**Journal of Cardiovascular Disease Research**

**ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022**

**RESEARCH ARTICLE**

# Preliminary Phytochemical Screening of Leaves of *Ficus arnottiana Miq.*

**Vinay Kumar Gupta*[1], Manoj Gahlot[2], Asha Pathak[3], Chandra Veer Singh[3], Alok Dixit[3]**

1) Lecturer, Department of Pharmacology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India.
2) Professor, Department of Pharmaceutical Sciences, Shri Guru Ram Rai Univgersity, Patel Nagar, Dehradun, Uttarakhand, India.
3) Professor, Department of Pharmacology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India.

**Corresponding Author[1*]:** Dr. Vinay Kymar Gupta

**Article submitted on: 02/01/2022| Article revised on: 17/01/2022| Article accepted on: 02/02/2022**

## ABSTRACT

**Introduction:**

*Ficus arnottiana* belonging to family *Moraceae* is commonly known as the Indian rock fig and has also other common names like Paras pipal in Hindi, as Crown (Ceylon) in English, as Kallal in Malyali, as Pipli in Marathi, as Kallaravi in Telugu and as Parisah and Plaksha in Sanskrit. It is widely distributed in India. Bark and leaves extract of this plant is being used in the traditional medicine as astringent, aphrodisiac, demulcent, depurative and emollient. It is also useful against inflammation, diarrhoea, diabetes, burning sensation, leprosy, scabies, wounds and skin diseases.

**Material and Method:**

The extraction was done using soxhlet apparatus. 500 gm shade dried leaves of both plants were taken and crushed to powder. The powdered material was subjected to soxhlet extraction with various solvents ranging from non- polar to polar. The solvents used in were petroleum ether, chloroform, acetone and methanol. The qualitative chemical tests for various phytoconstituents were carried out for all the extracts. TLC profiling of all extracts (Pet-Ether extract, Chloroform extract, Acetone extract and Methanol extract) were carried out by attempting with several solvent systems.

**Result:**

The TLC of all fractions was carried out and $R_f$ values of major spots were calculated. The percentage yield of all fractions with respect to acetone extract reveals maximum for Ethyl acetate fraction (26%) followed by *n*-butanol fraction (22%) and hexane fraction (20%). Least percentage yield among all fractions was found in chloroform fraction (18%).

**Discussion:**

The results of qualitative phytochemical analysis showed presence of flavonoids, phenolic compounds, tannins, alkaloids, glycosides and carbohydrates in a good entity in acetone extract of *F. arnottiana*.

**Key words:** Preliminary phytochemical screening, extract, TLC, Rf value.

ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022

**INTRODUCTION:**

Medicinal plants have been used for mankind for its therapeutic values since the beginning of human civilization. From thousands of years, nature has been a source of medicinal agents and significant number of modern drugs have been researched and isolated from natural sources. In traditional medicine, many of these isolations were based on the uses of medicinal agents.

In the recent years, there is revival of interest in the traditional system of medicine, where medicinal plants are major source of biodynamic compounds of therapeutic values. The knowledge of traditional medicinal plants is the fundamental basis for the selection of various plants species for further phytochemical, pharmacological, toxicological and ecological studies. If man starts living with nature then health will not be a problem.[1]

The genus *Ficus* is exceptionally large pantropical genus with over 700 species[2] of freestanding trees, hemi-epiphytes and shrubs primarily occurring in subtropical and tropical regions world-wide and belongs to the family *Moraceae*. It is retained as a single large genus because it is well defined by its unique reproductive system, involving *Syconia* fig and specialized pollinator wasps.[3]

*F. arnottiana* belonging to family Moraceae is commonly known as the Indian rock fig and has also other common names like Paras pipal in Hindi, as Crown (Ceylon) in English, as Kallal in Malyali, as Pipli in Marathi, as Kallaravi in Telugu and as Parisah and Plaksha in Sanskrit. It is widely distributed in India. It has synonyms like *F. aegrophylla*, *F. caulobotrya*, *F. populeaster*, *F. populifolia*, *Urostigma arnottianum*, *Urostigma caulobotryum*, *Urostigma cordifolium* and *Urostigma courtallense*. Indian Rock Fig is a tree which is commonly mistaken for Peepal (*Ficus religiosa*).

