



Extraction and Isolation of Bioactive Compounds from *Ficus racemosa* Bark and *Cissampelos Pareira* Root by Chromatographic Techniques

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ABSTRACT

Since ancient times, plants have been an exemplary source of medicine. The bioactive constituents are generally found in most of the plant parts and used as traditional medicines. The study such as ethno medicine keenly represents one of the best avenues in searching new economic plants for medicine. The present study was carried out to find the phytochemical constituents in the *Ficus racemosa* bark and *Cissampelos pareira* root. The plant parts were collected from Malkangiri district of Odisha. They were shade dried, cut into pieces, grinded and passed through sieve number 60. The extracts were obtained with solvent methanol using Soxhlet apparatus. Preliminary phytochemical screening revealed the presence of alkaloids, tannins, phytosterols, flavonoids, triterpenoids and saponins in those two plant extracts. The phytoconstituents were isolated from the methanolic extracts of *Ficus racemosa* bark and *Cissampelos pareira* root by column chromatography. The solvent system for column chromatography was selected on the basis of separation achieved by TLC.

Keywords: *Ficus racemosa*, *Cissampelos pareira*, extraction, isolation.

INTRODUCTION

F*icus racemosa* Linn.
Morphological profile

Ficus racemosa Linn. Locally known as 'Dimiri' (Odia) belongs to family Moraceae. It is a tree, highly cosmopolitan in occurrence, grows all over India especially in habitats like forests and hills. The tree is of medium height up to 10-16 meters; bark reddish grey, often cracked at outer surface with easily removable translucent flakes, greyish to rusty brown; uniformly hard and non-brittle. Many ancient scriptures of Ayurveda like Susruta Samhita described the properties of its bark as astringent, promotes healing process of fractured wounds by formation of callus (bhagnasandhaniya), alleviates hematemesia (Rakta pitta), burning sensation, obesity and useful in vaginal disorders.

Phytochemical profile

Bark of *Ficus racemosa* contain chemicals like new tetracyclic triterpene-glaunol, Sitosterol, unidentified long chain ketone, Cerylbehenate, lupeol and its acetate, amyirin acetate, stigmasterol, sitosterol, glucoside and friedelin as per report outstanding¹.

Cissampelos pareira L. var. *hirsuta* (DC) Forman:

Morphological profile

Cissampelos pareira locally known as 'Akanabindi'- Odia; belongs to family Menispermaceae. Herbal of softly tomentose, herbaceous climbers; petiole to 2.5 cm long; lamina ovate to orbicular; inflorescence dioecious, subtended with many conspicuous bracts imbricate arranged; pistillate inflorescence longer than staminate ones; flowers greenish white.

Phytochemical profile

Cissampelos pareira contains a group of phytochemicals called isoquinolinealkaloids². Out of thirty-eight alkaloids so far discovered; one, called tetrandrine is the most well documented³. Protoberberine alkaloids have been found in the roots.

MATERIALS AND METHODS

Selection of plants

Ficus racemosa Linn. And *Cissampelos pareira* L. were duly authenticated by taxonomist S.K.Dash and preserved in College of Pharmacy (Poly), Pandharpur, Solapur dist. for future reference.

Extraction

The powdered bark of *Ficus racemosa* and root of *Cissampelos pareira* were subjected to Soxhlet apparatus with 90% methanol for extraction separately.

Phytochemical investigation

The phytochemical investigation^{4,5} was carried out as per standard extraction procedure^{6,7} for the presence or absence of different chemical constituents⁸ present in bark of *Ficus racemosa* and root of *Cissampelos pareira*.

Isolation

Fractionates of both the plant extracts were obtained by column chromatography^{5,9} on the basis of TLC¹⁰.

Bioactivity against four microbes

The bioactivity study¹¹ of those two isolated compounds was also carried out and both were compared with activity.



RESULTS

Extraction

The plant materials were thoroughly washed with water to remove adhering debris followed by rinsing with distilled water. The plant materials were then spread in thin layers in stainless steel trays and dried under shade. After drying, the plant materials were pulverized in a mechanical grinder at Berhampur and the resulting powder was passed through sieve No. 60 to obtain uniform coarse powder. The coarse powder was kept in polythene jars and used for extraction¹².

The crushed barks of *Ficus racemosa* and roots of *Cissampelos pareira* were extracted successively with methanol. The crushed plant materials were soaked with methanol. The soaked plant materials were packed in a closed container. The solvent was poured on the above material till it forms a layer above the plant material. The material was shaken at regular interval for 8 days. The extract was filtered and concentrated in evaporator. Further marc was treated with methanol and same as above extraction procedure was followed. The final concentrated extracts were packed in an air tight container. The above extracts were used for further studies for colour, consistency and quality of extract obtained.

Phytochemical investigation

The qualitative chemical investigation for the above extract was carried out to detect the presence of various phytoconstituents in the extracts. The extract showed the presence of different constituents such as tannins, flavonoids and phytosterols. Phytochemical investigations of extracts were carried out as per the standard procedure.

