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PHARMACOGNOSTICAL INVESTIGATION OF AERIAL PARTS OF THE *POLYGONUM PLEBEIUM* R. BR.

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Keywords:

Polygonum plebeium, Pharmacognostic, Phytochemical evaluation, FE-SEM, EDX, FT-IR

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ABSTRACT: Background: Medicinal plants are gaining popularity due to their reputation for being safe and effectively addressing fundamental health care demands. Standardizing medicinal products is the most recommended option because herbal therapy is becoming more and more popular worldwide. **Objective:** The present study examines various pharmacognostic characteristics that ensure the safety, efficacy, and purity of *P. plebeium*. **Materials and Methods:** The present work aimed to investigate the pharmacognostical and phytochemical screening of *P. plebeium* aerial parts by using energy dispersive spectroscopy (EDX), field emission scanning electron microscopy (FE-SEM), fluorescence, and Fourier transform infrared (FTIR) spectroscopy techniques. **Results:** The scanning electron microscope (SEM) analysis detected the presence of xylem parenchyma, phloem parenchyma, and calcium oxalate crystals. The physicochemical characteristics of *P. plebeium* indicate that the sulphated ash value (7.6%) is greater than the values of total ash, acid-insoluble ash, and water soluble ash. The fluorescence study of raw powdered drugs revealed a variety of colours substance present in it. The preliminary phytochemical profile identified secondary phytocostituents such as alkaloids, carbohydrates, flavonoids, proteins, and amino acids. The powdered medications' EDX examination showed that nitrogen, aluminium, silicon, chlorine, potassium, zinc, zirconium, and thallium were present in different amounts. The FT-IR investigation reveals the presence of several functional groups, including alcohols, amines, carboxylic acids, phenols, alkanes, and ketones. **Conclusion:** A comprehensive analysis of pharmacognostic as well as employing physicochemical, phytochemical, EDX, and FT-IR tests can be highly beneficial in identifying adulterants, ensuring the safety of the phytocostituents present in *P. plebeium*, and guiding future research.

INTRODUCTION: The World Health Organization (WHO) estimates that between 75 to 80% of people worldwide use medicinal herbs for their basic medical requirements. Moreover, around 60 to 65% of Indians utilize various traditional, conventional herbal, and Indian medicinal plant therapies to address a variety of health conditions [1].

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India boasts a vast diversity of over 4 million unique kinds of medicinal plants, but only half of them have undergone scientific research to determine their usefulness as potential medicines [2]. A healthy individual's pharmacological activity is dependent on the availability of trace, major, minor, and heavy constituents in both medicinal

plants and the human body [3, 4]. In addition, the World Health Organization (WHO) and the Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homoeopathy (AYUSH), operating under the Ministry of Health and Family Welfare, have focused on enforcing legal regulations and improving quality control and standard operating procedures in the production of medicinal plant drugs.

To determine the plant's standards of quality, a quantitative examination of pharmacognostic characteristics may be useful. Standardization is carried out by conducting pharmacognostic investigations and assisting in the identification and validation of plant materials [5]. Pharmacopoeias and regulatory standards often recommend conducting macroscopic and microscopic inspections, along with assessing the phytochemical qualities of medicinal plants, to assure accuracy and quality control. Different types of studies, such as microscopic, macroscopic, Field Emission Scanning Electron Microscopy (FE-SEM), physicochemical, and fluorescence analysis, can identify specific drugs and verify the validity of raw materials [6]. Through morphological and microscopical research, examining the structure and appearance of aerial parts may aid in accurately identifying and verifying the plant's authenticity. A phytochemical study reveals the secondary metabolites and bioactive chemicals that often contribute to the pharmacological effects of a plant-based medication [7]. Energy dispersive X-ray (EDX) microanalysis and scanning electron microscopy (SEM) are often employed techniques for obtaining data on the surface morphology and chemical composition of a material [6]. The Fourier transform infrared (FT-IR) is a rapid, dependable, and valuable technology for elucidating the molecular structures of biologically active substances [8]. The purpose of this research was to analyse *P. plebeium* extracts for functional categories, elemental composition, and morphological characteristics using FT-IR, EDX, and FE-SEM spectroscopic methods.

The Odia name "Muthisag" refers to *Polygonum plebeium* R.Br. (Family: Polygonaceae). It has a wide distribution in tropical and subtropical regions. Indigenous populations of the Asian subcontinent predominantly use it as a vegetable. There are over 250 plant species in the genus *Polygonum*, including annual and perennial varieties [9]. The plants grow in diverse conditions, spanning from the northern temperate to tropical and subtropical regions. Numerous scientific studies on *P. plebeium* have identified a variety of phytochemical constituents, including alkaloids. flavonoids, essential oils, tannins, triterpenoids, sterols, and steroidal sapogenins (saponins). The constituents and crude extracts of *P. plebeium* showed a range of therapeutic activities, including antinociceptive, antioxidant, anticancer, anti-inflammatory, and neuroprotective properties. The extract of *P. plebeium* possesses diverse pharmacological activities and is employed for the management of conditions such as diarrhoea, eczema, inflammation, liver disease, and ringworm. However, there hasn't been much previous work to evaluate the physicochemical, pharmacognostic and phytochemical characteristics of *P. plebeium's* aerial part. This study aims to thoroughly evaluate *P. plebeium* extract for pharmacognostic standards in order to establish standardization parameters for the plant. The present study performed an extensive evaluation of the physicochemical, pharmacognostic, and phytochemical characteristics of the aerial sections of *P. plebeium*. This analysis will aid in the plant's identification and subsequent standardization [10].

