

Research Article

Preliminary Pharmacognostical Evaluation of *Givotia Moluccana* Leaves

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ABSTRACT

Preliminary Pharmacognostical screening was studied in *Givotia moluccana* leaves to establish authenticity and possible to help and distinguish the drug from other species. The study includes preparation of extract by successive soxhlet extraction for detailed analysis. Macroscopic and microscopic analysis was carried out using powder leaves. Fluorescence analysis of the leaf extract and powder were noted under UV light and normal day light, which signifies there characteristics. Different physicochemical parameters such as percentage yield, extractive value, chemical evaluation were carried out as per WHO recommended physicochemical determinations and authentic phytochemical procedure's. Preliminary qualitative chemical tests for the extract shows the presence of alkaloids, carbohydrates, proteins and amino acids, flavonoids and tannins.

Keywords: *Givotia moluccana*, physicochemical, phytochemical, soxhlet extraction.

INTRODUCITON

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal values of these plants are found in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Natural are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials^[1].

The *Givotia moluccana* vernacular names are telugu-Tella poliki, yell dabba and English-White catamaran tree. They are common in dry deciduous belt, in the slopes of forest, tirumala, talakona, dhumukuralla, SVU Botanical garden in Tirupathi. Leave are alternate haracterous, broadly ovate or orbicular, coarsely dentate, acuminate, glabrous above yellowish tomentose below 5-nerved. Flowers are pale yellow, in axillary or subterminal recemes or paniculate cymes. Perianth lobes 10, biseriate, imbricate, stamens 8-20, exerted. Ovary are 2-3 locular, Ovules 2-3 per locule, pendulous; styles 2, fixed from base. Drupes ellipsoid, epicarp fulvous, tomentose; seeds globose, testa horny. Flowers and fruits are seen during the month of April to August. The plant parts are used as Bark-Rhumantism, Fruit- skin diseases, Seed-Dandruff and psoriasis, Stem-Stem, bark and leaves paste is applied as bandage during deep cuts, Leaves and Root also possess medicinal activity^[2]. It is of great interest to carry out the screening of these plants in order

to validate their use in folk medicine and to reveal the active principle by preliminary study of their constituents. Systematic screening of the plants may result in the discovery of novel active compounds. In this study, Hydro alcoholic extract of *Givotia moluccana* (leaves) and folklore medicine of India which had been described in herbal books were screened and to our knowledge, there is still no report of any studies. Therefore, taking into consideration the problem of unavailability the screening of pharmacognostical evaluation is been carried out in developing of newer drugs even when the biologically active compounds are unknown.

