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### RESEARCH ARTICLE

## PHARMACOGNOSTICAL, FLUORESCENCE ANALYSIS, HEAVY METAL TESTING AND TLC PROFILING OF THE PLANT BOUGAINVILLEA GLABRA (CHOISY)

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### Abstract

**Objective:** The study's objective is to examine the plant Bougainvillea glabra (Choisy) efficacy by phytochemical, pharmacognostical, fluorescence analysis, heavy metal testing and TLC profiling.

**Methods:** A variety of solvents, including petroleum ether, chloroform, ethyl acetate, methanol, and water, were used to extract the plant powder. To identify different phytochemical ingredients, the various extracts were subjected to qualitative testing. Heavy metal testing and fluorescence examination in daytime and ultraviolet light (254 nm and 365 nm) were performed on the plant powder. For the identification of active phytoconstituents TLC procedure was performed by using various solvent systems.

**Results:** The water soluble extractive value was found to be greater than that of petroleum ether, methanol, ethyl acetate, and chloroform. The values of total ash were found to be greater than those of water-soluble and acid-insoluble ash. Numerous phytochemical ingredients, including alkaloids, glycosides, carbohydrates, flavonoids, saponins, terpenoids, tannins, and phytosterols, were found in the phytochemical screening results. Bougainvillea glabra leaf powder's fluorescence study revealed distinctive colours with different compounds. Heavy metal concentrations of cobalt, mercury, nickel, silver, and zinc were found to be negative. TLC of alcoholic and aqueous extracts of Bougainvillea glabra showed bands with R<sub>f</sub> 0.4–0.6, suggesting coumarin and flavonoids presence; further spectroscopic analysis is required for confirmation.

**Conclusion:** In conclusion, Bougainvillea glabra leaf extracts include bioactive natural compounds that can be utilized in the creation of novel medications that improve therapeutic use.

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**Introduction:-**

Plants have been utilized as a natural remedy for a number of ailments since ancient times. The knowledge of these plants has been passed down from generation to generation by shamans, healers, and sorcerers [1]. Over 80% of the world's population receives primary healthcare from approximately 20,000 medicinal plants, according to the World Health Organization (WHO) [2]. Because of their primary features, medicinal plants have been employed by phytochemistry and pharmacology to study novel ecological and biodegradable chemical entities that serve in the treatment of various diseases. Furthermore, because herbal treatments are inexpensive and widely accepted, the WHO advocates for their inclusion in national healthcare programs [3,4]. Plant tissue produces secondary metabolites, which are the main active components of the plant and have biological activity with a range of qualities like antibacterial, anti-inflammatory, antioxidant, and analgesic qualities. These active components are extracted from the plant's leaves, stems, flowers, and fruits, among other sections [5-6].

One of the major health issues with high morbidity and mortality rates globally is infectious diseases brought on by bacteria [7-8]. It has been chosen to acquire extracts from medicinal plants as potential antimicrobial agents because of the resistance of bacteria to current antibiotics, which is generated by the indiscriminate use of medications [9-12]. Although hundreds of plant species have been utilized medicinally throughout history [13-16], little is known about their active constituents. For instance, the striking "inflorescences," which are formed by the involucre, which is composed of a set of colorful bracts and the true flower, make *B. glabra* (Choisy), a climbing plant native to Brazil that belongs to the Nyctaginaceae family and thrives in warm climates, of great ornamental and horticultural importance [16-17].

In traditional medicine, *B. glabra* is used to treat gastrointestinal issues including diarrhoea and dysentery as well as respiratory conditions like the flu, cold, cough, bronchitis, and asthma [19-20]. It is also believed to have antimicrobial qualities since it includes active ingredients like flavonoids, tannins, alkaloids, phenols, betacyanins, terpenoids, glycosides, and essential oils. The botany, traditional applications, ecology, toxicity, phytochemistry, antibacterial properties, and antibiofilm of *B. glabra* bracts and flowers—plant organs that are frequently employed in traditional medicine but have received little research—are all thoroughly covered in this paper.

