

Research Article

Pharmacognostic and Phytochemical Evaluation of *Gynocardia odorata* Leaves

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Abstract: According to World Health Organization (WHO) medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants”. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties. In this section the results of various investigations carried out were compiled. All the pharmacognostic and phytochemical evaluations are performed in order to make an attempt to discuss these results, in order to provide convincing reasons for the studies performed. The TLC studies showed in naked eyes and under different wavelength the presence of some compound like, Flavonoid, Triterpinoids and their significant Rf values.

Keywords: Pharmacognostic, medicinal plant, evaluations, alkaloids

INTRODUCTION

“A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs”[1]. This definition of medicinal plant has been formulated by WHO (World Health Organization). The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants”. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties[2]. Medicinal and aromatic plants of high altitude region are an invaluable resource not only to local communities and the nation, but also to the global community at large. They have high ecological values as well as poor rural communities are highly dependent on them for their health and economic benefit derived from harvesting for trade. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease.

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate

illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Furthermore; many western drugs had their origin in plant extract. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment / management of various diseases. Herbal drugs or medicinal plants, their extracts and their isolated compound (s) have demonstrated spectrum of biological activities. Such have been used and continued to be used as medicine in folklore or food supplement for various disorders. Ethnopharmacological studies on such herbs/medicinally important plants continue to interest investigators throughout the world [3,4].

MATERIALS AND METHODS

This section deals with the detailed description of methods employed for carrying out different studies categorized into following headings.

Pharmacognostical evaluations

Collection and authentication of the plant material

The plant *Gynocardia odorata* is collected in the month of August-September from Singtam and Rangpo of Sikkim. The plants were identified and

authenticated by Botanical Survey of India, Gangtok zone Sikkim. The leaves were collected and cleaned thoroughly to remove the earthy matters or adherent impurities. The dried leaves were powdered by means of mechanical grinder. The resulting powdered material was stored in air tight glass container for further studies.

Macroscopical examination

The macroscopical observation were carried out as per performed by the standard methods to determine the shape, size, taste, color, odour [5].

Microscopical examination

Microscopic evaluation of leaves part was carried out by taking the transverse section of leaves [5].

Powder microscopy

The plant material were properly cleaned and dried first in the shade and then artificially in an oven at 60^o C for approximately 24 hours. The dried leaves were then subjected to size reduction to coarse powder using mixture grinder. The powder was then passed through sieve no 60. This was further treated with different reagents like Phloroglucinol and conc. HCl (1:1), iodine solution for the presence of the constituents like lignin, starch and calcium oxalate crystals.

Proximate analysis

Proximate analysis of the crude drug (leaves) was carried[6]. Following determinations were made using the procedure described under each heading:

- a) Extractive Value
- b) Fluorescence Analysis
- c) Moisture Content
- d) Ash Value

Extractive values

Extractive values of a crude drug determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which no suitable chemical or biological assay exists.

Water soluble extractive

This method is applied to a drug which contains water soluble active constituents of crude drugs such as tannins, sugars, plant acid, mucilage, glycoside etc.

Method

Macerate about 5gm of the coarse powdered air dried leaves of *Gynocardia odorata* with 100ml of chloroform water (2.5ml in 1000ml water) in a stopper flask for 24 hours, shaking frequently during first six hrs. Filter through filter paper taking precaution against excessive loss of solvent. Evaporate 25ml of water extract to dryness in a tarred flat bottomed shallow dish. Dried at 105^oC and weighed. The percentage w/w of water soluble extractive value was calculated with reference to the air dried drug.

Alcohol soluble extractive

This method is frequently employed to determine the approximate resin content of drug.

Method

Macerate about 5gm of the coarse powdered air dried leaves of *Gynocardia odorata* with 100ml of alcohol (95% ethanol) in a stopper flask for 24 hours, shaking frequently during first six hrs. Filter through filter paper taking precaution against excessive loss of alcohol. Evaporate 25ml of water extract to dryness in a tarred flat bottomed shallow dish. Dried at 105^oC and weighed. The percentage w/w of alcohol soluble extractive value was calculated with reference to the air dried drug.