Leaves are typical peepal like, but with wavy margins. One of the common ways of recognizing *F. arrnottiana* from *F. religiosa* is to examine the colour of the leaf-stalk and the veins which are bright Pink to red in colour. The leaf tip of *F. religiosa* are tapering, acuminate and long as against the leaf tip of *F. arnottiana* which are pointed and acuminate but not long.

The tree grows abundantly throughout India, mostly in the rocky hills up to an elevation of about 1350 m. It is also wide spread in Sri Lanka.[4-5] Paras pipal is a glabrous tree or shrub without aerial roots, reaching up to 20 m in height; leaves subcoriaceous 5-15 cm., broadly ovate, alternate, narrowed upwards to the shortly caudate-acuminate apex, with entire margins, base usually cordate; bark pale, smooth; petioles 5-15 cm; lamina simple approximately heart shaped with broadly ovate base and shortly caudate-acuminate apex; fruit achenes. [4-5]

*F.arnottiana* Miq. grows wild in the forests of Dehradun district of Uttarakhand mainly on rocks. Natural regeneration is done by seed. It grows on rocks, chiefly on dry rocks, inside shoals, sometimes grows on tress as an epiphyte wild. Bark and leaves extract of this plant is being used in the traditional medicine as astringent, aphrodisiac, demulcent, depurative and emollient. It is also useful against inflammation, diarrhoea, diabetes, burning sensation, leprosy, scabies, wounds and skin diseases. Hypoglycemic and antioxidant effect of an isolated compound (Ficanone) from *F. arnottiana* bark has been reported recently.[6]

The fruit of the plant contain β- sitosterol, gluacol acetate, glucose and friedelin.[7] Various phytochemical are present in bark extracts like Sterols, alkaloids, carbohydrates,

ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022

tannins and phenols.[6] Leaves of the plant are used for controlling fertility. The bark of *F. arnottiana* Miq. is used as astringent, demulcent and emollient, it is also used in, wounds and inflammation, diabetes, diarrhoea, burning sensation, pruritis, leprosy, scabies, as ulcer protective and in vaginopathy.[8] The root of the plant is used as astringent.[9]

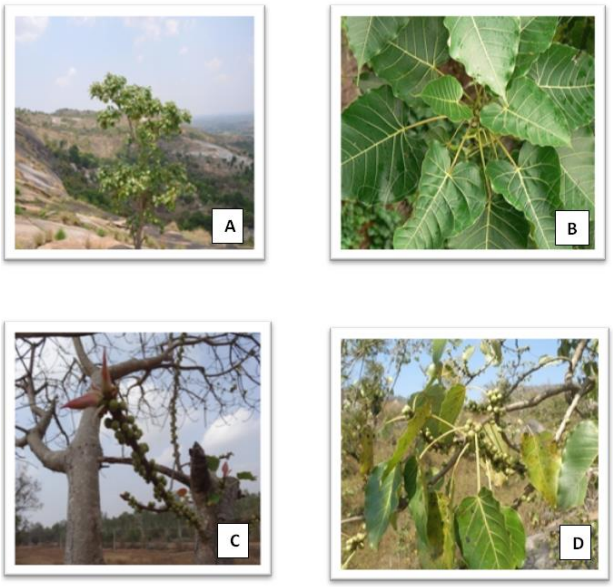


**Figure 1:** *F. arnottiana* Tree (A), Leaves (B), Fruits (C) and (D).

## MATERIALS AND METHODS:

### Plant collection and Authentication

The leaves of *F. arnottiana* were collected from region of Shastra Dhara valley, Dehradun, situated at latitude $30^0$ 17' 19.356" N and longitude $78^0$ 3' 33.904" E, elevation about 3051 ft and identified by Dr. H. C. Pandey (Scientist – D, Botanical Survey of India, Dehradun) and a voucher of specimen of plant (Acc. No. 114133) has been deposited in herbarium of BSI for further reference. The leaves were dried in shade and preserved in air tight container. The coarsely powdered dried leaves were used for the phytochemical screening and physicochemical evaluate- on. The leaves were extracted with petroleum ether, chloroform, methanol and acetone. The extracts were subjected to percentage yield calculation and phytochemical screening.