**Traditional uses and importance of *B. glabra*:-**

The involucre of *B. glabra* is widely used in traditional medicine to treat a variety of illnesses, including respiratory conditions. In Mexico, *B. glabra* is known by a variety of names, including butitis, purple buganbilia, paper flower, Santa Rita, and indigenous names like jukua, katsjoxhuan (Popolac), and shpupukuishonat (Mixtec) [21-22]. The most popular ingredient in Mexican traditional medicine is the bougainvillea bracts, which are often confused with flower petals. They are used in a number of recipes to treat lung pain, whooping cough, acne, urine sickness, drowning, and wounds, as well as gastrointestinal problems like diarrhoea and dysentery and respiratory ailments like cough, asthma, flu, and bronchitis [23-24].

In Nigeria, it is used to treat inflammation and as an analgesic. Flowers are frequently ingested in Thailand as a remedy for stomach aches and nausea [24]. Hepatitis, blood vessels, leucorrhea, sore throats, and heartburn are all treated with bougainvillea in Mandsaur [25-26], India. *B. glabra* formulations are used to treat digestive disorders in Africa. Furthermore, it has been demonstrated that *B. glabra*, also known as the "glory of the garden," has antioxidant, antimicrobial, antiviral, insecticide, larvicide, anti-diabetic, anti-lipidemic, anti-hyperglycemic, hepatoprotective, antiulcer, anthelmintic, antipyretic, anti-fertility, and anticancer properties in addition to boosting collagen production and suppressing tyrosinase and TNF activity [27-30].

Other studies have focused on employing bougainvillea betalains as culinary, cosmetic, textile, and medicinal pigments due to their antioxidant and non-toxic properties [31]. Although there are already some natural bougainvillea-based syrups available to treat respiratory tract pain, these products are usually only used as supplements because there are no scientific studies to confirm their efficacy and safety. In addition to its ethnobotanical function, *B. glabra* is considered one of the most important plant species for horticulture worldwide due to its ramifications and abundance of vivid inflorescences that give garden walls, gates, or pergolas a surprising appearance. Although *B. glabra* has several traditional uses, little is known about its chemical and pharmacological properties.

**Materials and Methods:-****Collection and authentication of plant material:-**

The *Bougainvillea glabra* (Willd) plant's leaves were collected in February at the GRY Institute of Pharmacy's medicinal garden in Borawan, MP. An ex-botany professor at the government postgraduate institution in Khargone validated the plant's herbarium specimen, which was kept in the pharmacognosy department. In order to reduce their size, fresh leaves were gathered, cleaned, shade-dried, and then mechanically pulverized. Following its passage through the fine power, it was collected and used for the preparation of the extract and the powder microscopy analysis. A fresh leaf sample was used for microscopy identification. Powdered dried leaves were used for phytochemical studies, ash values, and extractive values. All of the analytical-grade chemicals and reagents were purchased from Kasliwal Brothers in Indore, India.

**Pharmacognostical studies:-****Macroscopic analysis and Micro-scopic evaluation:-**

Shape, size, color, and fracture were evaluated for the macroscopic inspection using the unaided eye and a simple hand lens. For microscopic examination, sections of the fresh leaves were cut with free hands. The resulting slices were boiled in chloral hydrate for 10 minutes in order to eliminate any interfering pigments from the tissues. The sections were treated with phloroglucinol and strong hydrochloric acid for ten minutes before being inspected under a binocular microscope. Surface constants such stomatal number, stomatal index, veins, vein-islets, and vein terminations were investigated in quantitative microscopy using camera lucida. Using a computerized micro-ocular eye piece and a trinocular microscope, microphotographs were captured.

**Physicochemical Constants:-**

Extractive values, moisture content, acid insoluble and watersoluble ash values, and the percentage of total ash value were all measured.

**Ash value Determination:-**

Ash value determination was carried out in accordance with established protocol for total ash, acid insoluble ash, and water soluble ash content [32].