MATERIALS AND METHODS:

Collection of Plant Materials: The aerial parts of the *P. plebeium* plant were collected in the month of April 2023 in Mulugaon Village area, Jagatsinghpur, Odisha. Dr. K. Karthigeyan, Scientist-E at the Botanical Survey of India, Calcutta, West Bengal, authenticated the species. A voucher specimen (CNH//2316Tech.II/2023/94) was deposited with the Pharmacology Department at the Faculty of Pharmacy, SOA University, Bhubaneswar.

Preparation of Plant Materials: The collected plant materials were throughly washed with flowing tap water and left to dry in a shade area at the normal temperature. The dry plant material was crushed to powder using mechanical grinding. The coarse powder then passed through a sieve with a mesh size of 60. After sieving, the plant material was stored in a tightly sealed container.

Macroscopical Characters: The aerial section of the *P. plebeium* plant in **Fig. 1**, was evaluated macroscopically using the WHO Quality Control techniques. The colour was observed by visual examination. For determining the odour, small quantity of dry coarse powder of *P. plebeium* was maintained in a beaker and examined by breathing in. Taste was determined by organoleptically [11].

FIG. 1: *POLYGONUM PLEBEIUM* AERIAL PARTS

Microscopically Characters: The powdered aerial parts of *P. plebeium* shown in **Fig. 2,** was treated with glycerine, after staining with safranin, the specimen underwent a compound microscope examination [12].

FIG. 2: *POLYGONUM PLEBEIUM* POWDER DRUG

Pharmacognostical Studies:

Analysis with FE-SEM: FE-SEM allows for the precise observation of plant materials, especially powdered samples. This approach was used to examine the size, shape, and surface characteristics of *P. plebeium* powder. When using FE-SEM to analyse plant powders, useful information regarding the perceived texture of the plant material can be obtained.

Physicochemical Character: The coarse form of *P. plebeium* was determined for its physiochemical characteristics such as ash value, extractive value, loss on drying, foaming index, swelling index [13, 14, 15].

Ash Value: Ash values are used for evaluating the character of a powdered form of a crude drug. The drug's ash content refers to the inorganic ions that remain after combustion. Purity, authenticity, and identification of adulteration of sample was determined using the ash value.

Total Ash: The total amount of ash was used to calculate the entire amount of residual ash that remains after burning. External substances adhering to plant surfaces generate non-physiological ash.

Procedure: A tar-coated nickel crucible was heated to 450°C in a muffle furnace for approximately 15 min. After cooling in a decanter for nearly an hour, weighed the crucible. The crucible was filled with a 3 g powder sample, which was heated gradually until all of the moisture disappeared and the plant material was completely burned. After gradually raising the temperature until the carbon disappeared, 650°C produced a residue free of carbon. As a result, the powder sample turned gray, giving it a white, ash-like appearance. After the crucible was removed with crucible tongs, it was placed in a desiccator to cool before being weighed once again.

Water Soluble Ash: The remaining ash from the container, obtained from the total ash, was put into a 100 ml beaker. In the beaker, add 25 ml of water, and boil the mixture for 5 min. After passing the combination through ashless filter paper, the residual material and the filter paper were both dried in an oven. The residue on the ashless filter paper was made coarse powdered into the crucible and allowed to heat at temperature to 450°C until the ashless filter was completely burned. Using crucible tongs, the crucible was removed, Allow the sample to cool in a desiccator before weighing it again.

Insoluble Ash in Acid: The residue that remains after heating the resultant insoluble material and mixing it with dilute (dil.) hydrochloric acid is known as acid-insoluble ash. This quantifies the amount of residual silica that is still there, especially in sand and siliceous soil.

Procedure: The ash taken from the whole ash was put into a 100 ml beaker. Next, added 25 ml of dil. hydrochloric acid (HCl) drop by drop to the ash. Next, allow the mixture to boil for 5 min.

They filtered the solution using an ash less filter paper, then washed the residue twice with hot water. Once again, the insoluble residue was collected into a crucible and heated to 500 °C in a muffle furnace until it contained no carbon. Once the residual substance cooled down in a desiccator, measured its weight. A dried drug sample was used as a reference.

Sulphated Ash:

Procedure: The silica crucible was heated at 450°C for a duration of 30 min and subsequently cooled and weighed. 2 g of *P. plebeium* powder were placed in the crucible and slowly heated until it was thoroughly charred and cooled in a desiccator. The remaining mixture was treated with a dil. solution of sulfuric acid and slowly heated until it appeared from releasing any white fumes. The igniting process persisted until all the black particles vanished at a temperature of 800°C and then cooled down. A little amount of dil. sulfuric acid was added, heated up, and then allowed to cool before being weighed. The entire procedure is iterated until the disparity in weight between two successive measurements is less than or equal to 0.5 g.

Identification of Extractive Values: Extractive values were examined for assessing phytocostituents, especially when there are no direct methods available to test the contents of the crude drug being evaluated. Moreover, this data discloses the exact active components found in a natural medicinal product.

Identification of the Water-Soluble Extractive: For the maceration method, 5 g of carefully measured powder was placed in a 250 ml stoppered conical flask with 100 ml of chloroform water (95 ml of distilled water and 5 ml of chloroform) and allowed to stand for 24 hours.

The powdered drug combination was agitated nonstop for 6 hours, then allowed to macerate for a further 18 hours before filtering. A volume of around 25 ml of filtrate was gathered in a small porcelain dish, allowed to dry in a water bath, and then dried in oven at 100°C. After cooling the material in a desiccator, proceed to measure its weight. Determine the residue's water-soluble extractive value as a percentage of the drug dried in the air.

