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Qualitative Assessment of Bioactive Compounds from a Very Rare Medicinal Plant *Ficus dalhousiae* Miq

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ABSTRACT
Ficus dalhousiae Miq of Moraceae family is widely used in the traditional and Unani medicine for the liver and skin diseases. *F. dalhousiae* being very rare medicinal plant found scarcely distributed in India. In present study, the plant parts were shade dried and phytochemicals are extracted with solvents of different polarity like hexane, chloroform, acetone, methanol, water. These extracts showed great variation in terms of phytochemical composition. Of all the extracts, methanol was found to have more phyto constituents, whereas hexane and chloroform showed fewer compounds than the other extracts. Acetone and water extract showed moderate range of extract solubility which was in accordance with previously published reports. *F. dalhousiae* has wide range of chemical constituents and these can be useful for drug discovery and development of various new formulations. Further study of these compounds, their separation and purification will provide way for future research.

Keywords: *Ficus dalhousiae* Miq, phyto constituents, medicinal activities, solvent extraction.

1. Introduction
Several people have been using plants for the treatment of various ailments from thousands of years [1]. Plants are used medicinally in different Countries and are the sources of many potential and powerful drugs [2].
Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day to day life. These medicines are relatively safer and cheaper than synthetic or modern medicine [3, 4].
Herbal molecules are safer and overcome the resistance produced by the pathogens as they exists in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell [5, 6]. According to world Health Organization over 80% of the global populations relies chiefly on traditional medicine [7] 1992, since they cannot afford the products of western pharmaceutical industries [8] and lack of health care facilities [9].
Secondary metabolites are synthesized in a specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products (e.g. steroids, quinines, alkaloids, terpenoids and flavonoids) which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and the molecular weights are generally less than 2000.
Approximately 119 pure chemical compounds were extracted from higher plants are used in medicine throughout the world [10]. A survey of current pharmaceutical use revealed that out of the total prescription drugs dispensed, 25% are plant derived [11, 12]. Of the various pharmaceuticals used in modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quine, reserpine and tubocurarine serve as examples of drugs discovered through observations of indigenous medicinal practices [13]. Eloff in 1999 [14] Stated that the antimicrobial compounds from plants may inhibit by a different mechanism than presently used antibiotics and may have clinical value in the treatment of resistant microbial stains.
Medicinal Plants are known to have good immune modulatory property. They act by stimulating both nonspecific and specific immunity. These plants may promote host resistance against infections by reestablishing body equilibrium and conditioning the body tissue [15, 16, 17, and 18].

1.1 Description: *Ficus dalhousiae* Miq is a small spreading tree; Young branches are softly pubescent; Leaves are Ovate-elliptic to broadly elliptic to broadly ovate, white pubescent below; apex shortly acuminate; base more or less deeply cordate, up to 30 cm long and 20 cm long ,lateral nerves 10-14 pairs. Receptacles in pairs, Unisexual, Yellow, shortly peduncled, obovoid, pubescent, about 1.5 cm in diameter, with three apical scales and three bifid basal bracts

1.2 Origin: This species is endemic to peninsular area [19] and a very rare species [20, 21] first described this species as *Urostigma dalhousiae* based on Wights' collection from India and later [22] (Miquel 1867) he named it as *Ficus dalhousiae*. Subsequently, [23, 24, 25] recorded this species from the Nilgiri Mountains in the altitudinal range of 605-1370 m. Wherever it grows , its population size is very small and probably that is the reason for its inclusion under the very rare category in the threatened plant list [20].

1.3 Classification
Domain: Eukaryota
Kingdom: Plantae
Sub kingdom: Viridaeplantae
Phyllum: Tracheophyta
Sub Phylum: Euphyllophytina
Infraphylum: Radiatopses
Class: Spermatopsida
Sub Class: Rosidae
Family: Moraceae
Tribe: Ficeae
Genus: *Ficus*
Specific epithet: *dalhousiae* -Miq.

2. Materials and methods

2.1 Collection of sample:
The medicinal plant (*Ficus dalhousiae* Miq) material was collected from Talakona Forest of Andhra Pradesh, India. Plant was identified and authenticated by taxonomist (BT130010).

