Phytochemical Screening and Thin Layer Chromatography of *Ficus carica* Leaves Extract

Manik Sharma¹, Abid R¹*, Mehak Sajgotra²

¹Department of Zoology, Bhoj Mahavidyalaya, Bhopal (M.P.)-462016, India
²Indian Institute of Integrative Medicine (IIIM-CSIR) Jammu, Canal Road, Jammu-180001, (J&K), India

Abstract

*Ficus carica* leaves are traditionally used in the treatment of various diseases namely vitiligo, diabetes, coughs, asthma, constipation and gingivitis. The aim of the present study was a preliminary phytochemical and thin layer chromatography (TLC) analysis of the various extracts of *Ficus carica* leaves. Phytochemical analysis was carried out using the standard phytochemical assays. TLC analysis of the chloroform and ethanol extract of the leaves was carried out using the solvent system Ethanol: Chloroform (9:1) and Ethanol: Hexane (3:7), respectively. The findings of the preliminary phytochemical screening revealed the presence of various chemical compounds like alkaloids, glycosides, flavonoids, carbohydrates, tannins, phenols, fixed oil and fats. The three *R*<sub>f</sub> value (0.59, 0.78 & 0.94) and six *R*<sub>f</sub> value (0.05, 0.21, 0.34, 0.51, 0.65 & 0.87) were found in TLC plate of chloroform and ethanol extracts, respectively. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

Keywords: *Ficus carica*, Phytochemical, TLC

1 Introduction

The substantial proportions of the population of India have been using traditional medicines since many centuries. The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. Medicinal plants are believed to be an important source of new chemical substances with potentialtherapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on use of plants and plant extracts. Plants are being an effective source of both traditional and modern medicines are genuinely useful for primary healthcare. The medicinal plants produce wide range array of bioactive molecules and rich source of medicines.

Plants have been found to be the source of energy for the animal kingdom. Additionally, plant synthesizes large number of chemical substances that are therapeutically effective. The chief component produce by plants are alkaloids, glycosides, flavonoids, polyphenol, saponin, steroids, tannins etc. Since last two centuries, there have been serious investigations into the chemical and biological activities of plants and these have yielded compounds for the development of synthetic organic chemistry and the emergence of medicinal chemistry as a route for the discovery of more effective therapeutic agents.

*Ficus carica* (commonly known as Fig / Anjeer) constituted one of the largest genera of medicinal plants with about 750 species of woody plants, trees, and shrubs primarily occurring in subtropical and tropical regions throughout the world. The genus is remarkable for the large variation in the habits of its species⁷. The genus *Ficus* (Moraceae) was first published in Systema Naturae by Carolus Linnaeus in 1735. Ficus is one of the largest genus among angiosperms. Among the genera of seed plants it ranked as the twenty-first³. It is a small or moderate sized deciduous tree, 3-10 m high with broad ovate or nearly orbicular leaves, more or less deeply 3-5 lobed, rough above and pubescent below; fruits axillary, usually pear shaped, variable in size and colour. The fruit of *Ficus carica* like those of other species of Ficus, is a syconium a fleshy hollow receptacle with a narrow aperture at the tip. The bark is a cylindrical and
pale grey coloured. The plant leaves reported to contain furanocoumarins such as psoralen, bergapten, xantho toxin, triterpenes such as calotropenyl acetate, lupeol acetate, isoschaftoside and certain sterols. *Ficus carica* leaves have been traditionally used in the treatment of vitiligo, diabetes, coughs, asthma, constipation and gingivitis. The other reported pharmacological activities of fig leaves include cytotoxic, hypoglycemic and antihelminthic activity. A reliable pharmacological or clinical study must employ well-authenticated plant material. Thus the present investigation was aimed to investigate the pharmacognostical features and phytochemical analysis for identification and authentication of the plant.

2 Materials and Methods

2.1 Preparation of plant material

The leaves of the plant of *Ficus carica* were collected from the local surroundings at Kunzer area of Baramulla, Jammu and Kashmir during the month of August and September 2014. The plant was authenticated by Dr. Bikrama Singh, Scientist at taxonomy department Indian Institute of Integrative Medicine (IIIM-CSIR) Jammu and Kashmir, India. The voucher specimens (RRL-22990) are kept in the herbarium of Indian Institute of Integrative Medicine (IIIM)-CSIR Jammu and Kashmir for future reference. The fresh leaves of *Ficus carica* were collected and washed thoroughly under running tap water. The leaves were allowed sun dried after rinsed with distilled water. The dried plant material were coarsely powdered and subjected to extraction.

2.2 Preparation of extract

The extract was done by maceration using petroleum ether, Chloroform and ethanol. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. The powder extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extracts.

2.3 Preliminary Phytochemical studies

Preliminary phytochemical tests of various extracts of leaves powder of *Ficus carica* were performed for phytochemical analysis of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids.

2.3.1 Test for alkaloids

(a) Dragendorff’s test: To 1 ml of the extract, add 1 ml of dragendorff’s reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer’s test: To 1 ml of the extract, add 1 ml of mayer’s reagent (Potassium mercuric iodide solution). Whitish yellow or cream colour precipitate indicates the presence of alkaloids.

