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Pharmacognostic and preliminary phytochemical screening of ficus arnottiana miq.

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ABSTRACT

Objective: The study is aimed at development of physicochemical parameters and to investigate the active principle present in *Ficus arnottiana* Miq. (Moraceae). The fruits, leaves and bark are used in medicine. This is used as astringent, demulcent and emollient, it is also used in diabetics, wound healing and inflammation. From extensive literature survey it was revealed that no reports were available on microscopic evaluation, standardization parameters of *Ficus arnottiana* Miq.

Materials & Methods: The present work comprises the investigations carried out to establish methods for quality control of drugs, botanical evaluation which comprises of macroscopy, physicochemical parameters like loss on drying, extractive values, Ash values and to investigate the phytochemicals present in the extracts in the preliminary level with respect to thin layer chromatography were also carried out for the quality control of the drug.

Results: Thus it was thought worthwhile to explore this endangered plant on the basis of its standardization parameters. The study will provide referential information for the correct identification of the crude drug.

Conclusion: These physicochemical data and phytochemical analysis of different extracts of *Ficus arnottiana* Miq. in ethanolic extract and chloroform extract is useful for further studies of Pharmacological parameters.

Keywords: *Ficus arnottiana* Miq. Moraceae, Preliminary phytochemical screening, TLC, Rf value. Total extractive value. Moisture content. Ash value.

1. INTRODUCTION

Ficus arnottiana Miq. is a glabrous tree belonging to family Moraceae also known as paras pipal. It is widely distributed in India. The leaves of the plant are used for controlling fertility. The bark of *Ficus arnottiana* Miq. is used as astringent, demulcent and emollient, it is also used in diabetic, wounds and inflammation as per Kirtikar et.al. Though the plant and its extracts have been used in the folk medicine extensively, but no scientific evidence for such activities is available in established scientific journals of repute.

2. MATERIALS AND METHODS

The bark of *Ficus arnottiana* Miq. Was collected from Dehra dun (U.K.), India and identified by the Botanist Dr. Zea Ul Hasan, Department of botany, Saifia Science College (Barkatulla University) Bhopal (M.P.) and a voucher specimen of plant (No.276/Bot/Safia/2011) has been deposited in herbarium for further reference. It belongs to the family Moraceae.

The bark is separately dried in shade and preserved in air tight container. The coarsely powdered dried bark was used for the phytochemical screening and physicochemical evaluation. The physical constants like Ash value, Alcohol and water soluble extractive value, Moisture content were also determined. The bark was extracted with petroleum ether, chloroform, ethanol and water. The extracts were subjected to percentage yield calculation and phytochemical screening.

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2.1 Pharmacognostical studies

Morphological studies were carried out by using simple determination technique, shape, size, colour, odour of the leaf.

2.2 Physicochemical parameters

The parameter was done to evaluate the percentage of total ash as per Indian Pharmacopoeia. Extract of the powdered leaf was prepared with different solvents for the study of extractive value.

2.3 Preliminary phytochemical Analysis

For preliminary phytochemical analysis, extract was prepared by weighing and the dried powdered bark and was subjected to hot successive continuous extraction with different solvents as per the polarity petroleum ether, chloroform, ethanol and finally with water. The extracts were filtered in each step, concentrated and the solvent was removed by vacuum distillation. The extracts were dried over desiccators and the residues were weighed. The presence or absences of the primary and secondary phytoconstituents were detected by usual chemical testing methods as per Harbone, JB.et.al.& Oguymi, A.O.et.al.

3. Ash value

Dried leaves were incinerated to determine the ash content as per Kokate C.et.al.

3.1 Extractive values

3.1.1 Alcohol soluble extractive value

Accurately weighed 5 gm coarse and air dried drug material was macerated with 100ml ethanol (99%) in a stoppered flask for 24 hrs. with frequent shaking for 6 hrs. It was then filtered rapidly through filter paper taking precautions to prevent excessive loss of ethanol. The volume was made up to 100ml with ethanol. The residue was evaporated in a flat bottom shallow dish, dried at 105 °C, weighed and kept in a desiccators. Average extractive value in percentage w/w (on dry basis) was calculated with reference to air dried drug (Table-2).

3.1.2 Water soluble extractive value

5 gm coarse and air dried drug material was macerated with water in a stoppered flask for 24 hrs. with frequent shaking for first 6 hrs. The extract was filtered rapidly through filter paper taking precaution to prevent excessive loss of solvent. The residue was evaporated in a flat bottom shallow dish, dried at 105 °C weighed and kept in a desiccators. Average extractive value in

Percentage w/w (on dry weight basis) was calculated with reference to air dried drug (Table-2).

3.2 Determination of total phenolics by spectral analysis

Phenolic substances all absorb UV light, and all of them have some absorbance at 280 nm. This property can be used to determine phenolics by spectral analysis. One problem with this method is that each class of phenolic substances has a different absorptivity (extinction coefficient, ϵ) at 280 nm. Thus, the results cannot be related to any specific standard and are reported directly in absorbance units (AU).

The value of this method as per Andrew L.et.al. is that it is extremely simple and rapid, requiring only filtration and, in some cases, dilution.

