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Research Article

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Preliminary Phytochemicals Investigation and TLC Analysis of Ficus racemosa Leaves

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ABSTRACT

Ficus racemosa Linn belongs to the family Moraceae is used as herbal medicine from ancient times. It is an evergreen, moderate sized, deciduous tree found throughout India. Present study reports the phytochemical properties which have been carried out on the leaves extract using ethanol, methanol, ethyl acetate, acetone and n-hexane. The phytochemical analysis shows the presence of Phenols, Flavonoids, Quinones, Saponins, Cardinolites, Steroids, Tanins and Terpenoids in various extracts. Thin layer chromatography (TLC) have been carried out on different extracts of leaves of Ficus racemosa, which show different R_f values and possible combinations of chromatography solvents for separation of these phytochemicals.

Key words: - Ficus racemosa Linn, Moraceae, Phytochemicals, TLC, Extract.

INTRODUCTION

Phytochemicals are those chemical compounds that occur naturally in plants and responsible for color and organoleptic properties, such as the deep purple of blueberries and smell of garlic. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients [1]. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome. These Phytochemicals are abundant in fruits, vegetables and herbs.

Ficus racemosa Linn is commonly known as *Gular fig* or *Cluster fig* (English), *Gular* (Hindi), *Umbar* (Marathi) and *Udumbara* (Sanskrit) belongs to the family *Moraceae*, is an evergreen, moderate sized, deciduous tree. Its leaves are dark green, ovate or elliptical. The leaves have traditional medicinal importance. They are useful as wash for wounds, ulcers and also in dysentery, diarrhea. The infusion of bark and leaves is also employed as mouth wash for spongy gums, internally in dysentery and menorrhagia. It is also an effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis and haemoptysis [2].

An extraction and phytochemicals test on the leaves of selected plant is already carried out by Ramila Devi et. al. [3]. There is the need to extent their work by using additional solvents which are commonly used for extraction. The aim of the present study is to extract and investigate the presence of commonly known Phytochemicals in Ficus racemosa Linn. The selection of solvents is on systematic order, from highly non-polar solvents to the polar solvent. The series of solvents in systematic order will help in understanding the effect of polarity on the extraction and extracted Phytochemicals. In present study uses ethanol, methanol, acetone, ethyl acetate and n-hexane solvents of various extractions and mixtures of these solvents for thin layer chromatography (TLC) profiling.

EXPERIMENTAL SECTION

Plant material

The leaves of F. racemosa were collected from Nagpur region, Maharashtra, India, washed properly and shade dried. The dried leaves then powdered. Leaves powder kept in moisture free container and used for further analysis.

Preparation of extract

Five solvents are selected for extraction purpose based on their polarity. They are ethanol, methanol, acetone, ethyl acetate and n-hexane. The dried leaves powder (30 gm.) was extracted in Soxhlet apparatus in 250 ml of each solvent separately. The extractions performed for 48 hrs and were concentrated by slow evaporation process.[4]. The obtained extracts kept in moisture free container and used for phytochemical analysis.

Phytochemcial investigation

Various phytochemical investigations performed using obtained extracts. The procedures are already reported by number of workers and used without any modification or alteration [4,5,6,7]. The phytochemical name and investigation methods are as follows:

Phenols

To test the Phenol phytochemical presence, in a test tube 1ml of extract and 2 ml of distilled water were added followed by few drops of 10% ferric chloride (FeCl₃). Appearance of blue or green colour indicates presence of phenols. The obtained result is depicted in Table number 1.

Flavonoids

To test the flavonoid phytochemical presence, in a test tube 1 ml of extract a few drops of dilute sodium hydroxide (NaOH) added. An intense yellow colour was produced in the plant extract which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoid. The obtained results for all extracts are depicted in table number 1.

Quinones

To test the quinone phytochemical presence, in a test tube 1ml of extract and 1ml of concentrated sulphuric acid (H_2SO_4) was added. Formation of red colour shows the presence of quinones. The results are depicted in table number 1.

Tannins

To test the tannin phytochemical presence, in a test tube 1 ml of 5% ferric chloride added to solvent free extract. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate [5]. The obtained results from various extracts are depicted in table no 1.

Saponins

To test the saponin phytochemicals presence in various extract, the extract was diluted with 20 ml distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponin [5]. The obtained results for various extracts are depicted in table no 1.

Cardiac Glycosides

To test the cardiac glycoside phytochemicals presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride (FeCl₃) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicates a de-oxy sugar characteristic of cardenolites. The results for studied extracts are depicted in table no 1.

Steroids

To test the presence of steroid phytochemicals, 1 ml of extract dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid (H_2SO_4) added by sides of test tube. The upper layer turns red and sulphuric acid layer shown yellow with green fluorescence. This indicated the presence of steroids. The obtained results for all extracts are depicted in table number 1.

Terpenoids

To test the presence of terpenoid phytochemicals, in a test tube 5 ml of each extract was mixed with 2 ml of chloroform. 3ml of concentrated sulphuric acid (H_2SO_4) was then added to form a layer. A reddish brown precipitate colouration at the interface formed indicated the presence of terpenoids. The obtained results for all extracts are depicted in table no 1.

