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PHARMACOGNOSTIC, PHYTOCHEMICAL, PHYSICOCHEMICAL AND TLC PROFILE STUDY OF LEAVES *BOUGAINVILLEA GLABRA CHOISY (NYCTAGINACEAE)*

Seema Yuvraj Mendhekar*, Pooja Sharad Bhujbal, Vishakha Vikas Shinde, S. L. Jadhav and D. D. Gaikwad

Assistant Professor, Pharmacognosy Department, VJSM's Vishal Institute of Pharmaceutical Education And Research, Ale, Pune412411, Maharashtra-INDIA.

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***Corresponding Author**
Seema Yuvraj Mendhekar
Assistant Professor, Pharmacognosy Department, VJSM's Vishal Institute of Pharmaceutical Education And Research, Ale, Pune412411, Maharashtra-INDIA.

ABSTRACT

This paper was deals with the detailed study of pharmacognostic, phytochemical, physicochemical and TLC profile study of leaves *Bougainvillea glabra Choisy*. This leaves were collected, dried in a shed, made a powder by grinding and Powdered of leaves were subjected to alcohol extraction by Soxhlet extraction method. The extract then subjected for chemical tests to investigate chemical profile of *Bougainvillea glabra*. Work comprises of collection, identification, microscopically, morphologically, phytochemical evaluation of leaves of plant and TS. Shows presence of epidermis, vascular bundles, trichomes (uniseriate, multicellular, bulbulous) and stomata actino-mycetes type. Extraction is carried out by using soxhlet extraction technique. Alcoholic exact of leaf shows presence of alkaloids, carbohydrates, flavonoids, tannins & saponins. Physicochemical analysis was carried out on this leaves, which include parameter such as water soluble ash, acid insoluble ash, sulphated ash of plant were determined. Tlc profiling of plant extract in different solvent system was performed & different rf values are obtained.

KEYWORDS: Bougainvillea glabra Choisy, Microscopical study, TLC, Physicochemical screening.

INTRODUCTION

Bougainvillea was named after the world traveler, Louis de Bougainville, who discovered it in Brazil in 18th century and brought it to Europe where it became both widespread and popular**,** due to its versatility, richness and suitability to thrive in degrading environmental conditions. With its sharp thick thorns it is avoided by cattle, goats, monkeys and even birds. It loves open sunshine and the colors of some varieties grow brighter and more attractive in hot dry climate. A postal stamp was issued by the Indian Postal Department to commemorate this flower.[1] Bougainvillea commonly name as the paper flower owing to bracts are thin and papery. They are thorny woody, vines growing anywhere, its length about 1-12 meters tall, scrambling over other than plants with their hooked thorns. They are evergreen where rainfall occurs all the year, or deciduous if there is a dry season. The leaves are alternate, simple ovate acuminate, 4-13 cm long. The actual flower of the plant is small and generally white but each cluster of three flowers are surrounded of three or six bracts with the bright colors associated with plant, including pink, magenta, purple, red orange, white or yellow.[2]

Bougainvillea glare choicy have been used by the traditional practitioner of Mandsaur in variety of disorders like diarrohea, reduce stomach acidity, cough and sore throat, decoction of dried flowers for blood vessels and leucorrohea and decoction of the stem in hepatitis. The main part used is leaves.[3] The leaves of Bougainvillea glare choicy are reported to have insecticidal activity[4], anti-inflammatory[5], anti-diarroheal activity[6], anti-hyperglycemic activity[7], anti-ulcer[6] and anti-microbial activity.[6] The present investigation is an attempt in this direction and includes morphological evaluation, determination of physico-chemical constants and the preliminary phytochemical screening of the hydro-alcoholic extracts. Herbs are supposed to be safe but many unsafe and fatal side effects have recently been reported. In spite the numerous uses and pharmacological activity attributed of Bougainvillea glabra choicy but less work on pharmacognostical, standardization and phytochemical information regarding the leaves of this plant cultivar Bougainvillea glabra. Hence, the present investigation is an attempt in this direction and includes morphological evaluation, determination of physico-chemical constants and the preliminary phytochemical screening of the alcoholic extracts.

Fig.1: Leaves and flower of *Bougainvillea glabra Choisy*.

