, Table 3, Table 4 and Table 5. Graphical representation for these results is given in Fig. 3, Fig. 4, Fig. 5 and Fig. 6.

Table 2: Optimization of amount of solvent and time for extraction of *Dendrophthoe Falcate* (Methanol)

Solvent	Wt. of sample in gm	Volume of sample cm ³	% Extract with respect to time in hours			
			1 Hour	2 Hour	3 Hour	4 Hour
Methanol	2	25	7.76	9.30	9.70	28.20
	2	50	13.56	27.12	30.65	29.04
	2	75	18.77	25.55	31.63	28.24
	2	100	25.03	27.20	31.50	29.04
	2	125	31.29	29.75	32.70	30.20

Each observation is mean of three readings.

The above results can be summarized as follows by graphical representation.

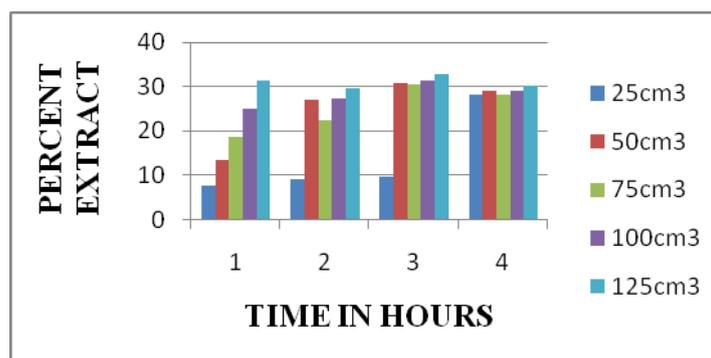


Fig. 3: Optimization of amount of solvent and time for extraction of *Dendrophthoe Falcate* (Methanol)

Table 3: Optimization of amount of solvent and time for extraction of Dendrophthoe Falcate (Chloroform)

Solvent	Wt. of Sample in gm	Volume of sample cm ³	% Extract with respect to time in hours			
			1 Hour	2 Hour	3 Hour	4 Hour
Chloroform	2	25	0.405	0.87	1.22	2.60
	2	50	0.81	1.58	2.5	6.24
	2	75	1.215	2.43	3.64	6.30
	2	100	1.62	3.25	4.84	6.45
	2	125	1.50	3.18	4.5	5.80

Each observation is mean of three readings.

The above results can be summarized as follows by graphical representation.

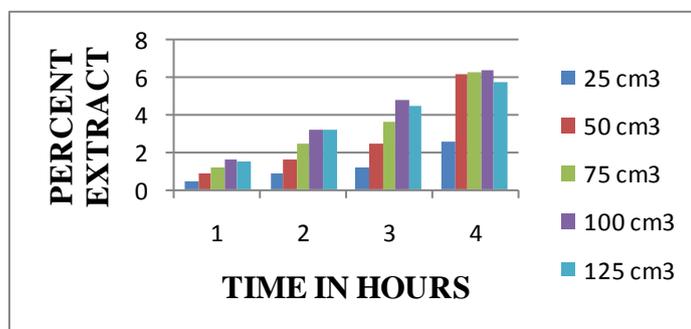


Fig. 4: Optimization of amount of solvent and time for extraction of Dendrophthoe Falcate (Chloroform)

Table 4: Optimization of amount of solvent and time for extraction of Dendrophthoe Falcate (Pet-Ether)

Solvent	Wt. of Sample in gm	Volume of sample cm ³	% Extract with respect to time in hours			
			1 Hour	2 Hour	3 Hour	4 Hour
Pet-Ether	2	25	0.10	0.20	0.22	0.25
	2	50	0.20	0.44	0.60	1.75
	2	75	0.28	0.56	1.20	1.76
	2	100	0.37	0.75	1.11	1.69
	2	125	0.46	0.90	1.34	1.79

Each observation is mean of three readings.

The above results can be summarized as follows by graphical representation.

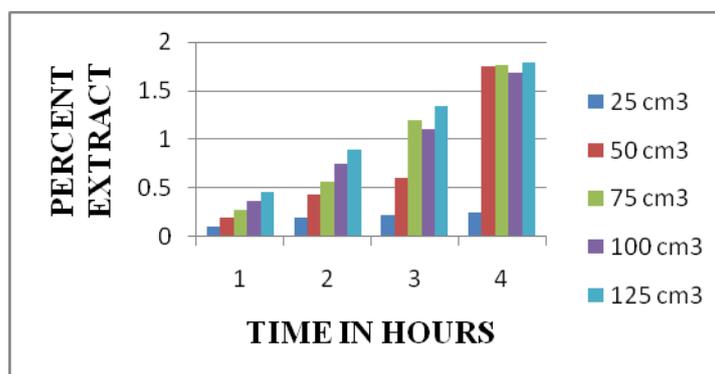


Fig. 5: Optimization of amount of solvent and time for extraction of Dendrophthoe Falcate (Pet-Ether)

Table 5: Optimization of amount of solvent and time for extraction of Dendrophthoe Falcate (Water)

Solvent	Wt. of sample in gm	Volume of sample cm ³	% Extract with respect to time in hours			
			1 Hour	2 Hour	3 Hour	4 Hour
Water	2	25	14	16	16	18
	2	50	15	20	22	22.6
	2	75	19	23	23	22.5
	2	100	22	22.5	24	22.8
	2	125	21	22	25.52	22.7

Each observation is mean of three readings.

