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PHARMACOGNOSTIC STUDIES OF *GREWIA TILIAEFOLIA* VAHL

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Keywords:

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ABSTRACT: The study was designed to study the macroscopic, microscopic, and also physicochemical parameters such as fluorescence analysis, extractive values, ash values, and loss on drying were performed. Therefore, this parameter is essentially used to control the quality of crude drugs and/or herbal drugs/drug products. The moisture content (% LOD) of the leaf powder was found to be 5.10± 0.14 (% w/w), total ash was found to be 12.27 ± 0.3 (% w/w). The amount of acid insoluble ash and water-soluble ash was found to be 14.5 ± 0.25, 6.23± 1.22 (% w/w), respectively. The extractive values of hexane extract were found to be 2.45± 0.26, ethyl acetate extract was found to be5.62±0.17, the water-soluble extractive value of the drug was found to be 9.142 ± 0.58 (% w/w), and the alcohol-soluble extractive value was found to be 14.64 ± 0.28 (% w/w). The results of physicochemical analyses lie within the acceptable limit, which in turn ascertains the quality and purity of leaf drugs.

INTRODUCTION: India has one of the oldest, richest, and most diverse cultural traditions associated with the use of medicinal plants. One-fourth of the world population, *i.e.* 1.42 billion people, depends on traditional medicines, particularly plant drugs, for curing ailments [1]. Correct knowledge of such crude drugs is an essential aspect of the preparation, safety, and efficacy of the herbal product. The process of standardization can be achieved by stepwise pharmacognostic studies [2]. Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect [3].

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Determination of extractive values, ash residues, and active components (saponin, alkaloids, and essential oil content) plays a significant role in the standardization of the indigenous crude drugs [4]. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plants. In the present investigation, the systematic pharmacognostic studies were carried out on the *Grewia tiliaefolia* Vahl.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The plant material was collected in 2016 from the Kambala Konda forest area, Visakhapatnam, Andhra Pradesh, India, and authenticated by Dr. S. B. Padal, taxonomist, Department of Botany, Andhra University, Visakhapatnam. The Voucher specimens A.U. (B.D.H), no. 22231 was deposited in the herbarium, A.U. College of Pharmaceutical Sciences, Andhra University.

Chemicals and Instruments: Electron Microscope (Eclipse Nikon 80i), Fluorescent UV Cabinet, Hot air oven, glass slide, coverslip, watch glass, and other common glassware was the basic apparatus and instruments used for the study.

Solvents such as hexane, ethyl acetate, methanol, distilled water, and reagents such as phloroglucinol, dilute hydrochloric acid, glycerol was used without further purification. All the solvents and chemicals used for pharmacognostic evaluation were analytical grades obtained from Merk India.

Experimental Methods:

Macroscopic Evaluation of *Grewia tiliaefolia* Vahl: Macroscopic evaluation of the selected plant was recorded as per visual observation organoleptic evaluation of the selected plant, color, odor, the taste was recorded separately. A small quantity of each powdered drug was spread on a TLC plate and physically examined for general appearance *i.e.*, color, taste, texture, *etc*.

Microscopic Evaluation of *Grewia tiliaefolia* Vahl: Microscopic evaluation is a step towards authentication of the internal structure of the natural drug to establish proper identification by revealing tissue arrangements. This is done by identifying internal structures such as epidermis, collenchyma, vascular bundles, types and arrangement of vascular bundles, sclerenchyma, types of stomata, calcium oxalate crystals, oil globules, trichomes, lignified fibers, stone cells, and any other specific features that lie therein. For this purpose, a transverse section or longitudinal section by either freehand section cutting were prepared as follows:

Freehand Sectioning: The small square piece of the leaf was cut in the midrib region (midrib including a small portion of the leaf lamina) using a sharp razor blade. The portion of midrib was put between the pith, and fine sections were cut with the help of a sharp razor blade. The sections so obtained were cleared using chloral hydrate solution.

Staining: The cleared sections were transferred to a watch glass containing staining solution phloroglucinol (0.2 g) 1 % solution was prepared in 80mL of 20% ethanol and 20ML of concentrated HCl (12N). The sections were allowed to stain for 2-3 min. The sections were then transferred into a watch glass containing plain distilled water to wash away the excess stain. The sections were then transferred into a clean micro slide with the help of a brush and add one drop of staining reagent, one drop of HCl, and add one drop of glycerol; place a coverslip gently on it to avoid air bubbles and remove the excess of reagent on the glass slide around the coverslip using a tissue paper and clean the glass slides back side using a tissue paper and then observed under a microscope at the magnifications 10x, 40x [5-6].