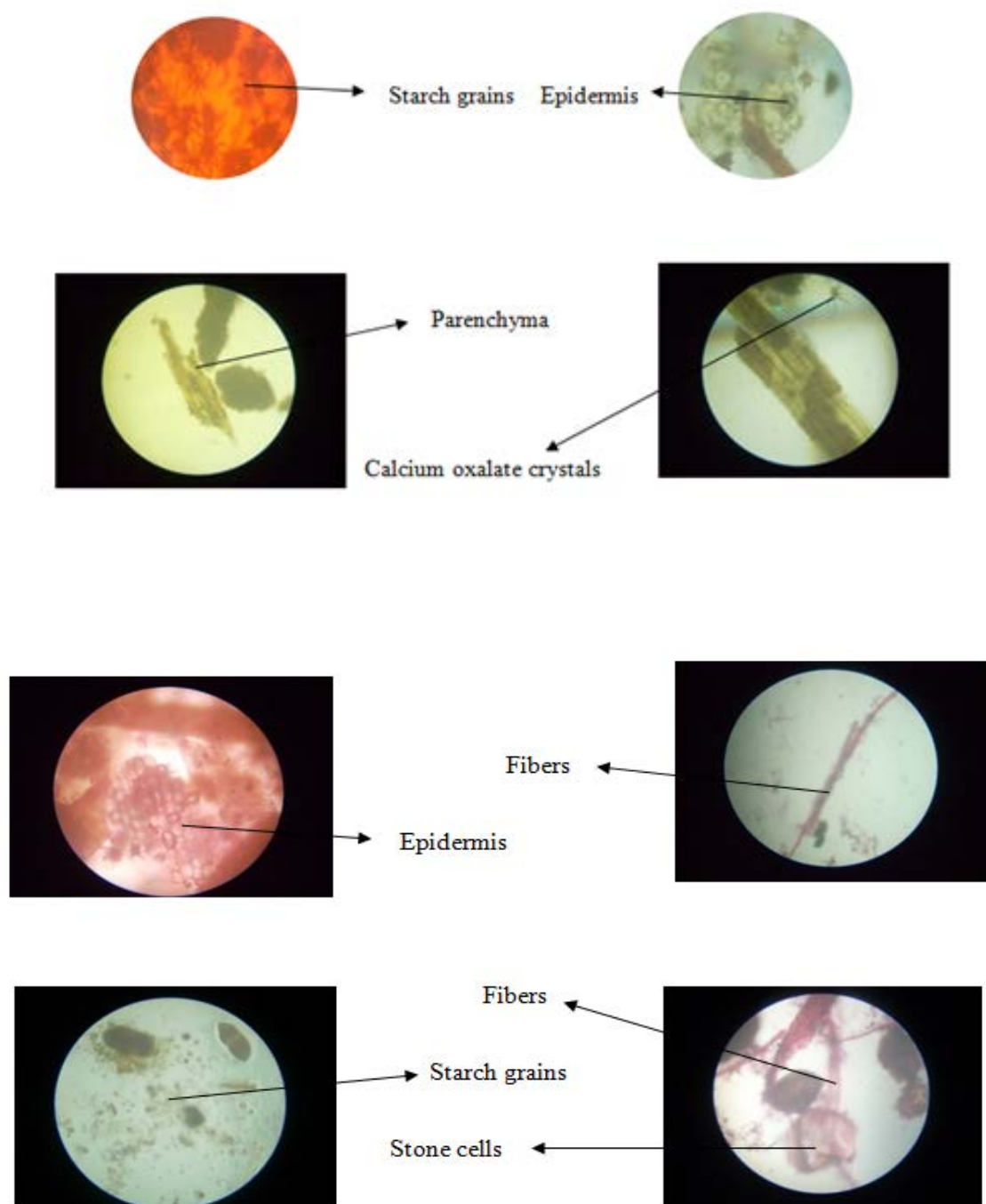
MATERIALS AND METHODS

Procurement of Plant Material: The leaves of *Givotia moluccana*.linn were collected from S.V.University and authenticated by Mr.K.Madhava Chetty,M.Sc, M.Ed, M.Phil, Ph D, PG DPD, Assistant Professor, Dept of Botany,S.V.U.Tirupathi, Andhra Pradesh, India. A voucher specimen (JCOP/Ph'cog/2011/05) was deposited at Jagans College of Pharmacy for future reference. The collected leaves were separated, shade dried, coarsely

Table 1. Percentage yield of extractive values

Extractive value	Leaves
Water extractive	soluble 0.53% w/w
Alcohol extractives	soluble 1.23% w/w
Ether extractives	soluble 0.024% w/w

Powder microscopic characteristic of *Givotia moluccana* leaves



powdered, passed through sieve no 40, and stored in a closed container for further studies.

Preparation of plant extracts: The powdered leaves of 500 mg were taken in 1 liter round bottom flask and extracted with 50% ethanol by hot decoction method (soxhlet) for 4hrs. The extract are cooled at room temperature and evaporated to dryness under reduced pressure in a rotary evaporator^[3,4].

Determination of Percentage yield: The percentage yield, extractive value and loss on drying with various reagents were determined as per the standard procedure.

Preliminary quantitative phytochemical test were performed for extract using specific reagents through standard procedure^[3,4].

Phytochemical studies: Preliminary phytochemical studies of the extract was conducted as per the standard procedure^{3,4,5,6,7}.

PHARMACOGNOSTIC EVALUATION

Organoleptic Evaluation: In organoleptic evaluation, various sensory parameters of the plant material, such as size, shape, color, odor, and taste of the leaves were

Table 2. Data showing the presence of phytoconstituents present in various extracts of *Givotia moluccana*.