**Determination of total ash:-**

3 grams of the powdered *Bougainvillea glabra* leaf sample were carefully weighed in a silicon crucible that had been heated at 350°C for an hour and weighed. The bottom of the crucible was covered with a thin layer of dried leaf materials. The crucible was burned in a muffle furnace at a temperature of no more than 450°C until it turned red hot then white, signifying that it was carbon-free. After cooling, the crucible was weighed. The air-dried powder was used to compute the percentage of total ash [33].

**Determination of acid insoluble ash:-**

The ash produced by heating 25 millilitres of 2 N hydrochloric acid for five minutes was analysed as part of the total ash determination. The portion of ash that remained insoluble was collected on ash-free filter paper, rinsed with hot water, and then transferred into a pre-weighed silica crucible. This crucible was heated for 15 minutes at a maximum temperature of 450°C. Finally, the percentage of acid-insoluble ash was calculated using the air-dried powder [34].

**Determination of water soluble ash:-**

To determine the total ash, the sample was boiled with 25 millilitres of water for five minutes. The insoluble residue was collected on ash-free filter paper, rinsed thoroughly with hot water, and transferred into a pre-weighed silica crucible. The crucible was then heated for 15 minutes at a temperature not exceeding 450°C. This process was repeated until a constant weight was achieved. The weight of the insoluble residue was subtracted from the total ash weight to obtain the amount of water-soluble ash. Finally, the proportion of water-soluble ash was calculated using the air-dried powder [35-36].

**Determination of extractive values:-**

The coarsely powdered leaf sample of *Bougainvillea glabra* was subjected to extraction. The extractive yield was expressed as [37]:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the Dry extract (g)}}{\text{Weight of the Sample used for extraction}} \times 100$$

**UV Fluorescence analysis:-**

For the UV fluorescence test, about 0.5 grams of plant powder was placed into clean, dry test tubes. Separate tubes were prepared by adding different reagents, including distilled water, ethanol, methanol, picric acid, diethyl ether, acetonitrile, ethyl acetate, acetone, chloroform, petroleum ether, benzene, ammonia, ferric chloride, acetic acid, 50% nitric acid, concentrated nitric acid, concentrated hydrochloric acid, 50% sulfuric acid, concentrated sulfuric acid, 1N hydrochloric acid, 1N alcoholic sodium hydroxide, and 1N aqueous sodium hydroxide. Each tube was shaken and then allowed to stand for 20–25 minutes. The resulting solutions were observed for their characteristic colors under visible light, short-wave UV light (254 nm), and long-wave UV light (365 nm) [38].

**Determination of Heavy Metals:-**

**COBALT:** 20 mg of the drug's ash were dissolved in around 0.5 ml of distilled water, and a few drops of dilute hydrochloric acid were added to acidify the mixture. A few drops of a diluted sodium hydroxide solution were added. There was no blue ppt, a sign that cobalt was not there [39].

**MERCURY:** 20 to 25 mg of the drug's ash were dissolved in 1 millilitre of distilled water, and then 2 M sodium hydroxide was added until the mixture became extremely alkaline. There was no formation of a dense yellow ppt, indicating the absence of mercury [40].

**NICKEL:** 20mg of the ash of the drug was added in about 0.5ml of water, acidified with a few drops of dilute hydrochloric acid, and then solution of sodium hydroxide was added drop by drop. No blue ppt was formed which indicate the absence of nickel [41].

**SILVER:** A quantity of 20–25 mg of the drug was dissolved in 2–3 ml of distilled water, followed by the addition of 0.2 ml of 7 M hydrochloric acid. This produced a curdy white precipitate, which dissolved upon adding 3 ml of 6 M ammonia. When a few drops of 10% w/v aqueous potassium iodide solution were added, no yellow precipitate appeared, confirming that silver ions were not present [42].

**ZINC:** Twenty to twenty-five milligrams of the ash were dissolved in 2–3 ml of distilled water, after which 0.2 ml of 10 M sodium hydroxide was added. This produced a white precipitate that dissolved upon adding 2 ml of 10 M sodium hydroxide. Subsequently, 5 ml of 2 M ammonium chloride and 0.1 ml of sodium sulphide solution were introduced. No white precipitate formed, confirming that zinc is absent [43].