### Preparation of Plant Extracts

The leaves of *F. arnottiana* was collected and dried in shade. The shade dried leaves were powdered. About 500gm of the dried leaves was extracted by using solvents of increasing polarity starting from Petroleum ether (40- 60ºC), Chloroform, Acetone and Methanol respectively. The extract were filtered and concentrated to dry mass by using vacuum distillation. The percentage yield of all four extracts is illustrated in table-1.

## PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening was performed for all four extracts to identify the phytoconstituents present in the extracts[10] are illustrated in table-2.

### 1. DETECTION OF ALKALOIDS

Small quantity of the extracts were separately treated with a few drops of dilute hydrochloric acid and filtered. The filtrate was treated with various alkaloids reagents such

ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022

as Mayer's reagent, Dragendroff's reagent, Wagner's reagent and Hager's reagent.

**1.1 Wagner's test:** To 1ml of the filtrate few drops of Wagner's reagent were added by the side of the test tube. Reddish brown precipitate if produced indicates the presence of alkaloids.

**1.2 Dragendorff's test:** To 1ml of the filtrate few drops of Dragendroff's reagent were added. Orange red precipitate if produced indicates the presence of alkaloids.

**1.3 Mayer's test:** To 1ml if the filtrate few drops of Mayer's reagent were added. Cream precipitate if produced indicates the presence of alkaloids.

**1.4 Hager's test:** To 1ml of the filtrate few drops of Hager's reagent were added. Yellow precipitate if produced indicating the presence of alkaloids.

## 2. DETECTION OF CARBOHYDRATES

The extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to Molish's, Barfoed's and Benedict's test to detect the presence of carbohydrates.

**2.1 Molish's test:** Filtrate was treated with 2-3 drops of 1% alcoholic alpha-naphthol and 2ml of concentrated sulphuric acid was added along the side of the test tube. Violet colour ring if formed at the junction of two liquids indicates the presence of carbohydrates.

**2.2 Barfoed's Test:** Test solution treated with Barfoed's reagent, on boiling on a water bath showed brick red precipitate.

**2.3 Benedict's test:** Test solution treated with Benedict's reagent and boiling on a water bath showed yellow or reddish brown precipitate.

## 3. DETECTION OF GLYCOSIDES

Small quantities of various extracts were hydrolyzed with conc. hydrochloric acid for 2hrs on a water bath and the hydrolysate was subjected to Legal's and Fehling's test to detect the presence of different glycosides.

**3.1 Legal's Test:** To the hydrolysate extract, 1ml of pyridine and few drops of sodium nitroprusside solution were added then it was made alkaline with 10% sodium hydroxide. Pink to yellow colour if produced showed the presence of glycoside.

**3.2 Fehling's Test:** Dissolve a small portion of the extract in water and treated with Fehling solution 1 and 2 equal volumes, the red precipitate showed presence of reducing sugar.

## 4. DETECTION OF PHENOLIC COMPOUNDS

**4.1 Ferric chloride test:** The extracts were dissolved in water individually and to this a few drops of ferric chloride solution were added. A green precipitate if produced indicates the presence of phenolic compounds.

**4.2 Vanillin Hydrochloric acid Test:** Test solution is treated with few drops of vanillin hydrochloride reagent gives purplish red colour.

ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022

## 5. DETECTION OF STEROLS

Small quantities of extracts were dissolved in 5ml of chloroform separately. Then these chloroform solutions were subjected to Salkowski and Liebermann-Burchard test for the detection of phytosterols.

**5.1 Salkowski test:** To 1ml of the above prepared chloroform solutions few drops of concentrated sulphuric acid was added. The chloroform extract if produces red colour in the lower layer indicates the presence of phytosterols.

**5.2 Liebermann-burchard test:** The above chloroform solution was treated with few drops of concentrated sulphuric acid followed by 1ml of acetic anhydride solution. Green colour if produced only in chloroform extract indicates the presence of phytosterols.

## 6. DETECTION OF FIXED OIL AND FATS

**Spot test:** Small quantities of various extract were separated between two filter papers. If oil stains were produced it indicates the presence of fixed oils.

## 7. DETECTION OF SAPONINS

The extracts were diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. If one-centimetre layer of foam was produced it indicates the presence of saponins.