2.2 Extraction Method: (Maceration)
Maceration is one of the well-known method of extraction process. In this process the plant material (10 gms) is mixed in the methanol solvent (100 ml) and it is continuously shaken for few days [26].

2.3 Maceration optimization:
Five conical flasks with 10 gms of plant material were taken and it is mixed in 100 ml of methanol and shaken continuously for three days. Components are taken in to pre weighed empty boxes after filtering with the Whatman no1 filter paper. The filtrate should be air dried completely then the component was weighed to know the crude extract content. The method was followed for successive four days (i.e. 4th, 5th, 6th, & 7th).

2.4 Phytochemical screening [27, 28, 29, 30]
Standard screening tests of five extracts were carried out for various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones using standard procedures.

Phytochemical screening of the extracts
Phytochemical examinations were carried out for all the extracts as per the standard methods.

I. **Detection of alkaloids:** Extract was dissolved individually in dilute Hydrochloric acid and Solution was clarified by filtration.
   a. **Mayer's Test:** Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicates the presence of alkaloids.
   b. **Wagner's Test:** Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
   c. **Dragendorff's Test:** Filtrate was treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
   d. **Hager's Test:** Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

II. **Detection of phenols**
   a. **Ferric Chloride Test:** The filtered solution of extract was treated with three drops of freshly prepared 1% Ferric Chloride and potassium ferrocyanide. Formation of bluish-green colour was taken as positive.
   b. The methanol extract was dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.

III. **Detection of flavonoids**
   a. **Alkaline Reagent Test:** The extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl acid, indicates the presence of flavonoids.
   b. **Lead acetate Test:** The extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

IV. **Detection of anthraquinones**
   a. **Free anthraquinones test: (Borntrager's test)** The extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered, and 5 ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red, or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.

   b. **Modified Borntrager's Test:** The extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

V. **Detection of phytosterols**
   a. **Salkowski's test:** The extract was dissolved in 2 ml chloroform in a test tube. Conc Sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown color at the interface indicated the presence of a steroid ring (i.e., the aglycone portion of the glycoside).

b. Liebermann Burchard's test: The Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

c. Detection of Terpenoids: The extract was added to 2 ml of acetic anhydride and conc H_2SO_4. Formation of blue, green rings indicate the presence of terpenoids.

VI. Detection of Fatty acids: The extract was mixed with 5 ml of ether. These extract was allowed for evaporation on filter paper and filter paper was dried. The appearance of transparency on filter paper indicates the presence of fatty acids

VII. Detection of Tannins

a. Ferric chloride test. The extract was dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in color to bluish black.

b. Lead acetate test. The extract was dissolved in water and to that 10% Lead acetate solution was added. The appearance of yellow precipitate confirms the tannins.

c. Pot. Dichromate Test. The extract was dissolved in water to that strong potassium dichromate solution was added, a yellow color precipitate indicates the presence of tannins and phenolic compounds.

VIII. Detection of saponins

a. Froth Test: Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of "honey comb" froth indicates the presence of saponins.

IX. Anthocyanins: The extract was added to 2 ml of 2N HCl and ammonia. Initial appearance of pink-red colour turning into blue-violet indicates the presence of anthocyanins

X. Leucoanthocyanins: The extract was added to 5 ml of isoamyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins

XI. Coumarins: 3 ml of 10% NaOH was added to the extract, formation of yellow colour indicates the presence of coumarins

XII. Emodins: 2 ml of NH_4 OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins

XIII. Detection of Reducing Sugars: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. Fehling's Test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

b. Keller - Kiliani test (for de-oxy sugars in cardiac glycosides): Methanol extract was obtained and the extract was dried. 50 mg of this was dissolved in 2 ml chloroform. H_2SO_4 was added to form a layer and the colour at interphase was recorded. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

3. Results and Discussion

Plants are the most fascinating ailments for the treatment of diseases [31]. These are the sources of many potential drugs [32]. Herbal medicines have been used for so many years that have lesser side effects and less priced than the synthetic medicine.