Powder Analysis: The shade dried leaves of the plant were powdered, and the powder was passed through the 80#sieve. A small amount of powder was taken onto a microscopic slide in 50% v/v glycerol in water. This was then observed under a microscope to study the characteristic features.

Physicochemical Parameters: The physico-chemical parameters such as fluorescence analysis, extractive values, ash values, and loss on drying were performed according to the official methods prescribed in [7-10].

Determination of Total Ash: About 5 g (accurately weighed) of leaf powder was taken in a silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat, not exceeding dull red heat (450 °C) until free from carbon, cooled, and weighed. The percentage of ash was calculated concerning the air-dried sample of the crude drug.

Total Ash = Weight of ash / Wt of the crude drug taken × 100

Determination of Water-soluble Ash: The total ash was boiled in 25ml of water for 5 min and was filtered through an ashless filter paper (Whatman No. 41). It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled, and the water-insoluble matter was weighed. The water-soluble ash was calculated by subtracting the water-insoluble matter from the total ash.

Water-soluble ash = Weight of ash / Wt of the crude drug taken × 100

Determination of Acid-insoluble Ash: The total ash obtained was boiled for 5 minutes with 25mL of 10 % dilute hydrochloric acid and filtered

through an ashless filter paper (Whatman No. 41). The filter paper was ignited in the silica crucible, cooled and acid insoluble ash was weighed.

Acid insoluble ash = Weight of ash / Wt of the crude drug taken × 100

Determination of Moisture Content by Loss on Drying: 5 g of the powdered drug was accurately weighed and placed in a Petri dish and dried in the hot air oven at 110 °C for 4 h. After cooling, it was placed in a constant weight was obtained and the percentage loss on drying was calculated concerning the dried powder [11].

Percentage loss in drying = loss of weight of sample / Wt of the sample × 100

Determination of solvent extractive values [7, 11-13]: 5 g of coarsely powdered air-dried plant material was macerated in 100 ml of different solvent separately (Hexane/ethyl acetate/methanol/distilled water) in a glass-stoppered conical flask with frequent shaking well and allow standing for 6 h and then allowed to stand for 18 h. Then filtered carefully, taking care against loss of solvent. About 25ml of the filtrate was evaporated in a tared flat bottomed dish to dryness rapidly through a dry filter. Then dried the extract at 105 °C for 6 h, cooled in a desiccator for 30 min and weighed.

Water-soluble extract = Weight of extract / Weight of the sample taken × 100

Determination of Swelling Index: The swelling properties of medicinal plants show specific therapeutic utility *e.g*. gums, pectin, or hemicellulose [14].

Procedure: 1 g of sample was taken into a 25 ml glass stoppered measuring cylinder. The measuring cylinder was filled up to a 20 ml mark with water. Agitate gently occasionally for 24 h and allowed to stand to measure the volume occupied by the swollen plant material.

Swelling Index= Final Volume – Initial volume / Final volume × 100

Determination of Foaming Index: Some of the medicinal plant materials contain saponins that cause persistent foam formation when a water decoction is stunned. The foam-forming capability of plant material and their extract is measured in terms of foaming index [15].

Procedure: 1 g of the plant material was weighed accurately, transferred into a 500 ml conical flask containing 100 ml, and boiled for 30 min. The extract was allowed to cool at room temperature and filter into a 100 ml volumetric flask and add sufficient water through the filter to dilute to volume. The decoction was poured into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml, *etc*. up to 10 ml and adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 min and measure the height of the foam. The results are assessed as follows: If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100. If a height of foam of 1 cm is measured in any tube, the volume of the herbal material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, similarly prepare an intermediate dilution to obtain a more precise result. If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case, repeat the determination using a new series of dilutions of the decoction to obtain a result. Calculate the foaming index using the following formula: 1000 a, where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

Determination of Fluorescence Analysis: The fluorescence characteristics of the leaf powders were examined in the daylight as well as under UV light (254 nm and 365 nm), after treatment with different reagents like ferric chloride, glacial acetic acid, hydrochloric acid, iodine, nitric acid, and sodium hydroxide, *etc.* [16-17].