Materials

Sample.

Filter membrane.

Cuvettes, transparent at 280 nm (e.g., quartz)

Spectrophotometer, set to 280 nm

1. Filter a sample or blank (deionized or distilled water) with a PTFE filter membrane or other material to achieve clarity. Nylon or other membranes that absorb phenolics should not be used. Membranes can be tested for phenolic absorption by comparing absorbance after single and double filtration.
2. Transfer an appropriate volume of sample to a quartz cuvette and measure absorbance at 280 nm in a spectrophotometer. If absorbance is not within the acceptable precision of the spectrophotometer (usually $A < 2$ AU), dilute sample as necessary and repeat.
3. Subtract absorbance of blank, and correct absorbance to original concentration and a 1-cm cuvette path length. Subtract 4 AU to report final value.

For instance, if a sample is diluted ten-fold with water and a reading of 0.85 AU is observed with a 2-mm cell, the correction would be as follows:

$$\text{Total phenol} = [A_{280} \times DF \times (1 \text{ cm}/b)] - 4$$

where DF is the dilution factor, b is the cell path length, and 4 is an arbitrary correction for non phenolic absorbance. result in **Table 3**

4. Phytochemical Screening

The fresh bark was collected and dried in shade and reduced to coarse powder. The powdered material was extracted with Petroleum ether, Chloroform, Ethanol and water in Soxhlet apparatus. The extract was filtered hot and solvent removed by distillation under reduced pressure as per Khandelwal Dr. K.R.et.al. The percentage yield was calculated and the extract was further subjected to Phytochemical tests for Alkaloids, Glycosides, Flavonoids, Carbohydrates, Tannins (Table-4).

5. Results and Discussion

Moisture content and Ash value are given in **Table-1(a and b)**. Extractive values of alcohol and water extracts are given in **Table-2**. Phytochemical screening shows the presence of carbohydrates, glycosides, Tannins and absence of alkaloids and flavanoids **Table-4**. R_f value given for pet. Ether in **Table-5**, chloroform in **Table-6**, ethanol **Table-7** and water **Table-8**.

Table: 1a Evaluation of bark of *Ficus arnottiana* Miq.

S/No.	Parameters	Bark
1.	Ash value	38

Table-1b Moisture content test by using Carl-Fisher Reagent:

S.No.	Extract	Quantity taken	Moistur content
1.	Pet. Ether	10mg	Nil
2.	Chloroform	10mg	Nil
3.	Ethanol	10mg	Nil
4.	Aqueous	10mg	1%

Table: 2 Extractive values of bark of *Ficus arnottiana* Miq.

S/No.	Solvent used	Average extractive value in % w/w on dry weight basis
1.	Ethanol	18
2.	(Absolute) Water	22

Table: 3 Phenolics content values of bark of *Ficus arnottiana* Miq.

S.No.	Extract	Phenolics content (AU)
1	Pet. ether	Absence
2	Chloroform	451
3	Ethanol	263.5
4	Aqueous	418.5

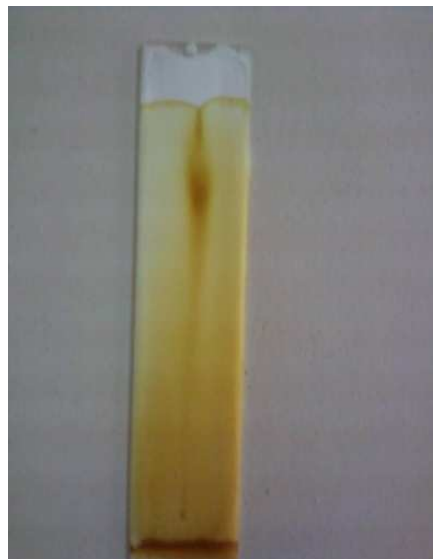


Fig.1 Pet. Ether extract.



Fig.2 Chloroform extract.



Fig.3 Ethanolic extract.



Fig.4 Aqueous extract.

The *Ficus arnottiana* Miq, bark was defatted with Petroleum ether then extracted with Chloroform and after that with ethanol solvent and Water in soxhlet apparatus.

Moisture content is zero in pet ether & chloroform, ethanol extract and 1% in aqueous extract found out by Karl Fischer Reagent. Extractive values of absolute alcohol is 18% and water is 22%. The total Ash value of the bark is 38.

Phytochemical Screening shows presence of carbohydrate in pet ether and chloroform extract extracts while absence in ethanol and aqueous extract, fats and oils are positive in chloroform extract absence in other extract Steroids, Glycosides, Saponins and alkaloids are present strongly in ethanol extract and chloroform extract while marginally in aqueous extract. These are not found in pet ether extract phenolic, tannin, resin compounds are strong l positive in ethanol extract. Thin Layer Chromatography of Pet.Ether, chloroform, ethanol & aqueous extracts have been performed in different solvent system of varying degree of polarity using silicagel G of TLC grade. In pet. ether and aqueous extract only one compounds are extracted while in Chloroform extract four compounds have been extracted and ethanol extract three compound have been extracted. This shows that chemical constituents of *Ficus arnottiana* Miq. is extractable in semi polar solvent ethanol extract.