Identification of Alcohol Soluble Extractive: A quantity of 5 g of the powder was combined with 100 ml of 90% ethanol and placed in a 250 ml conical flask with a stopper. The mixture was left to undergo maceration for a duration of 24 hours. The mixture was agitated incessantly for a duration of 6 hours, followed by a maceration period of 18 hours, and subsequently filtered. Approximately 25 ml of the liquid that passed through the filter were collected in a delicate porcelain container. The liquid was then allowed to evaporate completely by placing it in a heated water bath. Finally, oven temperature of 100°C was regulated to dry the remaining substance and cool in a desiccator. Then measure its final weight.

Moisture Content: The dried sample was transferred to an empty, tarred, lidded porcelain crucible (W1), and the crucible was weighed again (W2). The sample was exposed to a temperature of 65°C for a duration of 12 hours in an oven and thereafter transferred to a desiccator to cool down. The unused sample, after reheating in the crucible, was quantified by weighing (W3). The decrease in weight of the sample is typically referred to as loss on drying, and the moisture content was determined [16].

Determination of Foaming Index: Mix 1 g of powder with 100 ml of water, and then pour the resulting liquid into a 500 ml conical flask. The resulting mixture was heated to a temperature of 80 to 90°C and boiled for approximately 30 min. Subsequently, the solution was brought to cool and then filtered into a 100ml volumetric flask. Afterward, an adequate amount of water was added to the filtrate until it reached a final volume of 100 ml. The decoction was divided into 10 stopper test tubes, each with a diameter of 16 mm and a height of 16 cm. They incrementally added the decoction to the tubes in volumes ranging from 1 ml to 10 ml, increasing by 1 ml each time. 10 ml of water was added to each tube and tightly sealed the tubes and manually agitated for 15 sec. The tubes were shaking twice in a longitudinal position. The foam was allowed to stand for 15 min before its height was measured. The following criteria were used to evaluate the results: If the height of the foam in each tube is less than 1 cm, then the foaming index is below 100. Each tube measures 1 cm in height, and the volume of the plant material

decoction in each tube determines the index. To improve precision, it may beneficial to prepare an intermediate dilution if the specific tube is either the initial or second one in a sequence. When the foaming index exceeds 1000 and the height of the foam in each tube reaches 1 cm, now, it is important to continue the examination using new techniques of decoction dilution in order to produce a fruitful result.

Assessment of Swelling Index: 1 g of the powdered material was precisely measured and put into a stoppered 25 ml measuring cylinder. 20 ml of water were added to it. The mixture was frequently shaken for 23 hours. The mixture was then maintained its resting condition for 1 hour. The volume occupied by the swollen powder sample in stoppered measuring cylinder was calculated [17].

pH Measurement: The pH scale shows how acidic or basic a water-based solution is.

1. The pH of a 1% solution was determined by properly weighing 1g of coarsely powdered material that had been airdried and accurately weighed was dissolved in 100 ml of distilled water to get the pH of a 1% solution. Using a pH meter, the pH of the water-soluble fraction was determined at 25°C.

2. The pH level of the 10% solution was measured In 100 ml of distilled water, accurately dissolve 10 g of air dried material, ground into a coarse powder. Determine the pH of the water-soluble fraction at 25°C using a pH meter [18].

Fluorescence Analysis: The powder was subjected to fluorescence analysis following the specified protocols. The powder was treated by applying several chemical reagents, followed by examination and documentation using visible light and Ultra Violet light (namely, short wave length 254 nm and long wave length 366 nm) [19, 20].

Behaviour of Powder with Diverse Reagents: The powders were treated with a range of chemical reagents including ferric chloride ($FeCl_3$), glacial acetic acid (CH_3COOH), hydrochloric acid (HCl), nitric acid (HNO_3), potassium hydroxide (KOH), sodium hydroxide (NaOH), and sulfuric acid (H_2SO_4). Observations were made regarding the behaviour of powders, such as their ability to float or sink, as well as any changes in the colour of solutions [18, 21].

Phytochemical Analysis: A qualitative phytochemical study was conducted on the crude extract using standardized methodologies to determine the availability of different other metabolites in both the end product and the crude extract, which is of significance [22, 23].

Test for Acidic Compound:

- The alcohol extract of the powder was treated with sodium bicarbonate ($NaHCO_3$) to produce an effervescence, which indicates the presence of an acidic compound.

- Warm water was mixed with the alcohol extract of the powder and filtered. Filtrate was treated with litmus paper, this takes on a blue tint, indicating the presence of an acidic substance.

Test for Aleurone Grains: An aqueous solution obtained from the crude extract was treated with a few drops of alcoholic iodine (I) solution to appear a yellowish-brown to brown colour, this suggests that aleurone grains are present.

Test for Alkaloids: A mixture of 0.5 g of the powdered extracts and 5 ml of 1% hydrochloric acid was prepared and allowed to boil on water bath for 5 min. The solution was then filtration using Whatman's no. 1 filter paper. To 1 ml of the filtrate was added two drops of Dragendorff's reagent. The presence of alkaloids is indicated by an orange-red color.

Test for Carbohydrate (Molisch's Test): After adding 2 ml of distilled water to 0.1 g of powder, the mixture was heated to a boil and filtered. A small amount of Molisch's reagent (α-naphthol solution in ethanol (C_2H_5OH) was added to the filtrates. Next, carefully add a highly concentration (conc.) solution of sulfuric acid to the side of the test tube. A purple to violet ring has formed at the junction, indicating the presence of carbohydrates.

Test for Flavonoids: 10 ml of ethyl acetate and 2 g of the powder were combined to create a solution, which was then heated to boil in a water bath for 3 min. After cooling, the solution was filtered. The resulting filtrate was used in further experiments.

Ammonium Hydroxide Test: 4 ml of the previously described filtrate and 1 ml of dil. ammonia solution. The yellow color in the ammonia (NH_3) layer suggests the presence of flavonoids.