RESULTS: *Grewia tiliaefolia* (Dhanu vriksha in Sanskrit), belongs to the family Tiliaceae, and it is a medium-sized tree, up to 20 m in height, and grey to blackish brown fibrous bark, leaves simple, alternate flowers are yellow, small on thick axillary peduncles and fruits are globose drupes of the size of a pea, 2-4 lobed, black when ripe, seeds 1-2 the results were supported by Boydem and Brand *et al*., [18-19]. The arc-shaped vascular bundle is covered by a continuous sclerenchymatous Pericycle, 8-10 layered lamellar collenchyma, Mucilage canals

separated by walls of 1-2 cells in thickness, Mucilage cavities between palisade cells and upper epidermis. Reticulate arrangement of spongy tissue with large air spaces in between. Glandular trichomes with globose multicellular head and a unicellular stalk. Trichomes of different sizes and lumen sizes (2-6 pm). *Lacunar collenchyma* containing mucilage cavities. Both gelatinous fibers and libriform fibers. Parenchyma cells in the protoxylem region. A single layer of fiber covered this region. Heterogenous phloem rays having radial rows of elongated rectangular cells alternating with rows of square cells. The components present in the powder were lamellate collenchyma, double-layered palisade, prismatic crystals, and mucilage cavities, two types of stellate hairs one large and other small, mucilage cavities in palisade. The results of the physicochemical determinations are presented in **Table 1**.

The leaf powder's moisture content (% LOD) was found to be 5.10± 0.14 (% w/w), which indicates that the drug was properly dried and stored. Determining moisture content is important for plant drugs because insufficient drying may lead to possible enzymatic deterioration of active principles [20]. Therefore, this parameter is essentially used to control the quality of crude drugs and/or herbal drugs/drug products. The purity of crude drugs could also be evaluated by determining ash values, which represent the content of foreign matter such as inorganic salts or silica present as a form of adulterant in the drug sample. An analytical result for total ash was found to be 12.27 ± 0.3 (% w/w). The total ash includes both 'physiological ash' which is derived from the plant tissue itself and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. The amount of acid insoluble ash and water-soluble ash was found to be 14.5 ± 0.25, 6.23± 1.22 (% w/w), respectively. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth [20]. The results show that the amount of water-soluble ash is less than that of acid-insoluble ash, whereas the amount of total ash was almost double the quantity of water-soluble ash. The ash content gives an idea about the inorganic content of powdered leaves under investigation and thus the quality of the drugs can be assessed.

On the other hand, the hexane extract was found to be 2.45± 0.26, ethyl acetate extract was found to be5.62±0.17, the water-soluble extractive value of the drug was found to be 9.142 ± 0.58 (% w/w) which indicates the presence of water-soluble components such as sugar, acids, and inorganic compounds, *etc* and the alcohol-soluble extractive value was found to be 14.64 ± 0.28 (% w/w) which indicates the presence of polar constituents like phenols alkaloids steroids glycosides flavonoids. The results of physicochemical analyses lie within the acceptable limit, which in turn ascertains the quality as well as purity of leaf drugs.

TABLE 1: MACROSCOPY OF *GREWIA TILIAEFOLIA* VAHL

S. no.	Part of the plant	Colour	Taste	Odor
1	Leaf	Pale Green	Bitter	Characteristic
2	Stem	Pale Green	Bitter	Characteristic
3	Fruit	Red	Sweet	Sweet and aromatic
4	Flower	Yellow	No Characteristic	No Characteristic

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF *GREWIA TILIAEFOLIA* VAHL LEAF

S. no.	Parameters	% w/w (Average of three readings)
1	Total Ash	12.27 ± 0.3
2	Acid-insoluble ash	14.5 ± 0.25
3	Water-soluble ash	6.23± 1.22
4	Loss on drying	5.10± 0.14
5	Hexane soluble extractive value	2.45± 0.26
6	Ethyl acetate soluble extractive value	5.62 ± 0.17
7	Methanol soluble extractive value	14.64 ± 0.28
8	Water soluble extractive value	9.142 ± 0.58
9	Swelling Index	0.93 ± 0.05
10	Foaming Index	< 100