Fluorescence analysis

The organic molecules absorbs light usually over a specific range of wavelength, get excited to a high energy level and many of them emit such radiations while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence" [91].

Method

The powdered drug was examined under U.V. and ordinary light with different reagents. About 10 gm of the powdered drug was taken in a petridish and treated with different reagents viz., methanol, methanolic sodium hydroxide, 50% sulphuric acid, 50% nitric acid, 5% potassium hydroxide, 1N Hydrochloric acid, 1N methanolic sodium hydroxide. These were observed under different wavelengths i.e., visible rays and ultraviolet rays (254 nm and 365 nm). Various colour radiations emitted which were observed and noted. The powder was treated under visible and ultra violet rays. The results are presented in the table.

Determination of moisture content

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Drying plays a very important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and gives suitable condition, to the proliferation of living micro-organisms.

Method

About 5 gm of the air dried crude drug was accurately weighed in a tarred watch glass. The drug was kept in hot air oven at 105^oc and dried for a period until constant weight obtained. The difference in weight gives the moisture content of the drug.

Determination of ash values

Ash values are helpful in determining the quality and purity of a crude drug especially in a powdered form. The object of ashing vegetable drug is to remove all traces of organic matter that may otherwise interfere in an analytical determination. On incineration,

a crude drug normally leaves an ash consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Determination of total ash

About 3 gm of the powdered drug was accurately weighed and taken in a silica crucible, which was previously ignited and weighed. The powdered drug was spread in a fine even layer at the bottom of the tarred crucible. The crucible was kept inside the muffle furnace and the temperature increased to make it dull red hot until free from carbon. The crucible was cooled, kept in a desiccator and weighed. The procedure was repeated to get the constant weight. The percentage of total ash was calculated with reference to the air dried drug. The total ash value of the entire plant was noted.

Determination of acid- insoluble ash

The ash obtained (as described above) was boiled with 25 ml of dil. HCl for 5 minutes. The insoluble ash was collected in ash less filter paper and washed with hot water. The insoluble matter was transferred into tarred silica crucible, ignited and weighed. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of water soluble ash

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Qualitative phytochemical analysis

Preparation of plant extracts

The leaves of the plant were collected and washed thoroughly with water to remove any unwanted material. This was further dried in shade. After complete drying it was powdered and passed through sieve no. 60 and stored in an air tight container.

Extraction of crude drugs

Accurately weighed 500 gm of powdered leaves were taken and was extracted in a round bottom flask (cold maceration) with the solvent of increasing polarity as follows,

- Chloroform
- Methanol
- Ethanol
- Water

All the extracts were concentrated by distilling off the solvents and individual extracts were dried in an oven at 50°C. The consistency, color, appearance and percentage yield of the extracts were measured. Prior to perform the next extraction with the new solvent of different polarity, the materials were dried in hot air oven below 50°C. Finally the marc was macerated with chloroform water for 24 hours to obtain the aqueous extract.

Phytochemical screening

The concentrated extracts were subjected to chemical test as per the methods mentioned[7] below for the identification of the various constituents.

Qualitative chemical test

The extracts obtained in the successive solvent extraction process were then subjected to various qualitative tests to determine the presence of various phytoconstituents such as alkaloids, glycosides, saponin, flavonoids, carbohydrates, amino acids, sterols, tannins and mucilage etc. The concentrated extracts were subjected to different chemical test as per the methods mentioned below for the identification of the various constituents.