The Active compounds of many drugs found in plants are secondary metabolites [33, 34]. For a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases. In the early century of mankind, plant derived secondary metabolites have been used by humans to treat acute infections, health disorders and chronic illness. Only during the last 100 years natural products have been largely replaced by synthetic drugs [35]

The Arial parts of the *Ficus dalhousiae* Miq were extracted with the appropriate five solvents and these were evaluated for the qualitative phytochemical analysis and these can be further studied for the novel compounds and their biological activities. The hexane and chloroform extracts have much lesser compounds when compared with the other three extracts. When compared to the five extracts methanol and water extracts have high affinity towards the biological activities. These extractions were performed as successive extraction method and due to this almost all the compounds have been isolated and extracted efficiently. The qualitative phytochemical analysis mainly focused on different chemical compounds which can be useful for the drug discovery and effective medicine improvement from the natural resources. The hexane and chloroform extracts have none of the compounds according to these analysis only few compounds are soluble in the hexane and chloroform solvents due to its non-polarity nature. The acetone extraction contains alkaloids, phenolics, flavonoids, tannins, coumarins and reducing sugars. The Methanol extract has more phyto constituents than the remaining solvents due to its high polarity nature; methanol extract contains phenols, flavonoids, steroids, tannins, saponins and reducing sugars. Finally water is the universal solvent, due to its inorganic nature some of the compounds may not be dissolved, water extract contains phenols, steroids, saponins and reducing sugars.

More over flavonoids have admirable effects like antimicrobial, anti-inflammatory and anticancer activities [36]. Saponins have wide range of medicinal activities like anti-inflammatory, antioxidant, anti-cancer, hyperglycemic and also antifungal properties [37].

4. Conclusion

Ficus dalhousiae Miq is a very rare medicinal plant collected from Talakona forest of Andhra Pradesh, India. Plant parts were subjected to various solvents to extract their compounds and those are evaluated to assess their bio-activity. The methanol extracts of the plant parts were found to contain phenols, flavonoids, steroids, tannins, saponins and reducing sugars. Further study of these compounds and their separation and purification will provide way for future research.

Table 1: phytochemical Analysis of Whole Arial Part Extracts of *Ficus dalhousiae* Miq

S. No	Tests	Hexane Extract	Chloroform Extract	Acetone Extract	Methanol Extract	Water Extract
01.	**Alkaloids**					
	Mayer's	Negative	Negative	Positive	Positive	Negative
	Dragon	Negative	Negative	Positive	Negative	Negative
	Wagner's	Negative	Negative	Positive	Negative	Negative
	Hager's	Negative	Negative	Positive	Negative	Negative
02.	**Phenolics**					
	$Fecl_2$ Test	Negative	Negative	Positive	Positive	Positive
03.	**Flavonoids**					
	Lead Acetate Test	Negative	Negative	Positive	Positive	Negative
	NaOH Test	Negative	Negative	Positive	Negative	Negative
	Ethyl acetate Test	Negative	Negative	Positive	Positive	Negative
04.	**Anthraquinone Test**					
	Borntrager's Test	Negative	Negative	Negative	Negative	Negative
05.	**Steroids**					
	Salkowski's Test	Negative	Negative	Negative	Positive	Positive
06.	**Tannins**					
	$Fecl_2$ Test	Negative	Negative	Positive	Positive	Negative
	Lead acetate Test	Negative	Negative	Positive	Positive	Negative
	Pot. dichromate Test	Negative	Negative	Positive	Positive	Negative
07.	**Saponins**					
	Vigorous Shaking Test	Negative	Negative	Negative	Positive	Positive
08	**Anthocyanins**					
	Ammonia-HCl Test	Negative	Negative	Negative	Negative	Negative
09.	**Leuco- Anthocyanin**					
	Iso Amyl Alcohol Test	Negative	Negative	Negative	Negative	Negative
10.	**Coumarins**					
	NaOH Test	Negative	Negative	Positive	Negative	Negative
11.	**Reducing Sugars**					
	Keller-Kiliani Test	Negative	Negative	Positive	Positive	Positive

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