S.no	Name of the tests	REAGENTS	OBSERVATION LEAVES
1	Alkaloids	Drug + Dragondroffs reagent Mayer's reagent Hager's reagent Wagner's test	- Yellow ppt - Yellow ppt + Reddish brown +
2	Glycosides	Anthrone + H ₂ SO ₄ + Heat Coumarine Glycosides	Black - -
3	Carbohydrates	Drug + Molish's reagent+ conc.H ₂ SO ₄ Fehling's solution A&B Benedict's test	Purple color ring + Brick red + Brick red +
4	Phytosterols /triterpenoids	Liebermann Test Salkowski Test Noller's test	Orange - - -
5	ProteinsProteins & Amino acids	Biuret test Xanthoprotein test Millon's reagent test Lead acetate test Ninhydrin test	Dark green - Orange + Light brown - White ppt + Reddish orange -
6	Saponins (Foam test)	Drug + water + shaking	Formation of honey comb like froth -
7	Flavonoids	Shinodaw's Test n-HCl acid reduction Test Alkaline reagent test Lead acetate test	- - - White ppt +
8	Fixed oils & Fats	Spot test	Stains appear after drying +
9	Gums/Mucilage	Drug + water	No thickening of the Substance -
10	Volatile oil		-
11	Phenolics /Tannins	Drug + Fecl ₃ lead acetate + water Vanillin hydrochloride acid test Gelatin test	Black - White ppt + - -

recorded. It includes conclusions drawn from studies resulted due to impressions on organs of senses [3,4,5,6,7,8].

Microscopical Investigation: The qualitative features of *Givotia moluccana* leaves were determined using the methods of Evans and for quantitative study anatomical sections, and the microscopy & chemo-microscopy of powdered samples were carried out according to methods outlined by Brain and turner and Evans [3,4,5,6,7,8].

Powder Analysis: To a little quantity of powder taken onto a microscopic slide, 1-2 drops of 0.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added, mounted in glycerol, covered with a cover slip and observed under microscope with 10 × 10 magnification. The characteristic features of the powder viz., xylem fibers, calcium oxalate crystals, starch grains, stone cells etc. were recorded using standard techniques. Fibers and stone cells appear pink in color. Presence of starch grains was detected by the formation of blue color on addition of 2-3 drops of 0.01M iodine solution [3,4,5,6,7,8].

Determination of Alcohol Soluble Extractive Value: Accurately weighed powder (5 g) of leaves was taken and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was

filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight [3,4,5,6,7].

Determination of Water Soluble Extractive Value: Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration [3,4,5,6,7].

Determination of Ether Soluble Extractive Value: Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that ether was used for maceration [3,4,5,6,7].

Preliminary Phytochemical Screening: The extract were used for preliminary phytochemical screening with chemical tests viz., Molisch's, Fehling's, Benedict's and Barfoed's tests for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrin's test for amino acids; Salkowski and Liebermann-Burchard's reactions for steroids; Borntrager's test for anthraquinone glycosides; foam test for saponin glycosides; Shinoda and alkaline tests for flavonoid glycosides, Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids; and ferric chloride, lead acetate, potassium dichromate, dilute iodine tests for tannins and phenolics [3,4,5,6,7,8].

Fluorescence characters of the plant powder: Fluorescence characters of the plant powder and extract under ordinary light and UV light (UV 366 nm) were

Table 3. Data showing Fluorescence analysis of air dried drug with different chemicals

S.no	Particulates of the treatments	Leaves	
		Under ordinary light	Under UV light(366 nm)
1	Powder as such	Greenish yellow	Pale yellow
2	Powder + Acetic acid	Yellow ppt	Dark green
3	Powder + 5% FeCl ₃	Dark orange	Reddish brown
4	Powder + Iodine	Dark red	Greenish yellow
5	Powder + Ammonia	Dark yellow	Dark greenish yellow
7	Powder + 1N HCL	Light yellow	Dark yellowish green
8	Powder + H ₂ SO ₄ (1:1)	Dark red	Greenish black
9	Powder + HNO ₃ (1:1)	Light orange	Pale green
10	Powder + water	Yellow	Light yellow
11	Powder +1N NaOH (water)	Colorless	Light yellow
12	Powder + methanol	Greenish yellow	Greenish yellow

Table 4. Data showing Reagent characteristic of Leaves powder and extract of *Givotia moluccana*

S.no	Reagents	Leaves		Solubility
		Powder (Day light)	Extract(UV)	Powder
1	Petroleum	Pale Yellow	Colorless	Completely insoluble
2	Benzene	Pale Yellow	Pale yellow	Completely insoluble
3	Acetone	Yellow ppt	Yellowish ppt	Completely soluble
4	Ethanol	Pale Yellow	Greenish yellow	Completely soluble
5	Water	Dark brown	Brownish black	Completely soluble

determined wherein the powdered leaf sample and extract showed the visibility of varying colors which are as tabulated in table no. 3. The preliminary phyto-profiling for the leaves extract of *Givotia moluccana* was carried out wherein the consistency was found [9,10,11].