1% Aluminium Chloride Solution Test: The 4 ml of filtrate was mixed with a 1% solution of aluminum chloride ($AlCl_3$). The yellow color of the aluminum chloride layer indicates the presence of flavonoids.

Test for Saponins: A mixture of 2 g of the extract and 20 ml of distilled water was placed in a water bath and let to boil for a duration of 2 min.

After the mixture was filtered while still hot, it was allowed to cool. The following experiments were conducted using the filtrate of the solution.

Froth Formation Test: A volume of 15 ml of distilled water was combined with 5 ml of the filtrate. The mixture was quickly mixed until a steady froth formed. It signifies the existence of saponins.

Emulsion Test: The foaming solution was dil. with 2 drops of olive oil and then quickly mixed. The occurrence of an emulsion signifies the existence of saponins.

Test for Glycosides: In a test tube, 0.1 g of the powder and 5 ml of dil. sulfuric acid were added. After boiling the test tube in a water bath for 15 min, add a 20% potassium hydroxide solution to neutralize the solution.

A total of 10 ml of both Fehling's solution I and Fehling's solution II were combined and then warmed to boiling for a duration of 5 min. The formation of a more concentrated brick-red precipitate indicates that glycosides are present.

Test for Hydroxyl Anthraquinones Glycosides: 0.1 g of the powder drug was treated with a solution of potassium hydroxide. When a red color appears, it indicates the presence of hydroxy anthraquinones glycosides.

Test for Inulin: The test solution was put in a test tube containing a solution of α-naphthol and sulfuric acid. The brownish-red coloration provided evidence for the existence of inulin.

Test for Mucilage: The crude extract's pink coloration indicates the presence of mucilage when combined with a ruthenium red solution.

Test for Tannins: 20 ml of water was combined with 1 g of the powdered material, and the mixture was allowed to boil in a water bath. It was subsequently filtered. A few drops of $FeCl_3$ solution were added to the filter. Tannins are present when a greenish black precipitate forms.

Test for Proteins:

Warming Test: Boiling a test solution in a water bath while it's inside a test tube causes protein coagulation.

Biuret Test: A few drops of Biuret's reagent were added to 5 mg of extract. After properly mixing the resulting mixture, let it heat for 1–5 min. The development of a red or violet color indicates the presence of proteins.

Test for Starch: The aqueous extract was added to an aqueous iodine solution, appear a blue colour. The colour disappears during heating and reappears after cooling, indicates the existence of starch.

Test for Steroids: 5 ml of acetic anhydride and 2 ml of conc. H_2SO_4 were combined with 0.5 g of the powdered drug. Steroids are present when the color shifts from violet to blue.

Test for Terpenoids (Salkowski Test): The mixture of 2 ml of chloroform and 5 ml of crude extract was added. Next, gradually introduce 3 ml of conc. H_2SO_4 into the mixer. The interface becomes reddish-brown in color, signifying the presence of terpenoids.

Test for Naphthoquinones:

Juglone Test: 2 ml of chloroform ($CHCl_3$) extract and 2 ml of ethyl ether ($(C_2H_5)_2O$)) was mixed to a dil. ammonia solution. The pink coloration indicates the presence of naphthoquinones.

Dam Karrer Test: Plant extract containing chloroform was mixed with 10% potassium hydroxide. The presence of naphthoquinones is indicated by the emergence of blue colors.

EDX (Energy Dispersive X-Ray) Analysis: A sample of *P. plebeium* aerial parts of powder was subjected to elemental analysis using an energy

dispersive x-ray (EDX). The weight percentage of several elements, such as carbon, nitrogen, oxygen, aluminium, silicon, chlorine, potassium, zirconium, and thallium was determined using energy dispersive spectroscopy (EDX). The *P. plebeium* powder was prepared for EDX analysis by placing the powdered plant material onto copper specimen sticks using sticky carbon tape. The sample was then coated with a layer of gold using a sputter coater (JEOL, Japan). The samples were subsequently analyzed using a JEOL scanning electron microscope (JSM-6390, Japan) at an acceleration voltage of 10 kV [24].

FT-IR Analysis: The powdered substance of *P. plebeium* was combined with potassium bromide (KBr) salt, using a mortar and pestle, and compacted into a thin pellet. The infrared spectra were measured using a Shimadzu FT-IR Spectrometer 8000 series, within the range of 3500–500 cm^{-1}.

RESULTS:

Macroscopical Characters: The macroscopical characters of *P. plebeium* plant was observed as a green in colour, aromatic odour, and sour taste.

Microscopical Characters: The microscopical characteristics of the powdered plant's *P. plebeium* aerial parts were that the analysis detected calcium oxalate, epidermal cells, fibers, stomata, starch grains, and trichomes. Part of group of fibres and sclereids with an associated reticulate thickened vessel, steallet covering trichomes which are found entire an composed of seven elongated conical cell joined at their base. The prism of calcium oxalate which are found scattered with vary in size. The starch granules are simple spherical having different size. Sponge have characteristic irregular reticulations on the surface. Xylem parenchyma in longitudinal section, walls are lignified, moderately thickened in **Fig. 3**.