Test for alkaloids

50mg of solvent free extract is taken and it is then stirred with few ml of dilute hydrochloric acid & filtered. The filtrate is tested carefully with various alkaloidal reagents as follows:

- **Mayer's test:** To a few ml of filtrate add 1 or 2 drops of Mayer's reagent (potassiummercuric iodide solution) by the side of the test tube. A white or creamy precipitate indicates the test is positive for alkaloids.
- **Wagner's test:** To a few ml of filtrate, few drops of Wagner's reagent (solution of iodine in potassium iodide) are added by the side of the test tube. A reddish-brown ppt indicates test as positive.
- **Hager's test:** To a few ml of filtrate, 1 or 2ml of Hager's reagent (saturated solution of picric acid) are added by the side of the test tube. A prominent yellow ppt indicates test as positive.
- **Dragendorff's test:** To a few ml of filtrate, 1 or 2ml of Dragendorff's reagent (solution of potassium bismuth iodide) are added by the side of the test tube. A prominent reddish brown ppt indicates test as positive.

Test for carbohydrates

100mg solvent free extract is dissolved in 5ml of water & filtered. The filtrate is subjected to the following tests.

- **Molish's test:** To 2 ml of filtrate, 2 drops of alcoholic solⁿ of alpha-naphthol are added, the mixture is shaken well & 1ml of conc. H₂SO₄ is added slowly along the side of the test tube & allowed to stand. A violet ring indicates the presence of carbohydrate.
- **Fehling's test:** 1ml of filtrate is boiled on water bath with 1ml each of Fehling solution A (copper sulphate in distilled water) & Fehling solution B (alkaline sodium potassium tartrate in distilled water); a red ppt indicates the presence of sugar.
- **Benedict's test:** To 0.5ml of filtrate, 1ml of Benedict's reagent (combination of copper sulphate, sodium citrate and sodium carbonate) is added. The mixture is heated on a boiling water bath for 2 min. a characteristic colored ppt indicates the presence of sugar.
- **Barfoed's test:** To 1 ml of filtrate, 1 ml of Barfoed's reagent (copper acetate and acetic acid) is added & heated on a water bath for 2 min. Red ppt. indicates presence of sugar.

Test for saponins

50 mg extract is taken and diluted with distilled water and volume made up to 20ml. The suspension is shaken for 15 min. A layer of 2 cm of foam indicates the presence of saponins.

Test for phenolic compounds

- **Ferric chloride test:** 50mg of extract is dissolved in 5ml of distilled water. To this few drops of natural 5% ferric chloride solution is added. A dark green color indicates the presence of Phenolic compounds.
- **Lead acetate test:** 50mg of extract is dissolved in distilled water & to this; 3ml of 10% lead acetate solution is added. A bulky white ppt. indicates the presence of Phenolic compounds.

Test for glycoside

- **Legal's test:** 50mg of extract is dissolved in pyridine, sodium nitroprusside solution is added & made alkaline using 10% NaOH & presence of glycoside is indicated by pink color.
- **Borntrager's test:** 50 mg of extract is hydrolyzed with concentrated hydrochloric acid for 2hr on a water bath and then filtered it. To 2ml of filtered hydrolysate, 3ml of CHCl₃ is added & shaken, CHCl₃ layer is separated & 10% NH₃ solution is added to it; pink color indicates the presence of glycosides.
- **Keller-Killiani test:-** To the extract add glacial acetic acid and to this few drops of ferric chloride & conc. Sulfuric acids are added. A reddish brown color is formed at the junction of two layers & the upper layer turns bluish green.

Test for tannins

- **Gelatin test:** 50mg of extract is dissolved in 5ml of distilled water & add 1% solution of gelatin

containing 10% sodium chloride solution is added. White ppt. indicates the presence of Tannins.

- **Goldbeater's skin test:** Soak a small piece of goldbeaters skin (a membrane prepared by the skin of ox) in 2% hydrochloric acid rinse with distilled water and placed in the solution to be tested for 5 minutes. Wash with distilled water and transferred it to 1 percent solution of ferrous sulphate. A brown or black colour on the skin denotes the presence of tannins.
- **Phenazone test:** 50mg of extract is dissolved in 5ml of distilled water & add 0.5gm of sodium acid phosphate; warm, cool and filter. To the filtrate add 2% solution of phenazone. Bulky coloured ppt. indicates the presence of tannins.