The qualitative phytochemical test results of both the methanolic extract of *Cissampelos pareira* and *Ficus racemosa* are summarized in Table 1.

Table 1: The result of preliminary phytochemical screening of *F. racemosa* and *C. pareira*

Plants Constituents	<i>F. racemosa</i>	<i>C. pareira</i>
Carbohydrates	(--)	(--)
Alkaloids	(--)	(+)
Tannins	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(--)
Proteins	(--)	(--)
Fats	(--)	(--)
Steroids	(+)	(+)
Triterpenoids	(--)	(+)

(+) Indicates Present and (--) indicates Absent

Isolation of phyto constituents from *Ficus racemosa* bark

Fractionates of methanolic extract was done by column chromatography. The solvent system for column

chromatography was selected on the basis of separation achieved by TLC. TLC plates were prepared using silica gel G as the stationary phase. The plates were coated after thorough prior cleaning and rinsing with distilled water. The plates were kept in sunlight for some time and then activated in oven for 60 min at 120°C. The normal chamber was lined with filter paper and 100 ml of solvent was introduced in chamber and was saturated for 30 minutes. Various solvent systems were used and finally based upon the separation achieved petroleum ether (60-80o) was selected as the solvent system for column chromatography.

Column was packed using wet packing technique using silica gel (300 g) (# 60-120) as the adsorbent. Slurry was prepared using hexane and the slurry was poured in to the column (Figure 1). 13 g of extract in sticky state was added after mixing them with little amount of silica gel over the top of the column.



Figure 1: Packed column

Gradient elution technique was followed for column chromatography. The column was first eluted with petroleum ether (Figure 2) (60-80°C) and 7 fractions of 100 ml each were collected (Figure 3). The fractions collected were concentrated and TLC was performed to identify the presence of single compound.

After that the column was eluted with petroleum ether: chloroform in the ratio of 50:50 and 5 fractions were collected. Fractions were concentrated and TLC was performed.

Then solvent system was changed to chloroform: methanol (1:1) and fractions were collected and then the concentration of methanol was gradually increased in the solvent system and column was run in the ratio of (60:40), (70:30), (80:20), (90:10) of methanol: chloroform respectively.

In the 90:10 ratio of methanol: chloroform, 5 fractions of 100 ml each were collected and TLC was performed, in the 3rd fraction single spot was identified. This fraction was named as A. Chemical test was performed in the collected 3rd fraction and positive result was found for steroid.



Figure 2: Movement of Mobile Phase in Column



Figure 3: Collection of fractions

The last two fractions 4th and 5th collected showed similar TLC pattern so they both were mixed and weighed, it was found to be 700mg.

Isolation of alkaloids from *Cissampelos pareira* root

Total alkaloids from *C. pareira* was isolated using the general process of extraction of alkaloids (Figure 4), in which weighed quantity of methanolic extract (12g) was taken and dissolved in water containing a little dilute sulphuric acid. It was transferred to a separating funnel and Ammonia was added until the liquid was slightly alkaline in order to precipitate the free alkaloid.



Figure 4: Isolation of total alkaloids

The precipitated alkaloid was extracted with five successive quantities of chloroform. Mixed chloroform extracts were washed with water in a clean separating funnel and chloroform layer was transferred to a small beaker. It was evaporated on a water bath and 1.5 g of dried crude alkaloids was obtained.

To isolate various alkaloids from it column chromatography was used. The solvent system for column chromatography was selected on the basis of separation achieved by TLC. TLC plates were prepared using silica gel G as the stationary phase. The plates were coated after thorough prior cleaning and rinsing with distilled water. The plates were kept in sunlight for some time and then activated in oven for 60 min at 120°C. The normal chamber was lined with filter paper and 100 ml of solvent was introduced in chamber and was saturated for 30 minutes. Various solvent systems were used and finally based upon the separation achieved chloroform:methanol (1:1) was selected as the solvent system for column chromatography.

1.5g of dried alkaloidal fraction was loaded on top of the column and the column was eluted using the selected solvent system (Figure-5).



Figure 5: Separation of bands in Column

Nine fractions were collected, concentrated and weighed.

TLC was performed for 9 fractions after concentrating them in a water bath for identification of single spot using chloroform: methanol (50:50) solvent system.

Fraction 1st and 4th showed single spot in TLC, confirming the presence of single compound in the fractions.

Chromatographic studies

The separation and purification of plant constituent is mainly carried out using one or other, or a combination, of five chromatographic techniques, paper chromatography, thin layer chromatography, gas liquid chromatographic technique, high pressure liquid chromatography and high performance thin layer chromatographic technique. The choice of technique depends upon property and volatility of compound to be identified or separated.

Thin layer chromatography

TLC is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Retention of the component and their separation depends on the ability of the atom on the surface to remove the solutes from the mobile phase and adsorb them temporarily by means of electrostatic forces.