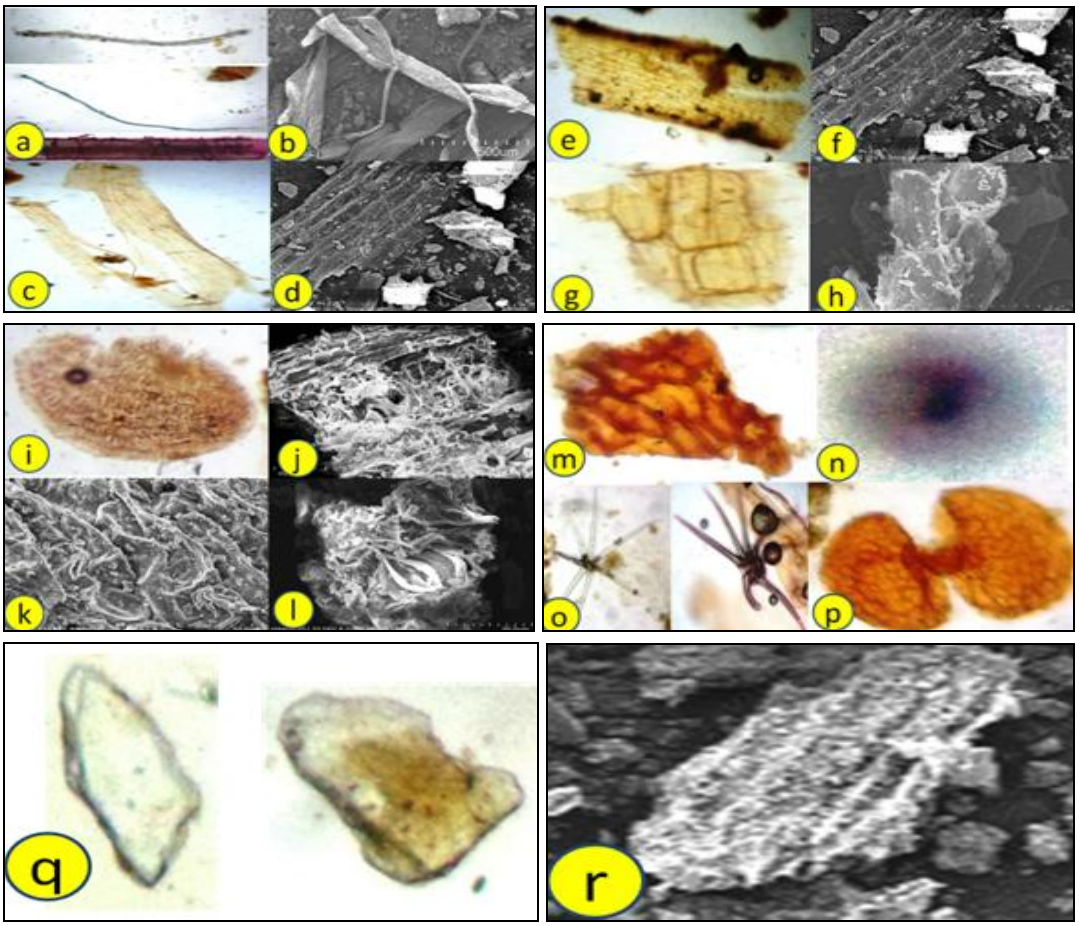

FIG. 3: POWDER MICROSCOPY OF PLANT *P. PLEBEIUM* AERIAL PARTS. a. fibres, b. FE-SEM of fibres, c. fibres and sclereids, d. FE-SEM of fibres and sclereids, e. xylem parenchyma in longitudinal section, f. FE-SEM of xylem parenchyma in longitudinal section, g. Phloem parenchyma, h. FE-SEM of Phloem parenchyma, i. Sponge, j. FE-SEM of brown mass k. & l. Stomata, m. Endocarp in surface view, n. Starch grain, o. Stellate trichome, p. Spore, q. Calcium oxalate crystals, r. FE-SEM of Calcium oxalate crystals.

Physicochemical Constant: In **Table 1,** displays the findings for several physicochemical parameters, including total ash, sulfated ash, water-soluble ash, and acid-insoluble ash. The value of sulfated ash (7.6%) was higher than that of total ash (6.5%). The levels of ash that were soluble in water and in acid were 4.5% and 1.3%, respectively. The extractive values of several solvents, namely petroleum ether, ethyl acetate, ethanol, and water, were 3.06%, 8.20%, 9.55%, and 12.05%, respectively shown in **Table 2**. The findings also revealed that the moisture content, swelling index, and foaming index were respectively, 12.5%, less than 100, and 1. While determination of pH 1% and 10% was found to be 5 acidic.

TABLE 1: ASH VALUE OF *P. PLEBEIUM* AERIAL PARTS

Sl. no.	Ash value	Result in %
1	Total ash	6.5
2	Water soluble ash	4.5
3	Acidic insoluble ash	1.3
4	Sulphated ash	7.6

TABLE 2: EXTRACTIVE VALUES OF *P. PLEBEIUM* AERIAL PARTS

Sl. no.	Extractive values	Colour reagent	Result in %
1	Petroleum ether	Light green	3.06
2	Ethyl acetate	Green	8.20
3	Ethanol	Dark green	9.55
4	Water	Dark green	12.05

The fluorescence properties of raw powdered drugs were assessed using both common visible light and UV radiation with both long and short wavelengths.

After introducing the powdered drug to different reagents and examining it under UV and visible light, it emitted diverse wavelengths of light, resulting in different colors.

The crude powder exhibited a clear and consistent color shift, indicating the solvent characteristics of the phytocostituents. In **Table 3,** contains the corresponding data. In **Table 4,** describes the sample's response to various chemical reagents.