Test for steroids and triterpenoids

- **Libbermann- Burchard test:** The extract is treated with few drops of acetic anhydride and boil cool then concentrated H₂SO₄ is added from the side of the test tube, brown ring is formed at the junction of two layers and the upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

Test for flavonoids

- **Alkaline reagent test:** An aqueous solution of the extract is treated with 10% NH₄OH solution. Yellow fluorescence indicates the presence of flavonoids.
- **Aqueous sodium hydroxide test:** An aqueous solution of the extract is treated with sodium hydroxide solution it gives blue to violet (anthocyanins), yellow (flavones), and yellow to orange (flavonones).
- **Concentrated sulphuric acid test:** An aqueous solution of the extract is treated with concentrate sulphuric acid it gives yellowish orange (anthocyanins), yellow to orange (flavones), orange to crimson (flavonones)
- **Magnesium & hydrochloric acid reduction:** 50mg of extract is dissolved in 5ml of alcohol & few fragment of magnesium ribbon & conc. HCl acid (drop wise) is added. If any pink to crimson color develops, presence of flavanol glycosides is inferred.

Test for proteins & amino acids

100mg of extract is dissolved in 10ml of distilled water & filter through Whattmann filter paper no.1& the filtrate is subjected to tests for proteins & amino acids.

- **Millon's Test:** To 2ml of filtrate, few drops of Millon's reagent (combination of mercury, fuming nitric acid and distilled water) are added. A white ppt. indicates the presence of proteins.
- **Biuret Test:** An aliquot of filtrate is treated with one drop of 2% copper sulphate solution. To this 1ml of ethanol (95%) is added, followed by excess

of potassium hydroxide palat. Pink color in the ethanolic layer indicates the presence of proteins.

- **Ninhydrin Test:** Two drops of Ninhydrin solution (10mg of Ninhydrin in 200 ml of acetone) are added to 2ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Test for fixed Oils & fats

- **Spot Test:** Press a small quantity of extract separately between two filter papers. Oil stains on the paper indicate the presence of fixed oil.
- **Saponification Test:** Add a few drops of 0.5 N alcoholic KOH to a small quantity of extract along with a drop of phenolphthalein. Heat the mixture on water bath for 1-2 hr. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils & fats.

Test for gums & mucilages

100 mg of extract is dissolved in 10 ml of distilled water & to this 25 ml of absolute alc. is added with constant stirring. White or cloudy ppt. indicates the presence of gums & mucilage.

Chromatography Technique

Chromatography is a technique of separation of mixture into individual component using a stationary phase and a mobile phase. For the separation of a component TLC technique is applied here[8,9]. The principle of separation is adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase (solvent) runs because of capillary action. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster. Thus the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

Practical requirements

- Stationary phase
- Glass plate
- Preparation and activation of plate
- Application of sample
- Development tank
- Mobile phase
- Development technique
- Detecting and visualizing agent

Stationary phase

There are several adsorbents which can be used as stationary phase. Some of the stationary phases, their composition and the ratio in which they have to be mixed with water or solvents to form slurry for preparing TLC plates. The stationary phases used are as follows:

- Silica gel H
- Silica gel G

- Silica gel GF
- Al₂O₃ G
- Cellulose powder
- Kieselguhr G

Glass plate

Glass plates which are specific dimensions like 20 cm X 20 cm (full plate), 20 cm X 10 cm (half plate) and 20 cm X 5 cm (quarter plate) can be used. These dimensions are used since the width of the commercially available TLC spreader is 20 cm.

Preparation and activation of TLC plate

After preparing the slurry the TLC plate can be prepared by using one of the following techniques: pouring, dipping, spraying and spreading.

In pouring technique, the slurry is prepared and poured on to a glass plate which is maintained on a labeled surface. The slurry is spread uniformly on the surface of the glass plate. After setting, the plate is dried in an oven. The disadvantage is that uniformity in thickness cannot be ensured.

In dipping technique, two plates are dipped into the slurry and are separated after moving from slurry and later dried. The disadvantage is that a larger quantity of slurry is required even for preparing fewer plates.