Fluorescence characteristics: When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study [9,10,11].

Behaviour of leaf powder with different chemical reagents: Behaviour of *Givotia moluccana* leaves with different chemical reagents was performed to detect the occurrence of phytoconstituents along with colour changes under ordinary daylight by standard method [9,10,11].

RESULTS AND DISCUSSION

Pharmacognostic Evaluation

A. Percentage yield: The percentage yield obtained by soxhlet extraction is 2.3 gms.

B. Organoleptic characters: In organoleptic evaluation, appropriate parameters like taste, odor, size, shape and color of the leaf were studied.

Plant parts: Leaves.

Colour: Green.

Odour: Faint and Characteristic.

Taste: Slight bitter.

Shape: Outer surface pubescent with pressure markings.

Texture: Soft.

C. Microscopical Characteristics

Powder Analysis – Leaf

Calcium oxalate crystals: Microsphenoidal crystals shaped calcium oxalate crystals are present and may be scattered in the powder.

Xylem vessels: These are large, well developed wide vessels with reticulate and spiral thickening and with numerous bordered pits and surface is lignified.

Xylem: Xylem vessels with annular thickening.

Epidermal trichomes: Unicellular, conical, thick walled with warty cuticle, curved at the base. (as in case of senna leaves).

Starch grains: Rounded or angular, simple or 2-5 compounds (radiate split).

Lignified and non-lignified fibers.

D. Physical Evaluation: Percentages of the extractive values were calculated with reference to air-dried drug are given in table 1.

E. Preliminary Phytochemical Screening: Freshly prepared leaves extract were tested for the presence of phytoconstituents using reported methods mentioned in the standard methods and results are given in table 2.

The different qualitative chemical tests were performed for establishing the chemical profile of the extracts. In the present investigation the extract of leaves was analysed for the presence of alkaloids, carbohydrates, glycosides, proteins, aminoacids, sterols, phenolic compounds, tannins and flavanoids using standard procedures. The hydroalcoholic extract of *Givotia moluccana* showed the presence of alkaloids, carbohydrates, proteins and amino acids, flavonoids and tannins. The results pertaining to this investigation were presented in table 2.

F. Fluorescence Analysis of Extract: The leaves extract are examined in daylight and UV to detect the fluorescent compounds by the reported method. The observations are given in below,

Table 5. Histochemical color reactions of *Givotia moluccana*.

S.no	Reagents	Constituent	Color	Histological zone	Degree of intensity
1	Phloroglucinol + HCl	Lignin	Pink	Xylem, Phloem fibers	+++
2	Weak Iodine solution	Starch	Blue	Starch grains	+++
3	Caustic alkali + HCl	Ca. Oxalate	--	Ca. Oxalate crystals	++
4	5% Aq. KOH	Antraquinone glycosides	--	--	--
5	Dragendorffs reagent	Alkaloids	--	--	--
6	Sbcl3	Steroids/Terpenoids	--	--	--
7	Ruthenium Red	--	--	--	--

+++ High, ++ moderate, + Slight, - Negative

Leaf extract

Under ordinary light – Dark green.

Under UV light (366 nm) – Dark black.

The treatment of powdered drugs with different chemical reagents reveals the presence of different chemical constituents present in the powdered drugs. Fluorescence analysis reveals the presence of chemical constituents with fluorescence character in UV light and color change observed in the visible light. The observations are given in table 3, 4 and the histochemical colour reaction is given in the table 5.

CONCLUSION

The present study on preliminary phytochemical, physicochemical evaluation of *Givotia moluccana* leaves could be used as the diagnostic tool for the standardization of medicinal plants for its bibliographic traditional support. WHO parameters as per WHO guidelines discussed here, can be considered as the identifying parameters to substantiate and authenticate the drug. The pharmacognostic parameters, which are being reported for the first time, could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

The present study on physicochemical characteristics and preliminary phytochemical screening of provide useful information which may help in authenticating the genuine plant along with nature of phytoconstituents present in it. The *Givotia moluccana* may be considered a rich source of natural antioxidants, which justifies its use in folk medicine. Furthermore, evaluation of in vitro antioxidant activity of these extract has also provided interesting results that might be beneficial for the pharmacological use of this plant in clinical trials.

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