All values were expressed as Mean ± SEM

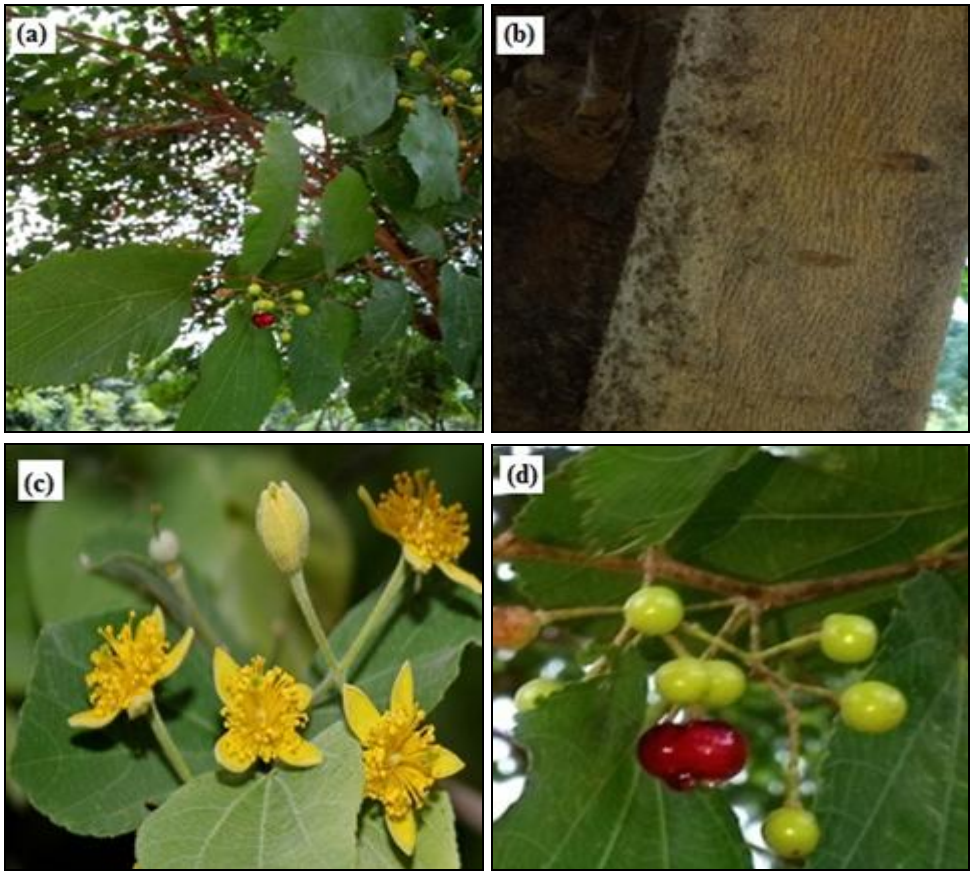

FIG. 1: PHOTOGRAPHIC IMAGE OF (A) LEAF (B) BARK (C) FLOWER (D) FRUIT OF *GREWIA TILIAEFOLIA*

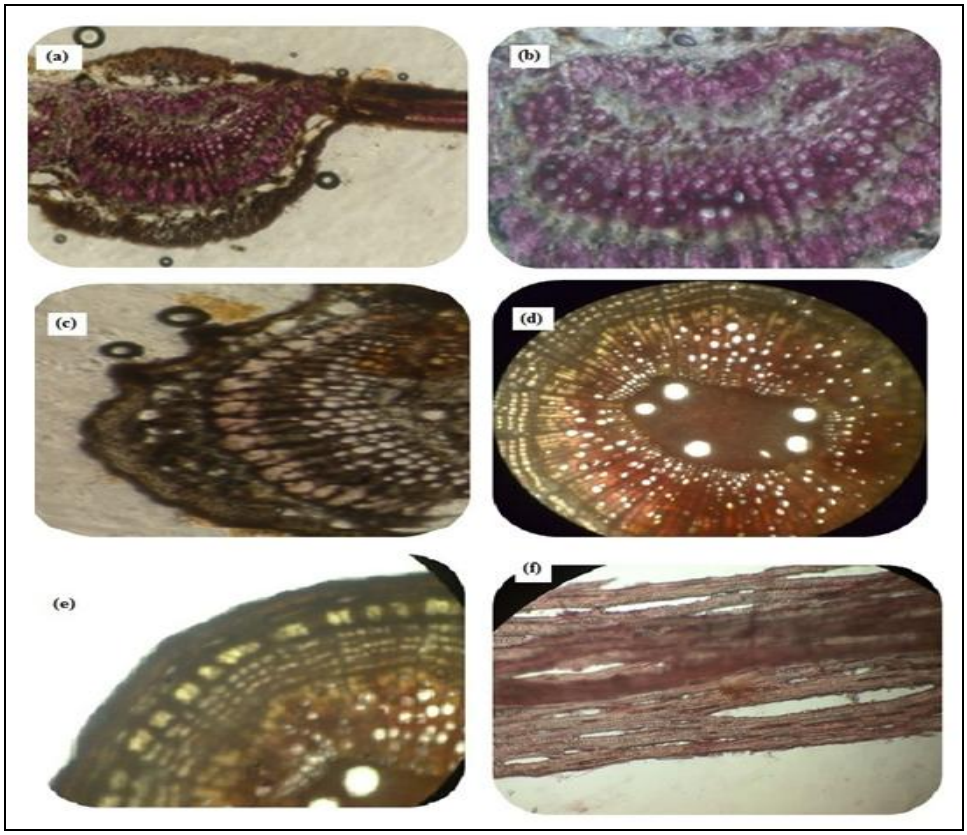

FIG. 2: (A) TRANSVERSE SECTION OF *GREWIA TILIAEFOLIA* LEAF (B) VASCULAR BUNDLES OF *GREWIA TILIAEFOLIA* LEAF, (C) OUTER SERRATE MARGINS OF *GREWIA TILIAEFOLIA* LEAF (D) TRANSVERSE SECTION OF STEM (E) VASCULAR BUNDLES ARRANGEMENT (F) STEM BARK OF *GREWIA TILIAEFOLIA* VAHL