**Preparation of the plant extracts and Phyto-chemical Screening:-**

A preliminary phytochemical screening was carried out to qualitatively identify the phytoconstituents present in the dried and powdered leaves. Using a Soxhlet apparatus, 50 grams of coarsely powdered leaf material was successively extracted with petroleum ether, chloroform, ethyl acetate, ethanol, and water through continuous hot percolation. The resulting concentrated extracts were evaporated to dryness, and the yield from each solvent was weighed. The proportion of each extract was calculated based on the weight of the initial air-dried plant material. All extracts were then subjected to qualitative phytochemical analysis using standard conventional methods to detect various active compounds.

**TLC (Thin layer chromatography):-**

Thin layer chromatography (TLC) is a chemical analysis procedure which is used for the separation of mixture into their components. This chromatographic technique was discovered in 1906 by M. Tswett. This technique involves spotting a sample solution onto a TLC plate, stationary phase is a solid or a liquid supported on a solid. TLC has been important for separation of secondary metabolites [44-45].

The ethanolic and aqueous plant extract was subjected to TLC analysis. TLC plates were created using iodine vapor, reagent was sprayed onto them, or TLC plates were inspected using a UV lamp. The plates to make it easier

to see. In order to determine which solvent system offered the optimum separation for TLC, it was customary to experiment with multiple systems. The TLC-based screening methods often target medicinal plants in the search for various different physio-logical properties of botanical material (e.g., the free radical scavenging, antimicrobial, and enzyme-inhibiting activity [46].

#### **Procedure of TLC:-**

Thin Layer Chromatography (TLC) was employed to analyze the phytochemical constituents of *Bougainvillea glabra* floral extract. Fresh flowers were collected, shade-dried, and coarsely powdered. The powdered material was subjected to methanolic extraction using a Soxhlet apparatus for 6–8 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator.

TLC plates pre-coated with silica gel 60 F254 were used as the stationary phase. The plates were activated by heating at 110 °C for 30 minutes to remove moisture. A small amount of the concentrated extract was dissolved in methanol to prepare the spotting solution. Using a capillary tube, the solution was carefully spotted 1 cm above the base of the TLC plate.

The mobile phase was prepared by mixing ethyl acetate:methanol: toluene in an 8:2:1 (v/v/v) ratio. The solvent system was poured into a TLC chamber lined with filter paper to ensure saturation and equilibrated for 15–20 minutes. The spotted TLC plate was then placed vertically in the chamber, ensuring the sample spot remained above the solvent level. The solvent front was allowed to ascend to approximately 75% of the plate height.

After development, the plate was removed, air-dried, and visualized under UV light at 254 nm and 366 nm. Additional visualization was performed using iodine vapor exposure. The retention factor (R<sub>f</sub>) values of the separated spots were calculated to aid in the identification of phytoconstituents such as flavonoids and coumarins [47].

For colourless compounds, techniques such as fluorescence, radioactivity, or chemical reagents can be used to produce visible coloured reactions, allowing for identification of the compounds' positions on the chromatogram. These colour changes can be observed under normal light or UV light. The position of each compound is quantified by calculating the ratio between the distance travelled by the compound and the distance travelled by the solvent. This value, known as the retention factor (R<sub>f</sub>), is used for qualitative analysis of the compounds.