Steps involved in TLC

1. Preparation of plates
2. Activation of plates
3. Preparation and saturation of chamber
4. Sample application and development
5. Detection and calculation of R_f value

Preparation of plates:

- Silica gel, the most frequently used stationary phase, was employed as such for adsorption TLC
- The particle size of the stationary phase were brought to uniform size in order to reduce the band broadening and also to provide a large surface area for interaction and a small void volume.
- Silica gel was mixed with water and made into slurry.
- The slurry was spread uniformly on the plate.
- The plates were air dried for some time and then kept for activation.

Activation of plates:

- By heating the plate in an oven at 100 to 110°C for 30 minutes.
- Activation is necessary for linear movement of solutes over stationary phase.

Preparation of solvent, saturation of chamber, sample application and development - Prepared the solvent system.

- Poured it into the chamber and saturated the chamber by lining the chamber with a piece of filter paper that has been wet with the mobile phase.
- The solvent system used for detecting alkaloids in extract of *C. pareira* was Chloroform: methanol (1:1), and the spraying reagent used was dragendroff's reagent. The number of spots was identified and R_f value calculated.
- The solvent system used for the detection of tannins in extracts of both the plants was chloroform. The developed plate was sprayed with ferric chloride solution. The spots appear dark bluish or green in color. The number of spots and R_f values were calculated.

Calculation of R_f value

- The R_f value (Table 2 and Table 3) of the spots were calculated using the formula

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Table 2: No. of spots and R_f values of alkaloids

Plant name	Spots	R_f value
<i>C. pareira</i>	1	0.14
	2	0.33
	3	0.41
	4	0.48
	5	0.56



Figure 6: TLC for Alkaloids in *C. pareira*

Table 3: No. of spots and R_f values of tannins detected in two plants.

Plant name	Spots	R_f value
<i>F. racemosa</i>	1	0.50
	2	0.74
	3	0.80
	4	0.90
<i>C. pareira</i>	1	0.21
	2	0.57

**Figure 7:** TLC for tannins in *F. racemosa* & *C. pareira***Table 4:** Comparative antimicrobial activity of *Ficus racemosa* and *Cissampelos pareira*

Drug/ Extract	Concentration ($\mu\text{g}/\text{disc}$)	Mean diameter of growth inhibition zones (mm)			
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
Ofloxacin	5	24	22	22	26
<i>C. pareira</i>	200	12	9	8	21
	400	18	12	12	18
<i>F. racemosa</i>	200	8	7	9	11
	400	10	8	10	13

Values are expressed in mean for zone of inhibition (n=3)

Disc diffusion method was used for the antimicrobial activity of the extracts. Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into petri dishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms, *Escherichia coli* (MTCC 1683), *Pseudomonas aeruginosa* (MTCC 4673), *Staphylococcus aureus* (MTCC 7443) and *Bacillus subtilis* (MTCC 6942), were spread over the media with the help of a sterile swab soaked in bacterium and is used for antibacterial study. Both the extracts were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 200 μg , 400 μg /disc and used for the study. Ofloxacin 5 $\mu\text{g}/\text{disc}$ was used as the standard. Then the sterile filter paper discs (6 mm) were immersed in

definite concentration of plant extracts and placed over the solidified agar in such a way that there is no overlapping of the zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated petridishes were incubated at 37°C for 24 hours. After the incubation period the zone of inhibition produced by the samples and the standard were measured (Table 4). All tests were performed in triplicate.

DISCUSSION

According to WHO, medicinal plants would be the best source to obtain a variety of drugs. In the present study, column chromatography and TLC eluted two compounds, an alkaloid and tannin¹¹.

Alkaloids often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Since the study was conducted in a controlled manner, the phytochemical results can be used for the standardization of the above mentioned drugs. A preliminary screening and more research has to be undertaken to explore the wonderful therapeutic properties of these medicines. It was found that most of the biologically active phytochemicals were present in the methanolic extract of *Ficus racemosa* bark and root of *Cissampelos pareira*.

Similarly, flavonoids (specifically catechins) are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants"¹³. The flavonoids in significant quantities are incorporated into the human systems through the regular diet. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". *In-vitro* studies show that flavonoids also have anti-microbial activity^{13,14}. The present study facilitates a step accordingly to establish more in it. Many phytochemicals too encompass phytosterols. The present study attests a step further to establish the antimicrobial activity of the extracts of *Ficus racemosa* and *Cissampelos pareira*.

CONCLUSION

From the above study it reveals that the presence of compounds like sterols, tannins and flavonoids¹⁵ found in these two plants supposed to play an active role to guard the integrated live systems against the microbes. However both the isolates showed the promising antimicrobial activity against different microbes and hence bioactive¹⁶. No conclusion can be drawn regarding these, unless the isolates are subjected to IR, NMR and mass spectroscopic study for establishing the structure.

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