Vernacular names

Common name: Bougainvillea, Lesser Bougainvillea

Hindi: Booganbel

Manipuri: Cherei

Bengali: Baganbilas

Marathi: Booganvel

Konkani: Bouganvila

Telugu: Kagithala Puvvu

Taxonomical Classification

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Order: Caryophyllales

Family: Nyctaginaceae

Genus: Bougainvillea

Species: B. glabra.

Part's used – The entired plant leaves, flowers, bark, stem etc. Are used in medicine.

MATERIALS AND METHODS

Collection Of plant material

Bougainvillea glabra plant leaves was collected from samiksha farm nursery in alephata pune. The leaves were dried in shed & then the leaves were powdered & powdered were stored in sterile container for further use.

Extraction preparation

The plant material was dried in shade and crush in the grinder. The dried powder was obtained. Then dried powder was taken into soxhlet apparatus for 72hr according to successive solvent extraction using alcohol as solvent. Afterwards, extract was concentrated and stored for further use.

Description

(A) Macroscopic Examination

Botanical description

Macroscopical studies were done by using simple compound microscope. The shape, size, taste and odour of leaves were determined.

(B) Microscopic Examination

The micropowder analysis was done according to the method of Brain and Turner (1975b)[8] and Kokate (1986a).[1] Epidermis layer is continuous and can be distinguish into upper and lower epidermis, without intercellular space and compact in nature. Upper epidermis is straight walled, single layer containing trichomes (uniseriate, multicellular, bulbulous) and stomata actinomycetes type. Lower epidermis is similer to upper epidermis but it contains trichomes and stomata. Cuticle present on above epidermis and lower to lower epidermis but it is wavier at lower side. Just below the epidermis collenchyma layers present this can be characterized by thick cellulosic deposition. It present in whole length of midrib but not in middle lamina. Cells of upper layers are small ans 4 to 5 layers but cells of lower are comparatively big and 2 to 3 layers. Mesophyll can be diffentiate in spongy and palisade parenchyma cells. Palisade is single layered readily elongated covering 1/10 of the lamina part. Spongy parenchyma is Thin layered loosely arranged containing intercellular spaces. Cells also contain starch (in large amount) and Ca.oxalate crystals (in small amount). It covers remaining part of lamina. Vascular bundles present in spongy tissues, usually 5 in bundles arc shaped, more prominent towards lower side. Each is surrounded by pericycle

single layer. Vascular bundles are surrounded by endodermal layer. Phloem present towards dorsalside while xylem toward ventral side.

Table 1: Morphology of Leaves of *Bougainvillea glabra Choisy*.

Sr.no	Characters	Appearance
1	Colour	Deep Green
2	Odour	Aromatic
3	Taste	Bitter
4	Shape	Oblong Lanceolate
5	Size	2-4.5 inches long and 1.5 – 3 inches in width

Table 2: Staining / Diagnosis/ Microchemical Test.

Sr.No.	Reagents	Observations	Characteristics
1	Phloroglucinol+Hcl(1:1)	Pink	Lignified tissues:xylem (vascular bundle)
2	Sudan Red III	Pink	Cutin/cuticle
3	Ruthenium red	pink	Mucilaginous cells of epidermis
4	Sulphuric acid	Needle shape crystals from calcium sulphate are formed.	Calcium oxalate crystals
5	Alcoholic Picric acid	Yellow	Aleurone Grains
6	Iodine	Blue	Starch
7.	Acetic Acid	Insoluble	Calcium oxalate crystals

Fig.2: Extraction preparation by soxhlet apparatus.