#### **Calculating the R<sub>f</sub> value of a compound:-**

The distance a compound travels relative to the solvent front is influenced by the molecule's structure, making thin layer chromatography (TLC) useful for both identifying and separating compounds. This relationship is quantified by the retention factor (R<sub>f</sub>), not "retardation factor." The R<sub>f</sub> value is calculated as the ratio between the distance travelled by the compound and the distance travelled by the solvent front, providing a standard measure for comparison. R<sub>f</sub> value is usually expressed as

**R<sub>f</sub> value** = Distance travelled by compound/ Distance travelled by solvent front

#### **Result and Discussion:-**

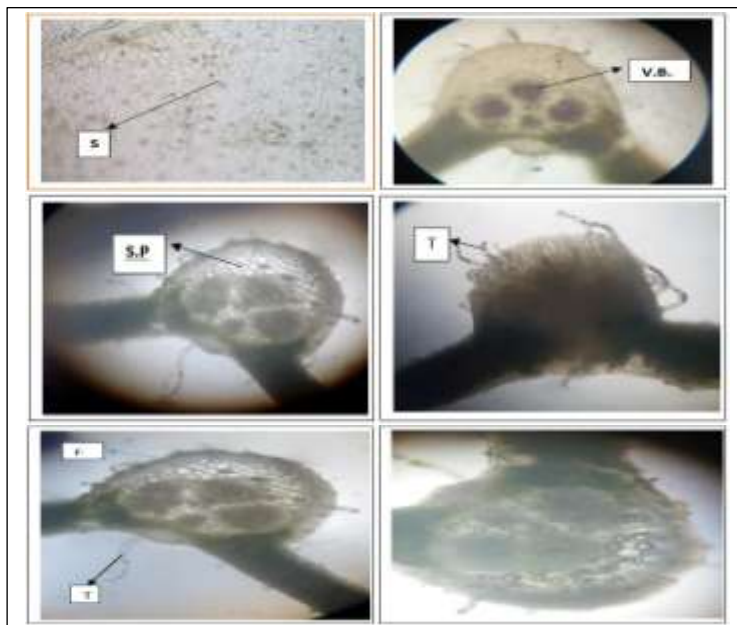
##### **Macroscopic studies:**

The macroscopical investigations revealed that the leaves are oblong lanceolate or simple ovate-acuminate to broadly ovate, measuring 2-4.5 inches in length and 1.5-3 inches in breadth. They have an undulating leaf with a complete margin, symmetrical, tapering, or wedge-shaped bases, and pinnate venation. Organoleptic characteristics include an evergreen hue, a slightly bitter taste, and no scent.

##### **Microscopic studies:-**

The method of Brain and Turner (1975b) and Kokate (1986a) was used for the micro-powder analysis. The epidermal layer is dense, has no intercellular gap, and can be divided into upper and lower epidermis. The upper epidermis is a single, straight-walled layer with actinomyces-type stomata and trichomes (uniseriate, multicellular, bulbous). Although it has stomata and trichomes, the lower epidermis is similar to the upper epidermis. There is cuticle on the upper and lower epidermis, although it is thinner on the lower side. Thick cellulose deposition is a characteristic of the collenchyma layers that are found just under the epidermis. It is present across the midrib, but not in the middle lamina. Lower layer cells are rather large and comprise two to three layers, while upper layer cells are small and comprise four to five layers. In spongy and palisade parenchyma cells, mesophyll can be differentiated. Palisade covers one-tenth of the lamina portion and is easily extended in a single layer. Thin, loosely

stratified parenchyma with voids between cells is called spongy parenchyma. Additionally, cells contain modest amounts of calcium oxalate crystals and huge amounts of starch. It covers the lamina's remaining portion. Vascular bundles are found in spongy tissues; they are typically five in number, arc-shaped, and more noticeable on the lower side. Each is encircled by a single layer of pericycle. The endodermal layer envelops the vascular bundle. Xylem is found on the ventral side and phloem on the dorsal side.



**Figure 1: Morphology and anatomy of Bougainvillea leaves.**

[Photomicrographs illustrating characteristic anatomical structures of *B. glabra*: (S) epidermal surface showing stomata; (V.B.) transverse section of the vascular bundle with distinct xylem and phloem tissues; (S.P.) secretory pore present in the epidermal region; (T) epidermal trichomes arising from the surface; (F) fibrous tissue providing mechanical strength; and (G) general microscopic view showing overall tissue organization. These microscopic features serve as important diagnostic characters for the identification and pharmacognostic evaluation of *Bougainvillea glabra*].