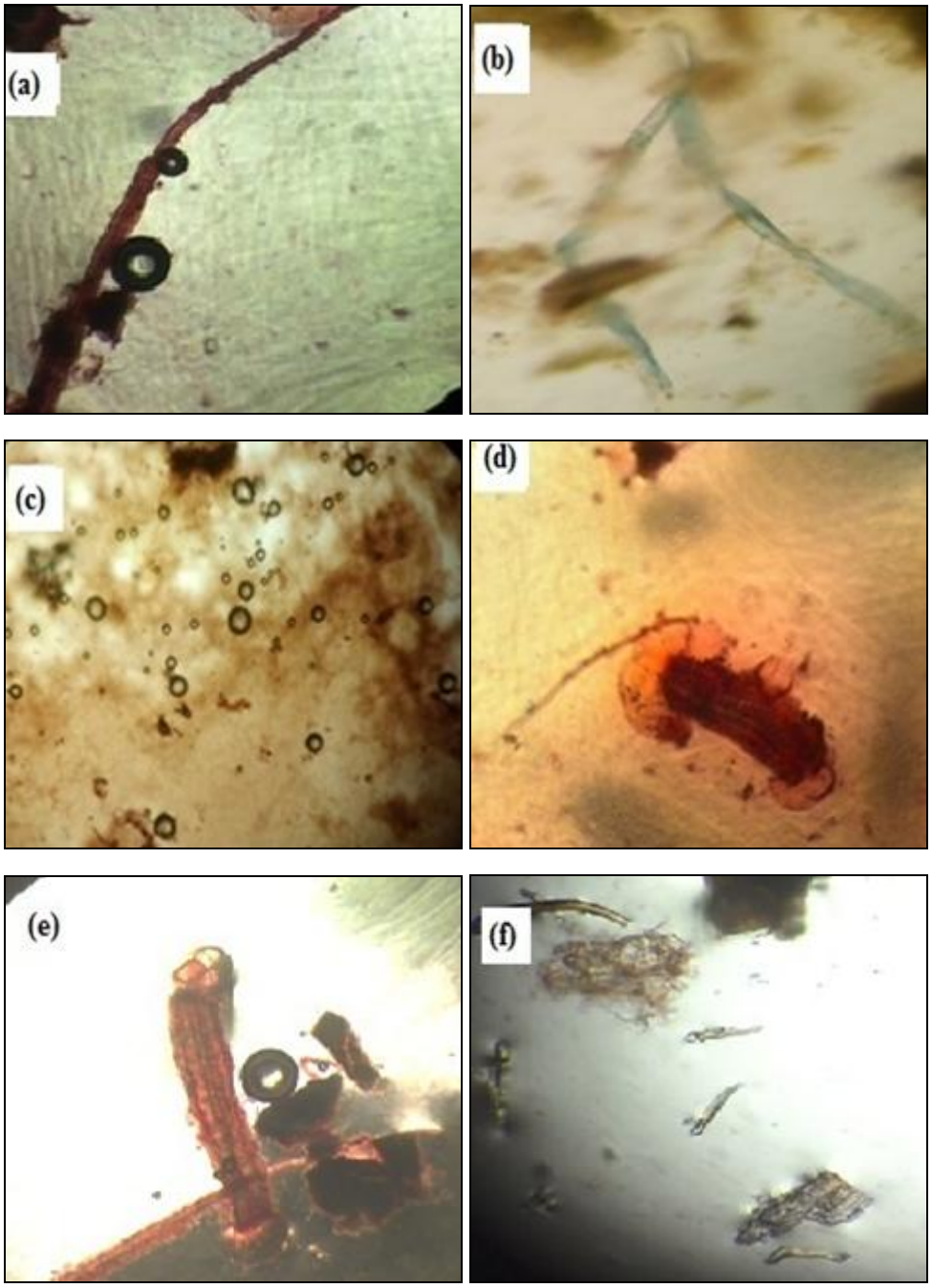

FIG. 3: (A) OIL GLOBULES (B) PARENCHYMA STAND (C) MULTICELLULAR UNISERIATE TRICHOME (D) PARENCHYMA CELLS OF *GREWIA TILIAEFOLIA* VAHL LEAF POWDER

TABLE 3: FLUORESCENCE ANALYSIS OF *GREWIA TILIAEFOLIA* VAHL LEAF POWER

Reagents	Visible	Short UV	Fluorescence
Powder + picric acid	Greenish-yellow	Yellow	Yellow
Powder + 1 % glacial acetic acid	Green	Yellowish-brown	Brownish black
Powder +10 % NaOH	Green	Dark brown	Yellowish-brown
Powder + dil. NH3	Dark green	Dark yellowish brown	Bluish brown
Powder + Conc. HNO_3	Green	Light brown	Brown
Powder+ Acetic acid	Green	Blackish brown	Dark brown
Powder +1M HCl	Dark green	Light brown	Blackish brown
Powder +1M H_2SO_4	Dark green	Dark brown	Reddish-brown
Powder + 10 % $FeCl_3$	Dark green	Brown	Dark brown
Powder + Bromine water	Light green	Light brown	Brownish-yellow
Powder +10 % Iodine	Light brown	Brown	Black

DISCUSSION: To ensure reproducible quality of herbal products, proper starting material control is of utmost importance. Thus, there has been an emphasis on the standardization of medicinal plants of therapeutic potential in recent years. Despite modern techniques, pharmacognostic studies' identification and evaluation of plant drugs is still more reliable, accurate, and inexpensive. According to World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [8]. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs [20]. The Organoleptic studies show the important characteristics of the drugs, the structure of the leaves, the hairy surface of the leaves, the typical tongue sensation, and the odor may screen the preliminary phytochemical constituents. The organized drugs are identified by microscopic examination to determine the histological characters and to distinguish possible adulterants [21-23].