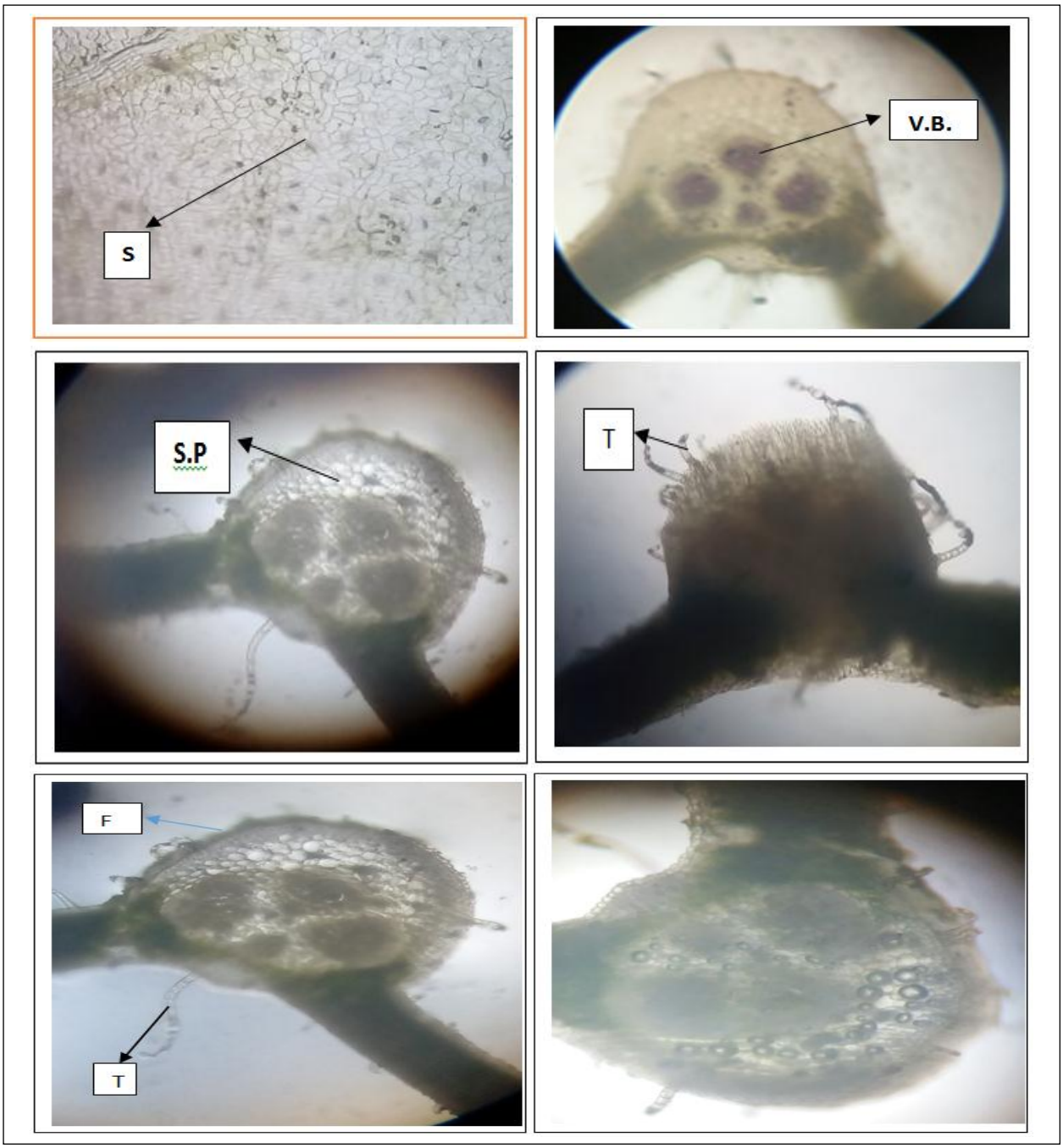

Fig.3: Microscopic Examination of Leaves Bougainvillea glabra Choisy. (S-Stomata, V.B- Vascular bundles, S.P.-Spongy parenchyma, T- Trichomes, E-Epidermis).

(C) Physico-Chemical Parameter

Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed[9] and the WHO guidelines on the quality control methods for medicinal plant materials.[10]

Table 3: Physico-Chemical constant of leaves of Bougainvillea glabra Choisy.

S.N.	Ash value	Observations
1	Total Ash	11.6
2	Acid insoluble Ash	3.9
3	Water soluble Ash	4.5
4	Sulphated Ash	21.2

(D) Phytochemical Screening of Leaves exatract

Phytochemical analysis involves the qualitative analysis of herbal plants. The preliminary qualitative tests have been attempted in Bougainvillea glabra leaves to find out the presence or absence of certain bioactive compounds. Following different qualitative chemical tests were performed to investigate the chemical composition of B.glabra extracts as describe by Kokate[11] and Harbone.[12] Preliminary phytochemical screening was carried out by using standard procedures described by Kokate (1986b)[11] and Harborne (1998).[12] The phytoconstituents present in the alcoholic extract of were expressed in the Table: 4 Phytochemical screening procedure.