#### Ash values:

The total ash measurement represents both physiological and non-physiological ash. Environmental contaminants are referred to as non-physiological ash, whereas physiological ash is the ash that a plant naturally creates as a result of biochemical activities. These could be various metals, such as carbonates, phosphates, nitrates, sulphates, chlorides, and silicates, that were taken up from the soil. When evaluating the purity of pharmaceuticals, the total ash value was extremely important. While a high percentage of total ash value indicated the presence of inorganic materials, a very low value of acid insoluble ash indicated the presence of very little siliceous particles. Acid insoluble ash was the percentage of total ash that was insoluble in diluted HCl. The percentage of total ash that dissolves in water is known as water-soluble ash. Water-soluble ash is a helpful indicator of the presence of exhausted material. In this analysis, *Bougainvillea glabra* had the highest total ash value, followed by acid-insoluble and water-soluble ash. The results were shown in Table 1.

**Table 1: Ash values of leaf powder of Bougainvillea glabra**

Parameters	Values % (w/w)
Total ash	24
Acid insoluble ash	7
Water soluble ash	9.67

**Extractive values of leaf extracts of *Bougainvillea glabra*:-**

Determining the extraction values helps evaluate the chemical components of the crude medication. This number also helps determine whether components are soluble in a particular solvent. The formation of the bioactive principle of medicinal plants is influenced by a variety of internal and external variables. High extractive values that are soluble in alcohol and water are indicative of polar compounds including phenols, tannins, and glycosides. In this experiment, the extraction yield has increased with the polarity of the solvent. The maximum yield was observed in the aqueous extract. This could be due to the stronger polarity of water. In the present study, the higher percentage of extractive values of crude drugs in water and ethanol extracts implied that water and ethanol are better solvents for extraction than petroleum ether, chloroform and ethyl acetate. The results were expressed in Table 2.

**Table 2: Extractive values of leaf powder of *Bougainvillea glabra***

Extract	Amount of extract (gms)	Values % (w/w)
Pet ether	3.9	15.6
Chloroform	4.5	18
Ethyl acetate	5.1	20.4
Ethanol	5.4	21.6
Water	6.2	24.8

**Fluorescence analysis of dried leaf powder of *Bougainvillea glabra*:-**

Fluorescence is a property exhibited by certain chemical constituents present in plant materials. When different reagents are applied to plant tissues, they may produce distinct colors. Some plant parts naturally display characteristic fluorescence in visible light during daylight. Under ultraviolet light, many substances that appear non-fluorescent in normal light begin to fluoresce. Additionally, non-fluorescent compounds can form fluorescent derivatives or degradation products when treated with specific reagents. Because of these reactions, fluorescence analysis serves as an important qualitative tool in the pharmacognostic evaluation of crude drugs. The colour changes produced by specific reagents were recorded, as they assist in assessing the quality and purity of the leaf powder. Powdered samples of *Bougainvillea glabra* leaves were treated with various chemical reagents for fluorescence analysis, and observations were made under visible light as well as UV light at 254 nm and 356 nm. When exposed to these reagents, the leaf powder exhibited characteristic colour variations. Under UV light, the crude drug showed distinct fluorescent reactions, which differed across wavelengths. These variations arise from the presence of multiple phytochemicals within the plant material.