## 8. DETECTION OF PROTEINS AND AMINO ACIDS

Small quantities of extracts were dissolved in few ml of water separately and they were subjected to Millon's, Biuret and Ninhydrin tests.

**8.1 Millon's test:** The prepared extracts were treated with Millon's reagent. A white colored ppt if produced indicates the presence of proteins.

**8.2 Biuret test:** 2ml of filtrate is treated with one drop of 2% copper sulphate solution. To this 1ml of ethanol was added, followed by excess of Potassium Hydroxide pellets. Pink colour in the ethanolic layer if produced indicates the presence of proteins.

**8.3 Ninhydrin test:** The above extracts were treated with Ninhydrin reagent. A characteristic purple colour if produced indicates the presence of amino acids.

## 9. DETECTION OF GUM AND MUCILAGE

The extract was dissolved in 10ml of distilled water and to this 25ml of absolute alcohol was added with constant stirring. White or cloudy if ppt. produced indicates the presence of gums and mucilage's.

**Table-1: Extractive values of *F. arnottiana* (for about 500gm powdered leaves)**

| Solvents | No. of Hrs (Extraction) | Extractive Value (grams) | % Yield |
|---|---|---|---|
| Petroleum Ether | 15 | 8.28 | 1.66 |
| Chloroform | 10 | 4.18 | 0.84 |
| Acetone | 10 | 16.23 | 3.25 |
| Methanol | 10 | 11.16 | 2.23 |

ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022

**Table-2: Qualitative Chemical Analysis of Extracts of *F. arnottiana***

**(+)** indicates the presence of phytoconstituents qualitatively.
**(-)** indicates the absence of phytoconstituents qualitatively.

| Test Performed | Pet. Ether Extract | Chloroform Extract | Acetone Extract | Methanol Extract |
|---|---|---|---|---|
| **Test for Alkaloids** | | | | |
| Mayer's Test | - | + | + | + |
| Dragendroff Test | - | + | + | + |
| **Test for Glycosides and Carbohydrates** | | | | |
| Fehling Test | - | - | + | + |
| Bendict's Test | - | - | + | + |
| **Test for Phenolic Compounds and Tannins** | | | | |
| Vanillin Hydrochloride Test | - | - | + | + |
| Dilute $FeCl_3$ Test | - | - | + | + |
| **Test for Sterols** | | | | |
| Salkowaski Test | + | - | - | - |
| Libermann Burchard's Test | + | - | - | - |
| **Test for Fixed Oils** | | | | |
| Test for Fixed Oils | + | - | - | - |
| **Test for Saponins** | | | | |
| Test for Saponins | - | - | - | + |
| **Test for Proteins and Amino Acids** | | | | |
| Ninhydrin Test | - | - | - | - |
| Biuret Test | + | + | - | - |
| **Detection of Gums and Mucilage** | | | | |
| Detection of Gums And Mucilage | - | - | - | - |

## CHEMICAL ANALYSIS OF LEAVES OF *F. arnottiana*

Phytochemical Screening shows presence of glycosides, carbohydrates, phenolic compounds and tannins in chloroform, acetone and methanol extract while absence in petroleum ether extract. Alkaloids are present in chloroform, acetone and methanol extract while absent in petroleum ether extract. Sterols and fixed oils are present in petroleum ether extract while absent in chloroform, acetone and methanol extract. Saponins are present in only methanol extract while proteins, amino acids, gums and mucilage are absent in all four extracts.

ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022

**THIN LAYER CHROMATOGRAPHY OF LEAVES OF *F. arnottiana***

TLC performed for all four extracts. TLC plates were coated with silica gel. The dried coated TLC plates were activated at $60^0$ C for 30 minutes and used. The spots of extracts are marked above 1cm and put in TLC chamber having different mobile phases (solvent system) depending upon the type of constituents to be analyzed. After the development of chromatogram *Rf* values of resolved spots were calculated and illustrated in table-3.