6. Conclusion

Effective formulations have to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials. The manufacture of plant products should be governed by standards of safety and efficacy. So finally we concluded that these physicochemical data and phytochemical analysis of different extracts of *Ficus arnottiana* Miq in ethanolic extract and chloroform extract is useful for further studies of Pharmacological parameters.

Table- 4 Phytochemical screening of *Ficus arnottiana* Miq. :

S.No.	CHEMICAL TEST	PET. ETHER ¹	CHLOROFORM	ETHANOL	WATER
1.	CARBOHYDRATE				
A	Molish test	-	-	+	++
B	Fehling test	-	-	-	-
C	Pholoroglucinol test	-	-	-	-
D	Tollen's test	-	-	-	+
E	Cobalt chloride	+	+	-	-
F	Iodine test	+	+	-	-
G	Tannic acid test	+	++	-	-
2.	PROTEIN				
A	Biuret test	++	+	-	-
B	Millon's test	-	-	-	-
C	Xanthoprotic test	+	+	-	-
3.	AMINO ACID				
A	Nihydrin test	-	-	-	-
B	Tyrosine test	-	-	-	-
4.	FATS AND OILS				
A	Filter paper test	-	+	-	-
5.	STEROID				
A	Salkowski reaction	-	-	-	-
B	Liebermann-Burchard reaction	-	-	-	-
C	Liebermann's reaction	-	-	-	-
6.	GLYCOSIDES				
A	Cardiac glycoside				
A	Legal's test	-	-	-	-
B	Keller-Killani test	-	-	-	-
B	Anthraquinone glucoside				
A	Borntrager's test	-	-	+	+
B	Modified Borntrager's test	-	-	-	-
C	Saponin glycoside				
A	Foam test	-	+	+	++
D	Flavonoids				
A	Shinoda test	-	+	+	+
B	Lead acetate test	-	-	+	+
7.	ALKALOIDS				
A	Dragendorff's test	-	++	+	-
B	Mayer's test	-	++	+	-
C	Wagner's test	+	-	-	+
8.	PHENOLIC COMPOUNDS				
A	5% FeCl ₃ solution	-	-	++	+
B	Lead acetate test	-	-	+	+
C	Acetic acid solution	-	-	+	++
9.	TANNINS				
A	Vanilline-HCl test	-	+	++	+
B	Gelatine	-	-	-	-
10.	RESINS				
A	FeCl ₃ test	-	-	++	+
B	Turbidity test	-	-	-	-

Key: strongly present ++**Present +****Absent □**

Rf value determination: Rf value calculated using different solvent system for different extracts of *Ficus arnottiana* Miq. by TLC.

Table 5 Rf Values for Petroleum ether extract by TLC:

S.No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	R _f Value
1.	Chloroform:Methanol (7:3)	5.5	1	5.4	0.98
2.	Methanol:Benzene (5:5)	5.2	-	-	-
3.	Benzene:Ethyl acetate (9:1)	5	-	-	-
4.	Benzene:Ethyl acetate (5:5)	5.9	1	5.7	0.96
5.	n-Butanol:Acetic acid (5:4)	5.5	1	5.3	0.96

Table-6 Rf Values for Chloroform extract by TLC

S. No.	Solvent system	Solvent front height (cm)	No.of spots	Spot height (cm)	R _f Value
1.	Chloroform:Methanol (7:3)	5.4	4	1.5, 3.6, 3.7, 5.3,	0.27, 0.66, 0.68, 0.98
2.	Methanol:Benzene (5:5)	5.7	2	4.2, 5.6	0.73, 0.98
3.	Benzene:Ethyl acetate (9:1)	5.7	3	4.3, 2.8, 2.3	0.75, 0.49, 0.40
4.	Benzene:Ethyl acetate (5:5)	5.6	2	1.1, 2.1	0.19, 0.37
5.	n-Butanol:Acetic acid (5:4)	6.0	1	5.1	0.85

Table-7 Rf Values for Ethanolic extract by TLC

S. No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	R _f Value
1.	Chloroform:Methanol (7:3)	5.7	2	0.9, 5.2	0.15
2.	Methanol:Benzene (5:5)	6	1	5.9	0.98
3.	Benzene:Ethyl acetate (9:1)	5.8	-	-	-
4.	Benzene:Ethyl acetate (5:5)	6.0	3	1.5, 2.1, 2.5	0.25, 0.35, 0.41
5.	n-Butanol:Acetic acid (5:4)	6.0	1	4.2	0.7

Table-8 R_f Values for Aqueous extract by TLC

S. No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	R _f Value
1.	Chloroform:Methanol (7:3)	5.7	1	1.3	0.22
2.	Methanol:Benzene (5:5)	5.6	-	-	-
3.	Benzene:Ethyl acetate (9:1)	5.7	-	-	-
4.	Benzene:Ethyl acetate (5:5)	5.5	-	-	-
5.	n-Butanol:Acetic acid (5:4)	6.0	1	5.2	0.86

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