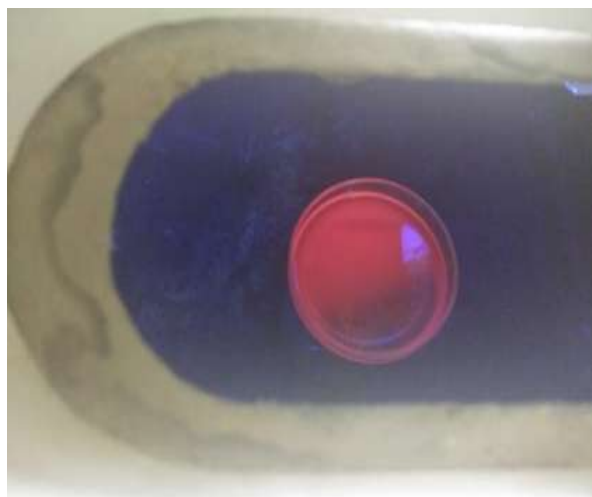






Figure 2: Fluorescence analysis of Bougainvillea glabra

Table 3: UV fluorescence analysis of the powder of Bougainvillea glabra

S. No.	Experiments	Visible light	UV Fluorescence	
			254 nm	365 nm
1.	Powder as such	Light green	fluorescent green	<b>brown</b>
2.	Powder + 1N Aqueous NaOH	Yellowish green	Dark green	Light Blue
3.	Powder +1N Alcoholic NaOH	Yellowish green	green	Blue
4.	Powder + 1N HCl	Yellowish green	green	Dark Green
5.	Powder + conc. H <sub>2</sub> SO <sub>4</sub>	brown	Dark green	fluorescent green
6.	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	brown	green	green
7.	Powder +conc. HCl	Light yellow	green	green
8.	Powder +conc. HNO <sub>3</sub>	brown	fluorescent green	green
9.	Powder + 50% HNO <sub>3</sub>	yellow	fluorescent green	violet
10.	Powder +Acetic acid	Yellowish green	green	Yellowish brown
11.	Powder +Ferric chloride	Yellowish brown	yellow	Fluorescent green
12.	Powder + NH <sub>3</sub>	Yellowish green	green	Fluorescent green
13.	Powder +Benzene	green	Dark green	blue
14.	Powder +Petroleum ether	Light brown	green	Brown
15.	Powder + Chloroform	green	Dark green	Brown
16.	Powder +Acetone	Yellowish green	Light green	Reddish brown
17.	Powder +Ethyl acetate	Yellowish green	green	Brown
18.	Powder +Acetonitrile	yellow	Dark green	Brown
19.	Powder + Picric acid	yellow	green	Dark green
20.	Powder +Methanol	yellow	Greenish yellow	Brown
21.	Powder +Ethanol	Light green	Fluorescent yellow	Reddish brown
22.	Powder +Water	yellow	yellow	Fluorescent yellow



**Table 4: Estimation of Heavy metal**

Si No.	Part of the Plant	Name of the Heavy Metal				
1.	LEAVES	Cobalt	Mercury	Nickel	Silver	Zinc
		-	-	-	-	-

**Table 5: Phytochemical Screening of Bougainvillea Glabra**

Si. No	Phytochemical Evaluation	Pet ether	Chloroform	Ethyle Acetate	Methanol	Aqueous
1	Alkaloids	+	+	+	+	-
2	Glycosides	-	+	-	+	-
3	Carbohydrate	+	-	-	+	-
4	Flavonoids	-	+	-	+	-
5	Saponins	-	+	+	+	+
6	Terpenoids	-	+	-	-	+
7	Taninns	-	-	-	+	+
8	Phytosterols	+	+	+	+	+

**Testing for Heavy metals:-**

Heavy metals—including cobalt, mercury, nickel, silver, and zinc—are known contaminants that can affect the purity of herbal materials. A standard limit test for heavy metals, as described in various pharmacopoeias, was performed on the sample. The results showed no detectable levels of heavy metals. The observations are summarized in Table 4.

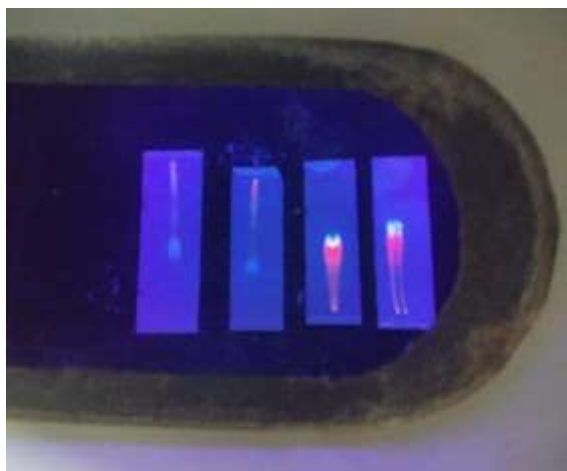
**Preliminary phytochemical screening:-**

Alkaloids, glycosides, flavonoids, saponins, polysaccharides, and phytosterols were found in significant amounts in the initial phytochemical screening of several leaf extracts. Table No. 5 presents the findings.