TABLE 3: FLUORESCENCE ANALYSIS OF POWDER *P. PLEBEIUM* AERIAL PARTS

Sl. no.	Reagent	Day light	Short wave length (254nm)	Long wave length (366nm)
1	Powder as such	Dull green	Dull green	Dark green
2	Powder+1N NaOH in methanol	Brown	Green	Dark green
3	Powder +1N NaOH	Yellowish green	Green	Dark green
4	Powder +Ethanol	Light green	Green	Dark green
5	Powder +HNO_3 +NH_3 solution	Orange	Fluorescent Green	Black
6	Powder +50% HNO_3	Yellow	Light green	Brown
7	Powder +1N HCl	Green	Green	Brown
8	Powder + HCl	Green	Green	Dark green
9	Powder +H_2SO_4	Black	Dark green	Dark green
10	Powder +50% H_2SO_4	Brown	Dark green	Dark green
11	Powder +Glacial acetic acid	Pale green	Light green	Dark green
12	Powder +HNO_3	Yellowish brown	Green	Black

TABLE 4: BEHAVIOUR OF POWDER *P. PLEBEIUM* AERIAL PARTS

Sl. no.	Acid/reagent	Observation
1	Powder as such	Dull green
2	Powder + Picric acid	Yellowish brown
3	Powder + Conc. HNO_3	Brown
4	Powder+ Conc. HCl	Brownish green
5	Powder + Conc. H_2SO_4	Black
6	Powder+ Glacial acetic acid	Green
7	Powder +5% $FeCl_3$ solution aqueous	Dark green
8	Powder +NaOH(5N)	Yellowish green
9	Powder +Iodine/20	Brownish green

The plant's phytochemical analysis revealed the presence of organic phytocompounds such as alkaloids, carbohydrates, flavonoids, proteins, amino acids, phenols, tannins, glycosides, and steroids. The information in **Table 5,** is presented.

TABLE 5: PHYTOCHEMICAL SCREENING OF *P. PLEBEIUM* AERIAL PARTS

Sl. no.	Phytocostituents	Petroleum ether	Ethyl acetate	Ethanol	Water
1	Acidic compound	-	-	-	+
2	Aleurone grain	-	-	-	+
3	Alkaloids	+	+	+	+
4	Carbohydrate	-	-	-	+
5	Flavonoids	-	-	-	-
6	Saponins	-	-	+	+
7	Glycosides	-	-	-	-
8	Anthraquinones glycosides	-	-	-	+
9	Inulin	+	+	-	-
10	Mucilage	-	-	-	+
11	Tannins	-	+	+	+
12	Protein	-	-	-	+
13	Starch	-	-	-	-
14	Steroids	-	-	-	-
15	Triterpenoids	-	-	-	-
16	Naphthoquinones	-	-	-	-

+= Present --= Absent

EDX Analysis: EDX, a non-destructive technique, generates data from a range of less than a few nanometers, enabling repeated sampling across various plant segments. Characterizing crystals and other inclusions, such as trace elements, is highly beneficial.

The **Fig. 4** displays the specific area where the EDX and sample were analysed. The spectrum of element analysis using EDX was recorded and shown in **Table 6**. Carbon had the highest weight percentage in the powder, accounting for 10.85%. It was followed by oxygen at 8.91%, and then nitrogen, aluminium, silicon, chlorine, potassium, zinc, zirconium, and thallium.

TABLE 6: EDX (ENERGY DISPERSIVE X-RAY SPECTROSCOPY) OF *P. PLEBEIUM* AERIAL PARTS

Element	Wt. (%)
Carbon	10.85
Nitrogen	1.56
Oxygen	8.91
Aluminum	0.28
Silicon	0.49
Chlorine	0.11
Potassium	0.22
Cobalt	0.00
Zinc	0.19
Selenium	0.00
Zirconium	0.22
Lead	0.00
Thallium	0.11

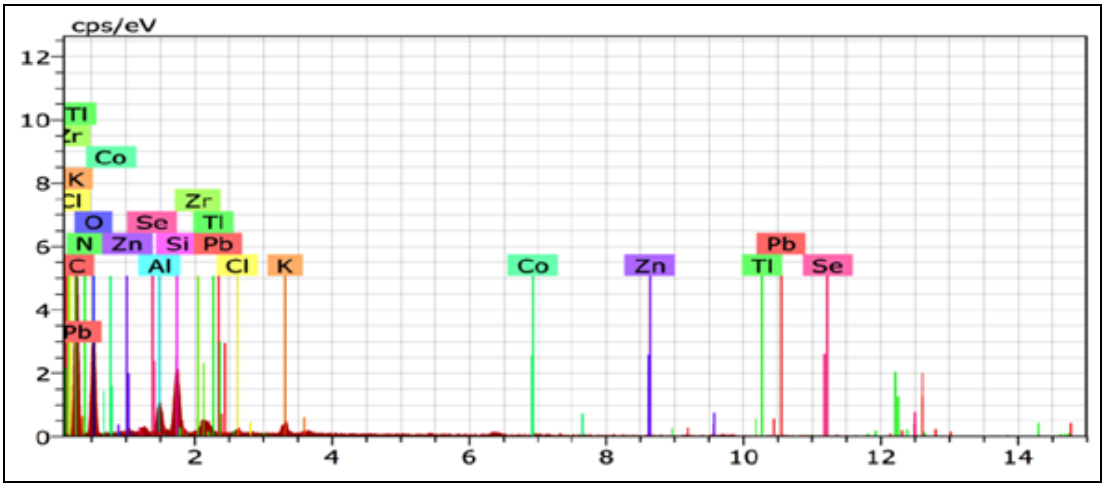

FIG. 4: EDX OF *P. PLEBEIUM* AERIAL PARTS OF PLANT POWDER

FT- IR of Powder Analysis: The presence of functional groups was subsequently verified using FT-IR analysis. The FT-IR spectrum depicted in **Fig. 5,** displays the values of transmittance and wavelength. Transmittance provides quantitative analysis by indicating the presence of functional groups. The strong absorption peaks at 3276.90 cm^{-1} & two medium peak is detected at 3689.11,

3611.12 cm^{-1}, indicating the stretching of O-H bonds. In addition, strong absorption bands are detected in the region of 518.86, 526. 87, 535.21 cm^{-1} indicating the stretching of C-L bonds. The strong absorption peaks at 758.87 cm^{-1} & medium peaks at 2850.70, 2916.43 cm^{-1} are attributed to the stretching of C-H bonds. A strong absorption peak has observed at 1312.83 & 1364.87 cm^{-1}, which suggests the presence of S=O & N-O bond. The strong peaks at 1011.65 and medium peaks at 1233.02 cm^{-1}, showed stretching vibrations of the C-F & C-N bond. The peaks at 1435.24, 1616.04 and 1726.22 cm^{-1}, indicated the bending of C-H bond and stretching of C=C and C=O bonds respectively. The medium type of peak at 3320.71 cm^{-1} represents stretching of N-H bond. Stretching & bending of various of function group powder drug of *P. plebeium* aerial parts as shown in **Table 7**.