1) Test for alkaloids

To the extract dilute hydrochloric acid will be added and filtered. The filtrate will be treated with various alkaloid reagents.

a) Mayer's test: The filtrate will be treated with Mayer's reagent: appearance of cream colour indicates the presence of alkaloids.

b) Dragendroff's test: The filtrate will be treated with Dragendroffs reagent: appearance of reddish brown precipitate indicates the presence of alkaloids.

c) Hager's test: The filtrate when treated with Hager's reagent, appearance of yellow colour precipitate indicates the presence of alkaloids.

2) Test for carbohydrates and reducing sugar

The small quantities of the filtrate will be dissolved in 4ml of distilled water and filtered. The filtrate will be subjected to

a) Molisch's test: A small portion of the filtrate will be treated with Molisch's reagent and sulphuric acid. Formation of a violet ring indicates the presence of carbohydrates.

b) Fehling's test: The extract will be treated with Fehling's reagent A and B. The appearance of reddish brown colour precipitate indicates the presence of reducing sugar.

3) Test for steroids

Liebermann bur chard's test

The extract will be treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green colour indicates the presence of steroids.

4) Test for proteins

a) Biuret test: The extract will be treated with copper sulphate solution, followed by addition of sodium hydroxide solution; appearance of violet colour indicates the presence of proteins.

b) Millon's test: The extract will be treated with Millon's reagent; appearance of pink colour indicates the presence of proteins.

5) Test for tannins

The extract will be treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

6) Test for phenolic compounds

a) The extract will be treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenolic compounds.

b) The extract will be treated with 10% sodium chloride solution; appearance of cream colour indicates the presence of phenolic compounds.

7) Test for flavonoids

a) 5ml of extract will be hydrolyzed with 10%sulphuric acid and cooled. Then, it will be extracting with diethyl ether and divided in to three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution will be added to the first, second and third test tubes respectively. In each test tube. Development of yellow colour demonstrated the presence of flavonoids.

b) Shinoda's test: The extract will be dissolved in alcohol, to which few magnesium turnings will beaded followed by concentrated HCL drop wise and heated, and appearance of magenta colour shows the presence of flavonoids.

8. Test for gums and mucilage

The extract was treated with 25 ml of absolute alcohol, and filtered. The filtrate will examine for its swelling properties.

9. Test for glycosides

When a pinch the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of conc. Sulphuric acid, formation of ring at the junction of two liquids indicates the presence of glycosides.

10. Test for saponins

Foam test

About 1 ml of the extract was diluted to 20 ml of with distilled water and shaken well in a test tube. The formation of foam in the upper part of test tube indicates the presence of saponins.

11. Test for Triterpenoids

The substance was warmed with tin and thionyl chloride. Pink colour indicates the presence of triterpenoids.

Table 4: Phytochemicals of Extracts of Leaves of *Bougainvillea glabra Choisy*.

Phytochemicals	Test	Ethanol
Alkaloids	Dragendorff's test Hager's test Mayer's test	+ + +
Tannins& Phenolics	Ferric chloride test	+
Saponins	Foam Test Lead Acetate Test	+ +
Flavonoids	Shinoda Test Lead Acetate Test	+ +
Carbohydrates	Fehling's Test Molisch Test	+ +
Proteins & Amino Acid	Million's Test Biuret Test	+ +
Phytosterols	Salkowaski test	+
Gum & mucilage	Ruthenium red test	+
Terpenoids	Libermann-Buchardt Test	+

(-)=Absent (+) =Present

Fig. 3: Phytochemical Screening of Leaves exatract.

(E) Thin layer chromatography

The ethanolic extract of plant was performed on thin layer chromatographic (TLC) plates, composed of silica gel g plate .the plate was developed in chamber was previously saturated by mobile phase. The mobile phase was Toluene:Ethyl acetate (93:7) as the solvent system and was seen in the U.V. light.[8] Rf value-0.5 & 0.7 was determined by formula.