**Table-3: Rf values of resolved spots of various extracts of *F. arnottiana***

| **Petroleum Ether Extract** Chloroform (90) : Pet. Ether (10) | **Distance Travelled by Solvent (cm)** | **Distance Travelled by Spot (cm)** | **Rf Value** |
|---|---|---|---|
| Spot A | 16.8 | 8.4 | 0.50 |
| Spot B | 16.8 | 11.6 | 0.69 |
| Spot C | 16.8 | 13.5 | 0.80 |
| Spot D | 16.8 | 14.8 | 0.88 |
| Spot E | 16.8 | 16.1 | 0.95 |
| **Chloroform Extract** | Chloroform (90) : Pet. Ether (10) | | |
| Spot A | 17.0 | 4.4 | 0.26 |
| Spot B | 17.0 | 5.0 | 0.29 |
| Spot C | 17.0 | 6.7 | 0.39 |
| Spot D | 17.0 | 11.2 | 0.66 |
| Spot E | 17.0 | 12.8 | 0.75 |
| **Acetone Extract** | Chloroform (95) : Methanol (05) | | |
| Spot A | 17.2 | 7.8 | 0.45 |
| Spot B | 17.2 | 14.6 | 0.85 |
| Spot C | 17.2 | 15.8 | 0.92 |
| **Methanol Extract** | Chloroform (80) : Methanol (20) | | |
| Spot A | 16.5 | 8.5 | 0.52 |
| Spot B | 16.5 | 9.2 | 0.56 |

**TLC of various extracts of *F. arnottiana***

## DISCUSSION

Qualitative phytochemical screening is an essential step towards discovery of new drugs as it provides the information regarding presence of a particular primary or secondary metabolites in the plant extract of clinical significance. The presence of any significant bioactive natural product indicates the necessity of separation of the compound from mixture of compounds through suitable chromatographic techniques. In the present study different phytochemical tests were performed on the extracts of *F. arnottiana* leaves shows presence of glycosides, carbohydrates, phenolic compounds, tannins, alkaloids, sterols, fixed oils and saponins. TLC analysis shows well separation of the compounds and also suggests the presence of different kinds of phytochemicals in leaves extract.

## CONCLUSION

The preliminary phytochemical screening of extracts of *F. arnottiana* leaves focus on the importance of separation of the natural compounds from their mixtures as they may be used for various lead compounds for further clinical studies. Further studies of *F. arnottiana* and its isolated compounds are necessary to elucidate the exact mechanism of action so as to develop it as a potent therapeutic drug.

ISSN: 0975-3583, 0976-2833 VOL 13, ISSUE 2, 2022

**ACKNOWLEDGMENT**

Authors are heartily thankful to the administration of SGRR University for providing necessary facilities and also pay thanks to authorities of BSI, Dehradun for identification of plant.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**REFERENCES**

1. Vijayan, K., Vetriselvi, J., Balu, S., 2003. Plants medicines for human ear disesse. *J Econ Tax Bot*., 27, 851-856.
2. Berg, C.C., 1989. Classification and distribution of *Ficus experimentia,* 45,605-611.
3. Novotny, V., Basset, Y., Miller, S.E., Drozd, P., Cizek, L., 2002. Host specialization of leaf chewing insects in New Guinea rainforest. *Journal of Animal Ecology*, 71, 400-412.
4. Warrier, P.K., *Indian Medicinal Plants*. Chennai, Published by orient Longman Ltd., 2, 3-30.
5. Kritikar, K.R., Basu, B.D., 1990. *Indian medicinal plants*. New Delhi, Periodical experts, 2nd ed., 2333.
6. Mazumder, P.M., Farswan, M., Parcha, V. Singh, V., 2008. Hypoglycaemic and antioxidant activity of an isolated compound from *Ficus arnottiana* bark. *Pharmacology online.* 3, 509-519.
7. Chopra, R.N., Nayar, S.L., Chopra, I.C., 1956. *Glossary of Indian Medicinal Plants*. CSIR, New Delhi, India, (First ed.) pp: 54.
8. Kritikar, K.R., Basu, B.D., 1988. *Indian medicinal plants*. Vol I, 195-196.
9. Bakshi, D.N.G., Sharma, P.S., Pal, D.C., 2001. A Lexicon of Indian Medicinal Plants. Nayaprakashan, New Delhi. 2, 190.
10. Kokate CK. Practical Pharmacognosy. New Delhi: Vallabh Prakashan; 1994.