TLC analysis of leaves extracts of Ficus racemosa

Each of the five extracts was to begin with, checked by Thin layer chromatography (TLC) on analytical plates over silica gel-G of 0.2 mm thickness. These plates were developed in *Toluene : Ethyl acetate : Formic Acid* having a ratio of *5:1.5:0.5* [3] and the spots were visualized by exposure of the plates to iodine vapour.[10,11]

In addition to that, TLC has also been carried out by using other mobile phases like *Chloroform: Ethyl acetate*, *Toluene: Ethyl acetate* etc; having different ratios whereas the results are not encouraging and hence discarded. [12,13]

RESULTS

The extract of leaves of *F. racemosa* were prepared in Ethanol, Methanol, Ethyl acetate, Acetone and n-Hexane. Various phytochemical tests performed on the extracts and the results are reported in Table 1. It is reported that phenol and flavonoids phytochemical presence reported in all extracts. Quinones and steroids are reported in all except ethyl acetate and n-hexane respectively. Tannins and cardiac glycosides are reported in ethanol and methanol solvents only. Terpenoids and saponins tested positive in ethyl acetate and acetone respectively.

Table 1. P	hytochemicals	analysis of different	extracts of F.	racemosa leaves.
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Phytochemicals	Extract				
Phytochemicals	Ethanol	Methanol	Ethyl acetate	Acetone	n-Hexane
Phenol	+	+	+	+	+
Flavonoid	+	+	+	+	+
Quinones	+	+	-	+	+
Steroids	+	+	+	+	-
Tannins	+	+	-	-	-
Cardiac glycosides	+	+	-	-	-
Terpenoids	-	-	+	-	-
Saponins	-	+	-	-	-
Note : $(+) =$ Present and $(-) =$ Absent					

TLC analysis also suggests the presence of different kinds of phytochemicals in leaves extract. Table 2 reports the R_f values for various extracts and Figure 1 shows photographs of the studied TLC slides. TLC of plant extract in ethyl acetate and acetone reports three spots for various phytochemicals. The reported spots are separated with enough space and having various R_f values showing the presence of atleast three phytochemicals in ethyl acetate and acetone solvent extracts.

Extracts in ethanol, methanol and n-hexane report only one spot. It is prominent and uniquely identified.

	R_f Values			
Plant Extract	Toluene: Ethyl acetate: Formic acid			
	Ratio 5:1.5:0.5			
Ethanol	0.85			
Methanol	0.57			
	0.88			
Ethyl acetate	0.73			
	0.54			
	0.97			
Acetone	0.84			
	0.79			
n-Hexane	0.74			

Table 2. R_f values of various extracts of *F*. racemosa leaves

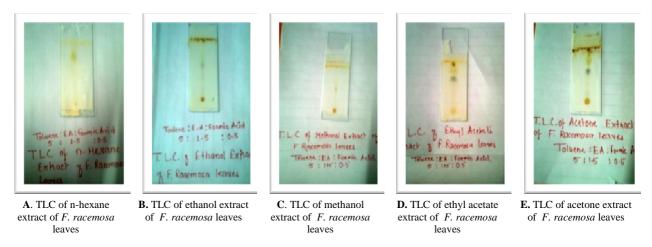


Figure 1. Photographs of TLCs of extract F. racemosa leaves

DISCUSSION

When a new drug to be discovered, qualitative phytochemical analysis is a very important step as it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. In any case, if any significant bioactive natural product is present, it is necessary to separate that compound from the mixture of compounds by using suitable chromatographic technique.

The different phytochemicals tests performed on the extracts of *Ficus racemosa* leaves show the presence of phenols, flavonoids, quinones, tannins, steroids and cardiac glycosides in ethanol extract, the presence of phenols, flavonoids, quinones, saponin, tannins, terpenoids and cardiac glycosides in Methanol extract. Ethyl acetate extracts reports the presence of phenol, flavonoids, terpenoids and steroids. Acetone extract reports the presence of phenol, flavonoids, quinones and steroids and in case of n-hexane extracts phenols, flavonoids and quinones show their presence. The findings are also in line of previous findings and reported literatures [3,7,8].

The TLC experiment was successful only with mobile phase of toluene: *ethyl acetate:formic acid*. Whereas the mobile phase of *toluene: ethyl acetate and chloroform* of varying ratios fail to give any acceptable results.

CONCLUSION

The phytochemical tests performed on the various extracts of *Ficus racemosa* leaves shows the presence of phenols, flavonoids, quinones and steroids in major quantities and reported in nearly all extracts confirm their presence in abundance. Tannins and cardiac glycosides presence in moderate amount and need slightly polar solvents like ethanol or methanol for their separation. Terpenoids and saponins are also reported in leaves of *Ficus Racemosa* and their separation is possible using moderate polar solvents like methanol and ethyl acetate respectively. The reported results also on the line of work or assumptions previously executed by number of workers [7, 8, 9] with clear understanding about the possibilities of separation medium.

Ficus Racemosa leaves can be further analyzed for qualitative and quantitative extraction of reported phytochemicals to explore the possibilities of using it as an herb medicine on scientific ground.

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