Spraying technique resembles that of a using a perfume spray on a cloth. The suspension of adsorbent or slurry is sprayed on a glass plate using a sprayer. The disadvantage is that the layer thickness cannot be maintained uniformly all over the plate.

Spreading is the best technique where a TLC spreader is used. The glass plates are stacked on a base plate. The slurry after preparation is poured inside the reservoir of TLC spreader. The thickness of adsorbent layer is adjusted by using a knob in the spreader. Normally a thickness of 0.25mm is used for analytical purpose and 2mm thickness for preparative purpose. Then the spreader is rolled only once on the plates. The plates are allowed for setting. This is done to avoid cracks on the surface of the adsorbent. After setting, the plates are activated by keeping in an oven at 100°C to 120°C for 1 hr.

Application of sample

Usually to get good spots, the concentration of the sample or standard solution has to be minimum 2-5µl of a 1% solution of either standard or test sample is spotted using a capillary tube or micropipette. The spots can be placed at random or equidistant from each other by using a template, with marking. The spot should be kept at least 2cm above the base of the plate. And spotting area should not be immersed in the mobile phase in the development tank.

Mobile phase

Pure solvent or mixtures of solvents are used. The following gives a list of solvents (of increasing polarity) – Petroleum ether, Carbon tetrachloride, Cyclohexane, Carbon disulphide, Ether, Acetone, Benzene, Toluene, Ethyl acetate, Chloroform, Alcohols, Water, Pyridine, Organic acids, etc.

Detecting or visualizing agent

After the development of TLC plates, the spots should be visualized. Detecting coloured spots can be done visually. But the detecting colourless spots, anyone of the following techniques can be used.

- a) **Non-specific method:** where the no. of spot can be detected, but not the exact nature or type of the compound.
 1. **Iodine chamber method:** where brown or amber spots are observed when the TLC plates are kept in a tank with few iodine crystals at the bottom.
 2. **Sulphuric acid spray reagent:** 70-80% v/v of sulphuric acid with few mg of either potassium dichromate or potassium permanganate or few ml of nitric acid as oxidizing agent is used. This reagent after spraying on TLC plates is heated in an oven. Black spots are seen due to charring of compounds.
 3. **UV chamber for fluorescent compounds:** when compounds are viewed under UV chamber, at 254nm or at 365nm, fluorescent compounds can be detected. Bright spot are seen under a dark background.
 4. **Using fluorescent stationary phase:** when the compound is not fluorescent, a fluorescent stationary phase is used. When the plates are viewed under UV chamber, dark spots are seen on a fluorescent background.
e.g. – Silica gel GF
- b) **Specific methods:** Specific spray reagents or detecting agents or visualizing agents are used to find out the nature of compounds or for identification purposes.
 - e. g..
 - i) Ferric chloride - for phenolic compounds, tannins and coumarin
 - ii) Ninhydrin in acetone – for amino acids
 - iii) Dragondroff's reagent – for alkaloid
 - iv) 3, 5 dinitrobenzoic acid – for cardiac glycosides

- v) Anisaldehyde-sulphuric acid reagent – for triterpenoids

Analysis

The Rf (Retardation factor) value is calculated for identifying the spots i.e. in qualitative analysis. Rf value is the ratio of distance travelled by the solute to the distance travelled by the solvent front.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent front}}$$

The Rf value range from 0 to 1 but ideal value are from 0.3 to 0.8. Rf value is specific and constant for every compound in a particular combination of stationary and mobile phase. When the Rf value of a sample is same, the compound is identified by its standard.^[94]

RESULTS AND DISCUSSIONS

In this section the results of various investigations carried out were compiled. An attempt has also been made to discuss these results, in order to provide convincing reasons for the studies performed.

Pharmacognostical Studies

Macroscopic Observation

The results of macroscopic observation of leaves of *Gynocardia odorata* were given in Table-1.

Microscopic Examination

T.S. of leaves of *Gynocardia odorata* under microscope

Transverse section of leaves of *Gynocardia odorata* by phlorogucinol: HCl (1:1) shown in Fig-1 and with weak Iodine solutions in shown in Fig-2.