(c) Hager’s test: To 1 ml of the extract, add 3 ml of Hager’s reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

(d) Wagner’s test: To 1 ml of the extract, add 2 ml of wagner’s reagent (Iodine in Potassium Iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for Protein

(a) Biuret test: Added 1 ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color was produced, and then added to the 1 ml of the extract. Formation of pinkish or purple violet color indicated the presence of proteins.

(b) Ninhydrin test: Added two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

2.3.3 Test for Glycosides

(a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Babiet test: To 1 ml of the test extract, add 1 ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1 gm of powdered drug is extracted with 10 ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10 ml of water and 0.5 ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5 ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3 ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2 ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager’s test: Add a few ml of dilute Sulphuric acid to 1 ml of the extract solution, Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1 ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

2.3.4 Test for carbohydrates and sugars

(a) Molisch’s test: To 2 ml of the extract, add 1 ml of a-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.
(b) Fehling’s test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

(c) Benedict’s test: To 5ml of Benedict’s reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5 Test for tannins and phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.6 Test for flavonoids

Shinoda’s test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

2.3.7 Test for steroids

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H₂SO₄. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.3.8 Test for fixed oils and fats

(a) Spot Test: Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.

(b) Saponification test: To 1ml of the extract, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

2.4 Thin layer chromatography (TLC)

The Chloroform and ethanol extracts were selected for TLC study. The extracts were prepared with the respective solvent ethanol and distilled water and made up to 10 ml in different test tubes. Then the extracts were taken in a capillary tube and it was spotted in preparative TLC plates coated with silica gel G. The plates were developed in TLC chamber previously saturated with different solvent systems. The different spots developed in each solvent system were identified by means of detecting agent and the Rₛ value are correspondingly calculated\(^4\).

3 Results

3.1 Phytochemical screening

Preliminary phytochemical investigations of the extracts of leaves of Ficus carica revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. The details are presented in table 1.

From the result of phytochemical screening, the petroleum ether extract of leaves of Ficus carica exhibited the presence of fats and oils. Alkaloids, flavonoids, tannins and polyphenol were found in chloroform extracts of leaves of Ficus carica. Further, glycosides, carbohydrates, flavonoids, tannins and polyphenol were present in ethanol extracts of leaves of Ficus carica. The maximum phytoconstituents were observed in ethanol extracts of leaves of Ficus carica (Table 1). Now chloroform and ethanol extracts of Ficus carica were selected for further TLC evaluation.

3.2 TLC

The retention factors (Rₛ) of chloroform and ethanol extracts in different solvent systems are shown in table 2. The chromatogram revealed 3 spots and 6 spots for chloroform and ethanol extracts, respectively.

4 Discussions

For the pharmacological study of novel drugs, the essential information’s regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study qualitative tests of extracts showed significant indication about the presence of metabolites. Preliminary phytochemical investigations tests are useful to isolate the pharmacologically active principles present in the plant. Plant derived natural products such as polyphenol and steroids have received considerable attention in recent years due to their diverse pharmacological properties. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots and barks\(^22,23\). Ficus carica is an important medicinal plant of the world. Its usage not only fulfills the nutritive need of the human being but due to the presence of different types of bioactive constituents makes this plant medicinally very important for the human being. Different types of phytoconstituents were present in the Ficus carica like flavonoids, phenolic compounds, alkaloids and glycosides which makes this plant potent to various types of ailments.
### Table 1: Phytochemicals present in leaves of *Ficus carica* extracts

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Pet. Ether</th>
<th>Chloroform</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dragendorff’s test</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Hager’s test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mayers</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Wagners</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Legal’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Balje test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Glycosides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keller-Killiani test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Borntrager’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Molish test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benedict’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fehling’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5%FeCl₃ solution</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Tannins and Phenolic compound</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead acetate solution</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Potassium ferric cyanide and ammonia solution</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shinoda test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Liebermann burchard test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Steroid test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salkowski test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Biuret test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ninhydrin test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Fat and oil test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponification test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spot Test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Present, - = Absent
TLC profiling of chloroform and ethanol extracts gives an impressive result that directing towards the presence of number of phytochemical. Various phytochemicals gives different $R_f$ values in different solvent system. This variation in $R_f$ values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the $R_f$ values of compounds in different solvent system\(^1\).

The TLC method is best choice for the identification of secondary metabolite present in plants. Here the different $R_f$ values indicate the presence of different nature of phytoconstituents in single extracts. Different $R_f$ values of the compound also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

5 Conclusion

The findings of study indicate that the extract of *Ficus carica* contained many phytochemicals as revealed by phytochemical studies and TLC analysis. The ethanol extract of *Ficus carica* incorporating maximum number of phytoconstituents. The present study on phytochemical screening and TLC of *Ficus carica* leaves will provide useful information in regard to its correct identity and help to differentiate from the closely related other species of *Ficus*. It is concluded from the data that extracts of *Ficus carica* leaves exhibited significant role in medicinal chemistry for formulation of life saving drugs.

5 Acknowledgements

Authors are thankful to the Director General MPCST, Bhopal and Director General Indian Institute of Integrative Medicine (IIIM-CSIR), JAMMU for supported financially to carry this research work.

6 Conflict of interests

No conflict of interest among all authors of this work

7 Author’s contribution

Research was designed by MS and AR. AR and MS handled data analysis, while MS handled manuscript writing and revising of content. All authors read and approved the final copy for publication.

8 References