TABLE 7: FT-IR ANALYSIS OF *P. PLEBEIUM* AERIAL PARTS OF PLANT POWDER

Sl. no.	V_{max} (cm^{-1})	Type of vibration	Appearance	Compound class
1	518.86	C-Lstr	Strong	Chloro compound
2	526.87	C-L str	Strong	Chloro compound
3	535.21	C-L str	Strong	Chloro compound
4	758.87	C-H str	Strong	1,2 disubstitued
5	1011.65	C-F str	Strong	Fluro compound
6	1233.02	C-N str	Medium	Amine compound
7	1312.83	S=O str	Strong	Sulfone compound
8	1364.87	N-O str	Strong	Nitro compound
9	1435.24	C-H ben	Medium	Methyl group
10	1616.04	C=C str	Strong	α,β unsaturated ketone
11	1726.22	C=O str	Strong	Conjugated anhydride
12	2850.70	C-Hstr	Medium	Alkane
13	2916.43	C-Hstr	Medium	Alkane
14	3276.90	O-H str	Strong	Carboxylic acid
15	3320.71	N-Hstr	Medium	Secondary Amine
16	3611.12	O-Hstr	Medium	Alcohol
17	3689.11	O-H str	Medium	Alcohol

Str: stretching; ben: bending;

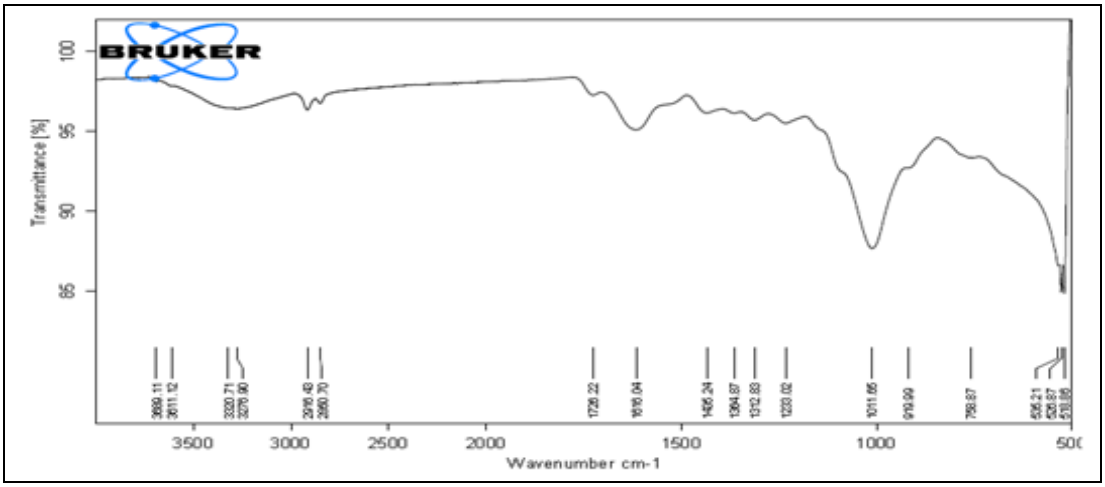

FIG. 5: FT- IR ANALYSIS OF *P. PLEBEIUM* AERIAL PARTS OF PLANT POWDER

Chemical and micro chemical studies with water or aqueous extracts reveal the presence of chemical elements such as saponins, tannin, anthraquinones glycoside, mucilage, and carbohydrates in the powder, as shown in **Table 8**.

TABLE 8: CHEMICAL AND MICRO CHEMICAL TESTS *P. PLEBEIUM* AERIAL PARTS OF PLANT POWDER

Sl. no.	Test	Observation	Inference
1	Test for oils	Oily stain persisting for long time	Fixed oil not present Volatile oil not present

2	**Test with water/aqueous extract**		
	Powder + water shake the test tube.	Frothing	Saponins present
	Powder + water boil in test tube.	Aromatic	Aromatic
	Test for tannins: Aqueous extract+$FeCl_3$.	Dark colouration precipitation.	Tannin present
	Test for anthraquinone: Aqueous extract + ether, shake separate etherical layer + Strong ammonia solution shake and keep aside.	Lower ammonical layers shows pink red colour.	Anthraquinones glycoside.
	Test for mucilage powder +1-2 drops of water on microscopic slide.	Mucilaginous mass/ swelling.	Mucilage present.
	Aqueous extract/ powder + Molisch's reagent	Violet blue colouration.	Carbohydrate present.

DISCUSSION: Macroscopic examination is the primary method employed to determine the authenticity and assess the quality of medicinal plants. Typically, this technique involves visually examining the physical characteristics like flavor, smell, and color of the specimen, which serves as a simple way to verify the authenticity of therapeutic plant constituents. The macroscopic process will be beneficial in the development of herbal monographs and pharmacopeia standards. Powder microscopy is a crucial technique for identifying the specific histological features seen in powdered crude pharmaceuticals. It serves as a valuable tool for identifying plants adulterants [25].