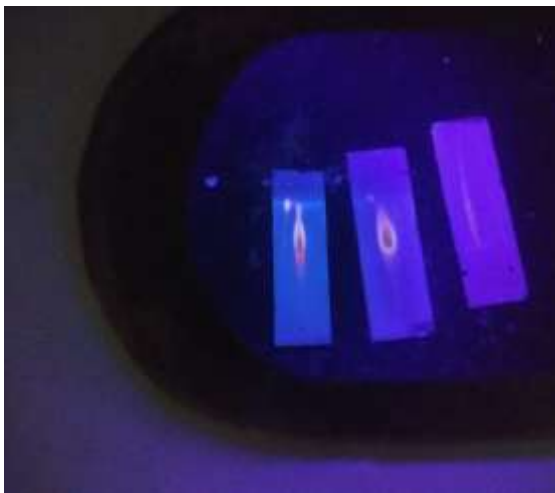
**TLC (Thin layer chromatography):-**

Thin layer chromatography (TLC) analysis of alcoholic and aqueous extracts of *Bougainvillea glabra* using the solvent system ethyl acetate: methanol: toluene (8:2:1) revealed distinct bands with R<sub>f</sub> values ranging from 0.4 to 0.6. These values correspond to the reported mobility of coumarin and certain flavonoid constituents, suggesting their possible presence in the extracts. The alcoholic extract exhibited clearer separation compared to the aqueous extract, reflecting differences in solubility and phytochemical composition. Visualization was performed under UV light and iodine vapours to enhance spot detection.

However, TLC provides only preliminary evidence, and further spectroscopic and chromatographic investigations are required for definitive identification of the compounds.



**Figure 3: TLC visuals of ethanolic extract**



**Figure 4: TLC visuals of aqueous extract**

### **Conclusion:-**

More than 80% of people in developing countries rely on traditional medicine, particularly plant-based remedies, for their primary healthcare needs. This study was conducted to evaluate key standardization parameters of the herbal product, including pharmacognostic, phytochemical, physicochemical, fluorescence behaviour, heavy-metal content, and extractive values. The formulation was found to be free from heavy metals, and the plant exhibited distinct fluorescence characteristics—an important criterion in herbal standardization. The extractive value was also assessed to identify the most suitable solvent for extraction and to understand the nature of the chemical constituents present. Physicochemical and phytochemical analyses of the leaves confirmed the plant's identity, quality, and purity. Overall, *Bougainvillea glabra* leaves proved to be a rich source of phytochemicals with potential therapeutic benefits. To effectively incorporate these chemicals into pharmaceuticals. Thin layer chromatography (TLC) of alcoholic and aqueous extracts of *Bougainvillea glabra* using the solvent system ethyl acetate: methanol: toluene (8:2:1) revealed a prominent spot with an  $R_f$  value ranging between 0.4-0.6. This mobility is consistent with the reported behaviour of coumarin derivative, certain flavonoid constituents, suggesting the possible presence of coumarin and flavonoid in the plant extracts.

However, TLC provides only preliminary evidence, and definitive identification requires further analytical characterization. Advanced techniques such as co-chromatography with authentic standards, spectroscopic profiling, or chromatographic separation are essential to confirm the compound's identity. These findings support the plant's potential as a source of bioactive flavonoid compounds for further pharmacological investigation. More research is needed to isolate and identify the molecules responsible for the various therapeutic effects with appropriate scientific evaluation and support.

### **Conflict Of Interest:-**

The authors declare no conflict of interest.

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