Powder Microscopy of leaves

The powdered leaves of *Gynocardia odorata* was mounted with iodine solution, conc. H₂SO₄, phloroglucinol and HCl (1:1) and saffranin. The following elements were observed and shown in Fig-3.

Flatted Starch grains, Fragment of vessels, Fibres, trichome, Calcium oxalate crystal, Xylem, Phloem.

Table 1: Macroscopic observation of leaves of *Gynocardia odorata*

Length: 14-15 cm	Width: 4-5 cm
Odour: Characteristic	Venation: Reticulate
Taste: Mild bitter	Base: Symmetrical
Margin: Entire	Colour: Green (upper surface)
Shape: Elliptical or oval	Apex: oblong
Surface of leaf: Coiraceous(texture of leather)	

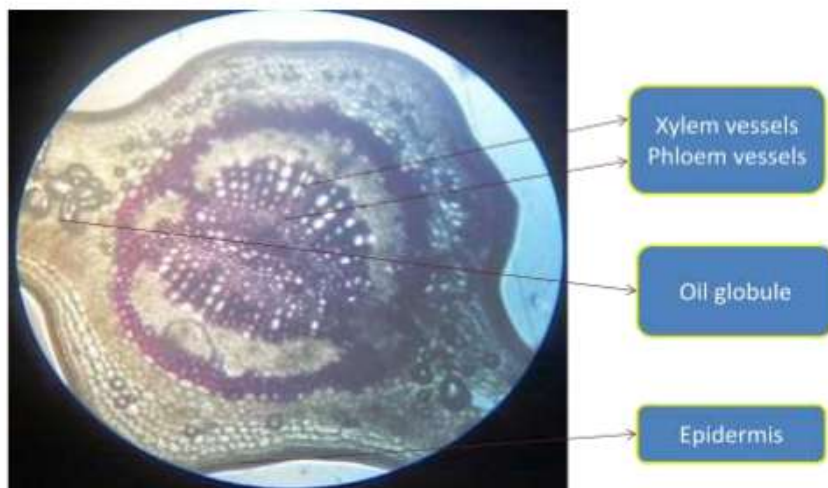


Fig-1: Transverse section of leaves of *Gynocardia odorata* by phloroglucinol: HCl (1:1)

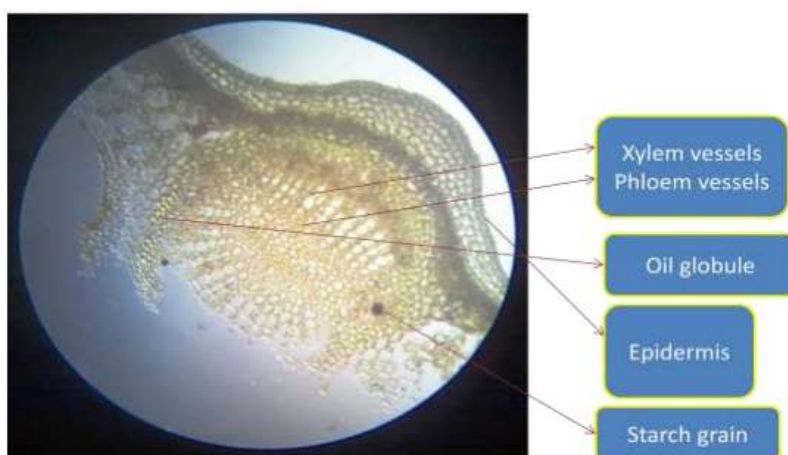


Fig-2: Transverse section of leaves of *Gynocardia odorata* by weak Iodine solutions