The electron microscope operates in a vacuum setting, where it concentrate the electron beam and improves the images by using electromagnetic lenses. This technology is highly efficient for analyzing the surface morphology of herbal remedies at high magnifications. It provides a three-dimensional representation of the specimen's surface. Therefore, it can serve as a benchmark for carrying out a detailed or concise examination of any plant component [26]. Because of its importance, the powder drug was the subject of a FE-SEM investigation. The investigation shows the microscopical characteristic including lignified fibers, cork and cortex, xylem channels, crystals, and starch grains.

The SEM study of *P. plebeium* is being presented for the first time. Scanning electron microscopy (SEM) is crucial for accurately defining the characteristics of an object at both macroscopic and microscopic levels. To gain the most comprehensive information from biological materials, it is common to use both light and electron microscopy together. In this case, an attempt was made to utilize scanning electron microscopy (SEM) [27]. Ash value refers to the inorganic remains that are left behind when a substance is completely burned. The presence of several harmful substances, such as carbonate, oxalate, and silicate, was indicated. Total ash value is the total of the components of physiological and non-physiological ash. Acid-insoluble ash value refers to the non-physiological components of ash, such as sand and soil. Water-soluble ash value, on the other hand, is the portion of total ash that may dissolve in boiling water [28]. The plant material was ash treated to remove any organic waste that might obstruct the analytical process.

The ash that is naturally present in plants as a result of biochemical processes is known as physiological ash, whereas environmental pollutants are known as non-physiological ash. These might include silicates of different metals that were absorbed from the soil, as well as carbonates, phosphates, nitrates, sulphates, and chlorides [29]. The assessment of the purity, quality, and identity of crude drugs in powder form relied significantly on the measurement of total ash value [30]. Ash that dissolves in water is a useful marker for the existence of exhausted material.

The extractive values are useful for determining appropriate solvents for extraction as well as evaluating specific elements soluble in a given solvent. Pharmacopoeia standards were consistent with the analytical standards for aerial components, which could serve as a reference tool for identifying and assessing their quality and purity. The constituents of *P. plebeium* aerial parts were soluble in petroleum ether, ethyl acetate, ethanol, and water. The solubility of water, alcohol, ethyl acetate, and petroleum ether is highest in decreasing order [31, 32]. The moisture content was measured to determine any weight gain resulting from moisture absorption. In the presence of air, the moisture content of 12.5% suggests that the active components would likely undergo enzymatic hydrolysis and breakdown. Since the amount of

water present determines how quickly plant matter decomposes, a high moisture content suggests that bacterial or fungal growth may occur [33]. The swelling index test yielded a negative result, suggesting the lack of mucilaginous components in the powder. The reported values for the swelling and foaming index were both below 1 and 100, respectively. While pH measurements at 1% and 10% revealed that the solution was acidic.

Moreover, fluorescence analysis of powdered drug with different reagents gave different characteristics colour under ultraviolet (254nm-366nm) and under normal ordinary light. Various chemical constituents present in plant, materials exhibited different fluorescence or behaviour as such or when react with various chemicals.

All the phytochemical properties were studied for the identification of the sample. As mentioned earlier, the idea about the quality and purity of the sample can also be observed by performing some tests mentioned in **Table 5**.

Medicinal herbs include a variety of trace elements that are crucial for the structure and function of metalloproteinases and enzymes in living cells. Each component performs numerous functions within the body. The transfer of components from soil to plant, and subsequently from plant to person, is the primary pathway by which these substances reach the human body. Humans have recognized lead (Pb), cadmium (Cd), aluminium (Al), and thallium (Tl) as hazardous, but the remaining elements are not toxic. Studies have shown that zinc (Zn) is an element that triggers the activation of various kinds of enzymes in physiological processes and is involved in the process of wound healing [34]. Potassium is useful for regulating cardiovascular disease and plays a significant capacity in the contraction of both skeletal and smooth muscles. It also stimulates hormone production, including the release of insulin, and contributes to the immunological response. It has a crucial role in promoting healthy gastrointestinal and muscle function. Additionally, it aids in the transportation of nutrients within cells while also preventing cell death [35, 36]. Silicon is a critical element that primarily protects vein and artery walls from hardening [37]. The FT-IR spectral technique is commonly adapted to identify the various functional groups present in the plant extract. This was done to identify the therapeutic compounds present in plant residue by analysing the characteristic absorption peaks in the fingerprint area [38]. The *P. plebeium* aerial part powder drugs were analysed using FT-IR in the frequency range of 3500–500 cm^{-1}.

The existence of major phyto metabolites such as alkaloids, amino acids, flavonoids, glycosides, phenolic acids, quinones, steroids [39] and sesquiterpenoids [40], has been shown by the unique peaks of different functional groups. Several types of phyto metabolites found in *P. plebeium* aerial part, along with their functional groups (O-H, S=O, N-O, C-N), have been observed to have therapeutic properties and they have also responsible for other medicinal benefits, including antimicrobial, anticancer, anti-inflammatory, hepatoprotective, antioxidant, and activities [40, 41, 42].

CONCLUSION: The quality and quantity of medicinal compounds present in medicinal plants determine their therapeutic effectiveness. Incorrect identification is the initial cause of the erroneous use of medicinal plant. Therefore, the establishment of the Pharmacognostical examination of medicinal plants of natural origin is crucial. The majority of research in Pharmacognosy has focused on the identification of erroneous plant species and the authentication of commonly used traditional medicinal plants through the investigation of their morphology, and physicochemical study.

This study is the first to report the pharmacognostic analysis for this plant species, providing an extensive pharmacognostic profile of *P. plebeium*. The present study will ensure the accurate identification and authenticity of medicinal plants of natural origin, and they may also aid in preventing their manipulation.

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