The physicochemical evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs [24]. The percentage of active chemical constituents in crude drugs is mentioned on an air-dried basis. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The test for loss on drying determines both water and volatile matter [8, 20].

The residue remaining after incineration of plant material is the ash content of ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods: total ash, acid-insoluble ash, and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially sand and siliceous earth. Water-soluble ash is the water-soluble portion of the total ash [20, 25]. These ash values are an important pharmacognostic tool for standardized crude drugs. The extracts obtained by exhausting plant materials with specific solvents are indicative of approximate measures of their chemical constituents extracted with those solvents from a specific amount of air-dried plant material [8, 26]. Extractive values are useful for the determination of exhausted or adulterated drugs and help estimate specific constituents soluble in a particular solvent [27]. Extractive values of *Grewia tiliaefolia* Vahl were given in **Table 2**. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products (*e.g.*, alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way, and it is an important parameter of pharmacognostical evaluation [16, 28]. Colour reactions of *Grewia tiliaefolia* Vahl leaf power in fluorescence analysis indicates the presence of different phytoconstituents **Table 3** reddish-brown with sulphuric acid indicates the presence of steroids or triterpenoids black-green color with aqueous ferric chloride solution indicates the presence of tannins yellow color indicates the presence of alkaloids, light yellow with aqueous sodium hydroxide solution indicates the presence of flavonoids [29].

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CONFLICTS OF INTEREST: Nil

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