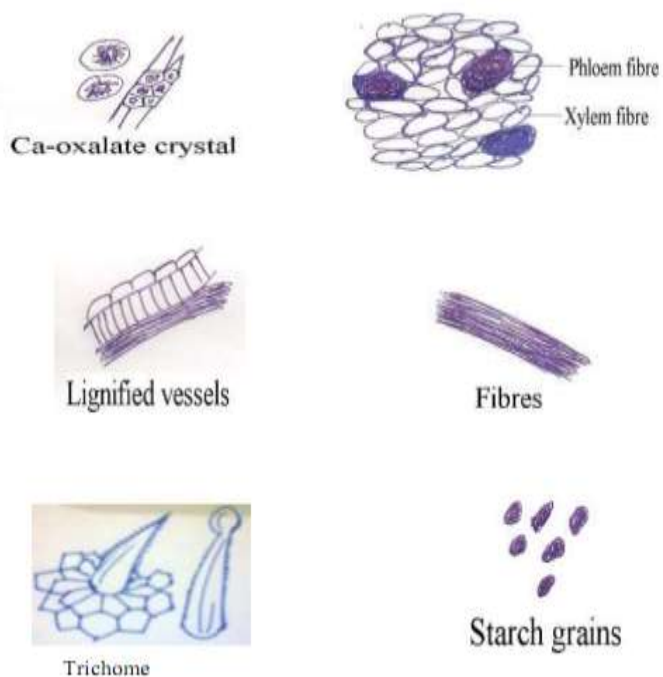


Fig-3: Powder microscopy of leaves of *Gynocardia odorata*

Proximate Analysis

The result of extractive values, moisture content, ash values, fluorescence analysis of the leaves

of *Gynocardia odorata* are shown in table no 2, 3, 4 and 5.

Table 2: Extractive value of leaves of *Gynocardia odorata*

Sl.No.	Type of extractive values	Percentage(w/w)
1.	Alcohol soluble extractive	5.520%
2.	Water soluble extractive	9.040%

Table 3: Moisture content of leaves of *Gynocardia odorata*

Wt. of drug	Initial wt of drug + petridish (gm)	Constant wt. after drying (gm)	Loss on drying (gm)	Moisture content
5 gm	40.668	40.109	0.559	11.18

Table 4: Ash value of leaves of *Gynocardia odorata*

Sl.no	Types of ash values	Percentage yield
1	Total ash	3.95%
2	Acid insoluble ash	0.37%
3	Water soluble ash	1.31%

Table 5: Fluorescence analysis of the powdered leaves of *Gynocardia odorata*

Treatment	Visible rays	Ultra -violet light	
		short wave length (254 nm)	long wave length (365 nm)
Powder	Light green	Light green	Deep blue
Powder + 50% H ₂ SO ₄	Light green	green	Dark green
Powder + 50% HNO ₃	Light green	green	Light green
Powder + 5% KOH	Light green	Light green	Deep blue
Powder + Methanol	Light green	Dark green	Dark green
Powder + 1N Hcl	Light green	Light green	Dark green
Powder + 1N NaOH	Light green	green	Light green

Treatment of the powdered drug with different reagents/solvents

The powdered drug was treated with different solvents/ reagents and the result are displayed in the table 6.

Table 6: Treatment of the powdered drug with different reagents/solvents

Sl. No.	Treatment	Observation
1.	Powder + distilled water	Yellowish green
2.	Powder + 5% FeCl ₃	Greenish black
3.	Powder + Glacial acetic acid	Brown
4.	Powder + 5% Aq. KOH	Brown
5.	Powder + 50% HNO ₃	Reddish brown
6.	Powder+ $\frac{N}{10}$ Iodine solution	Dark reddish brown
7.	Powder + con. H ₂ SO ₄	Brownish black
8.	Powder + con. HCl	Green
9.	Powder + Methanol	Light green
10.	Powder + 5% Aq. NaOH	Green

Phytochemical studies**Successive solvent extraction**

Successive solvent extraction was carried out and the results are presented in the table 7.

Phytochemical analysis

The extracts were subjected to phytochemical analysis and the results are displayed in the table 8.