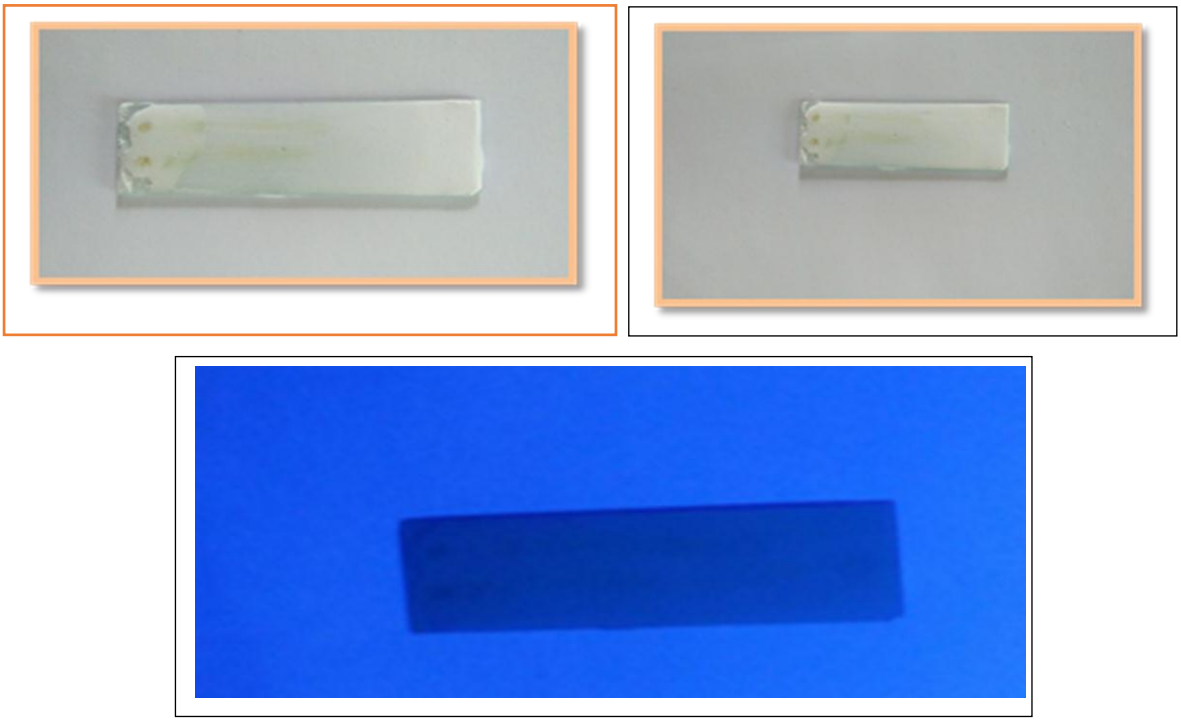

Fig.4: Thin layer chromatography of Leaves Extract.

RESULT AND DISCUSSION

Now-a-days there has been drastic increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the availability of modern techniques, it is more reliable to identify a plant drug by pharmacognostic evaluation. A complete and systematic study of a crude drug which comprises of collection, preservation, storage, macroscopical, microscopical, organoleptic characters, etc. is claimed to be the scientific or pharmacognostic evaluation. Standardization is an essential measure for quality, purity and sample identification. Standardization of herbal drugs is a very challenging task for herbal drug industry because of complex nature and variation of chemical constituents. Microscopical evaluation is one of the simplest methods for identification of drugs. According to WHO, the macroscopic and microscopic evaluation is the first step to be carried out to establish its identity and purity. The evaluation of physico-chemical constants is an important parameter in detecting adulteration or improper handling of drugs. The extractive values are immensely

useful to evaluate the chemical constituents that are present in the crude drug. These extractive values are also helpful in the estimation of specific constituents soluble in particular solvent. The total ash is particularly important in the evaluation of purity of drugs. Ash value varies within equitably wide limits and is therefore an important parameter for the evaluation of crude drug. All crude drugs were standardized for the active phytoconstituents. Here preliminary phytochemical studies confirmed the presence of saponins, flavonoids, glycosides, triterpenoids, carbohydrates and fats. These findings are not only helpful in the pharmacological and therapeutic evaluation of the leaves but also assist in standardization for quality, purity and sample identification. And all the evaluation parameter were mention in Table.1-4.

CONCLUSION

The present study i.e., Pharmacognostic, Phytochemical, Physicochemical And TLC Profile Study Of Leaves of crude drug. Physiochemical and phyto-chemical analysis of leaves confirm the quality and purity of plant and its identification. The leaves of B.glabra was screened for phytochemical constituents and found to be good source of medicinally active elements which can be further exploit to isolate and synthesize modern medicines. This work justifies the need to isolate and characterize the medicinally active compounds.

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