The above results can be summarized as follows by graphical representation.

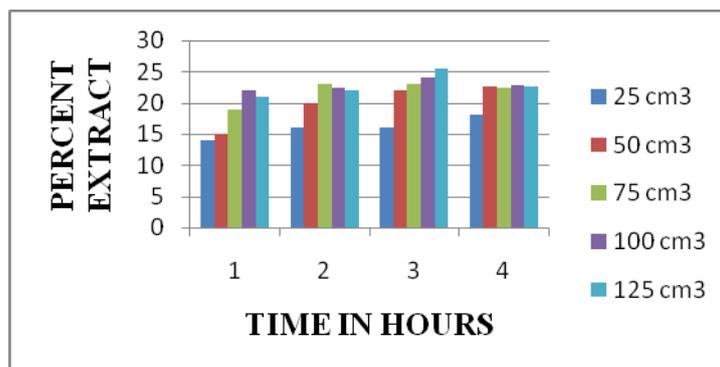


Fig. 6: Optimization of amount of solvent and time for extraction of Dendrophthoe Falcate (Water)

From the graph of number of extractions versus percentage extraction as shown in Fig. 7, it was observed that the percentage extraction levels after certain number of extraction remains

constant. The number of extractions and the corresponding percentage extraction are given in Table 6. The optimized extraction conditions for various solvents are given in Table 7.

Table 6: Optimization of number of extractions for extraction of Dendrophthoe Falcate Linn

Solvent	Wt. of sample	% Extract with respect to number of extractions			
		1	2	3	4
Methanol	2	34	35.63	35.63	35.63
Chloroform	2	5	6	7.28	7.28
Pet-Ether	2	1	1.5	1.98	1.98
Water	2	26	28.82	28.82	28

Each observation is mean of three readings.

The above results can be summarized as follows by graphical representation.

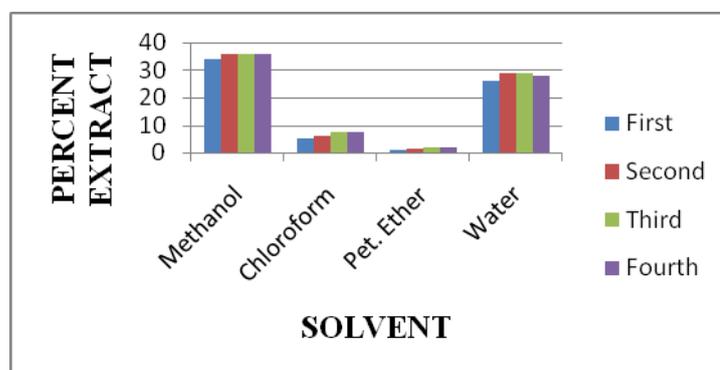


Fig. 7: Optimization of number of extractions for extraction of Dendrophthoe Falcate Linn

Table 7: Optimized extraction conditions for different solvents for Dendrophthoe Falcate Linn

Sr. no	Solvent	Optimized Extraction Conditions		
		Amount of Solvent in cm ³	Time in hours	Number of extractions
1	Methanol	125	3	2
2	Chloroform	100	4	3
3	Pet. Ether	125	4	3
4	Water	125	3	2

Among the seven metals analyzed in whole plant powder of *Dendrophthoe Falcate Linn*, the concentration of Fe was found to be maximum, whereas that of Cd was found to be minimum (Table 8). Thus, on the basis of the current study there is no precise evidence

to indicate pollution-related accumulation of heavy metals in this plant. It may suggest that the obtained levels could be related to the normal physiology of the plant.

Table 8: Metal concentration in ppm in *DENDROPTHOE FALCATE Linn*

Metals	Concentration (ppm)
Ni	0.0843
Cu	0.0767
Zn	0.0793
Pb	0.042
Fe	0.0975
Cd	0.0011
Cr	0.0137

Solvents of varying polarity were used in preparing the extracts of *Dendrophthoe Falcate Linn*. These extracts showed varying effects on the test organism (Table 9 and Table 10).

There seems to be an antimicrobial compound present in *Dendrophthoe Falcate Linn* powdered seed responsible for its antimicrobial action. More research has to carry out on seed powder to check the activity of the plant⁹.

Table 9: Antimicrobial activity of *Dendrophthoe falcate Linn*. against gram positive organisms

Parts Used	Organisms	Methanol	Chloroform	n-hexane	Acetonitrile
Stem	S.aureus	-	-	-	-
Leaves		-	-	-	-
Flowers		-	-	11mm	-
Stem	B.subtilis	7mm	-	8mm	-
Leaves		6mm	7mm	8mm	-
Flowers		-	-	12mm	-

Key '-' = No growth

Zone of inhibition (mm) includes zone diameter of disc = 5mm

Table 10: Antimicrobial activity of *Dendrophthoe falcate Linn*. against gram negative organisms

Parts Used	Organisms	Methanol	Chloroform	n-hexane	Acetonitrile
Stem	E.coli	-	-	-	-
Leaves		-	-	-	-
Flowers		-	-	10mm	-
Stem	S.typhi	-	-	-	-
Leaves		-	-	-	-
Flowers		-	-	7mm	-

Key '-' = No growth

Zone of inhibition (mm) includes zone diameter of disc = 5mm

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