Table 7: Successive solvent extractive values and nature of extracts

Sl. No.	Solvent	Colour	Consistency	Extractive value (% w/w)
1	Chloroform	Dark green	Sticky	2.81%
2	Methanol	Light green	Sticky	2.43%
3	Ethanol	Dark green	Sticky	0.77%
4	Water	Reddish brown	sticky	9.05%

Table 8: Preliminary phytochemical screening of leaves of *Gynocardia odorata*

Test	C	M	E	W
Alkaloids	—	—	—	—
Carbohydrates	—	—	—	—
Glycosides	—	+	+	+
Phytosterols	—	—	—	—
Fixed oil and fats	+	—	+	+
Phenolic compound and Tannins	+	+	+	+
Saponins	—	+	—	—
Proteins and Amino acids	—	—	—	—
Gums and Mucilage	—	—	+	+
Steroid and triterpenoids	+	+	—	+
Flavonoids	—	+	+	—

C = Chloroform, M = Methanol, E= Ethanol, W = Water, in pro = in progress

Thin Layer Chromatography:

TLC study confirmed that the presence of some compound in methanolic extract of leaf like flavonoids,

triterpenoids, cyanogenic glycoside. Result of TLC study for methanolic extract (leaf) is given in the table-9 and Fig-4.

Table 9: Rf values

Compound	Mobile phase	Ratio	Rf value
Flavonoid	N butanol: Acetic acid: water	3:6:9	0.66
Terpinoids	Toluene: Chloroform: Ethanol	11:9:1	0.35, 0.72

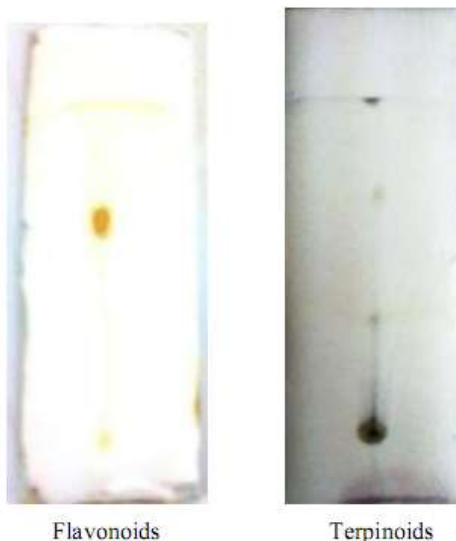


Fig 4: Thin layer chromatography

DISCUSSION

The powdered leaves of *Gynocardia odorata* is green in colour, mild bitter in taste and has characteristic odour. The powdered leaves of the plant was mounted with chloralhydrate, phloroglucinol, weak Iodine and HCl and stained with saffranin; it showed Flatted Starch

grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Trichome, Xylem and Phloem.

The physicochemical constants like ash value such as total ash, acid insoluble ash, water soluble ash, moisture content; extractive values such as water soluble

extractive value and alcohol soluble extractive value were determined. These helped in formulating Pharmacopoeial standards for the drug[10].

Fluorescence analysis of the powder material was also carried out. Powdered drug showed the brown colour in visible rays with different solvents / reagents whereas it was found to be, light green, dark green colour in short UV rays (254 nm) and blue and dark green under long UV rays (365 nm). Behaviors of powder of *Gynocardia odorata* with different reagents / solvents were also observed. This study helped in distinguishing the drug in powder form.

The extracts of the plant material were obtained by successive solvent extraction which was done by using Chloroform, Methanol, Ethanol and Water. Methanol and Ethanol gave reddish brown extract; Chloroform and Water extract gave brown colour extract. Natures of all the extracts were sticky in nature. The quantity of extract was found to be maximum in case of Water extract and minimum in case of Ethanol extract. The extracts obtained by successive solvent extraction were subjected to preliminary phytochemical analysis, which revealed the presence of Flavonoid, Glycosides, Tannins, Fixed oils and fats, Saponins, Steroids and Triterpinoids.

The TLC studies showed in naked eyes and under different wavelength. The presence of some compound like, Flavonoid, Triterpinoids and their significant Rf values were found.

CONCLUSION

Pharmacognostic and phytochemical evaluation of *Gynocardia odorata* leaves were performed and found important phytochemicals like flavonoid, glycosides, tannins, triterpinoids, etc. which may responsible for

various biological activities. Further pharmacological screenings for various activities are recommended.

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