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Original Article

Pharmacognostic and Phytochemical Evaluation of the bark of *Grewia tiliifolia* Vahl.

Jaya Kuruvilla[1], M. Anilkumar[2,*]

ABSTRACT

Introduction: *Grewia tiliifolia* Vahl. is an important ethnomedicinal tree widely distributed in the tropical and sub-tropical areas and has been used as a source of herbal shampoo by the local communities in many places of Kerala, India. It has been routinely used in the traditional Ayurvedic medicines against cough, ulcers, cancer, skin diseases, pruritus, wounds and urinary infections. **Objective:** The aim of this study was the pharmacognostical standardisation of *G. tiliifolia*. **Methods:** Pharmacognostic evaluation of *G.tiliifolia* bark was carried out by usual macroscopic and microscopic examinations and phytochemical screening. In addition, the quantification of major phytoconstituents such as alkaloids, flavonoids, phenols, tannins, saponins and carotenoids were carried out by standard procedures which can further throw light on the medicinal use of this ethnobotanically important plant. **Results:** Anatomical studies revealed the presence of prismatic crystals of calcium oxalate and druses in the stem and bark. Mucilage cavities were observed only in the stem. Histochemical studies revealed that the tissues of phloem parenchyma are the main localising region of various phytoconstituents. The physicochemical examinations along with the estimation of alkaloids, flavonoids, phenols, tannins, saponins and carotenoids will help in setting the pharmacopoeial standards of *G.tiliifolia*. **Conclusion:** The present study provides useful information that will help in the exact identification as well as assessment of purity of crude drugs of *G.tiliifoia*.

Key words: *Grewia tiliifolia*, Pharmacognostic studies, Physicochemical evaluation, Phytochemical screening, Quantification of phytoconstituents.

Jaya Kuruvilla[1], M. Anilkumar[2,*]

[1]Department of Botany, St. Xavier's College, Aluva-683102, Ernakulam, Kerala, INDIA.
[2]Department of Botany, Union Christian College, Aluva-683102, Ernakulam, Kerala, INDIA.

Correspondence

M. Anilkumar

Department of Botany, Union Christian College, Aluva-683102, Ernakulam, Kerala, INDIA.

E-mail: drmakumar@gmail.com

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INTRODUCTION

The search for natural compounds of medical importance has increased recently due to their minimal or no side effects, easy availability and affordable prize. However, in many instances natural drugs pose chances of adulteration or substitution. Thus, pharmacognosy is of great significance in the exact identification of raw drug samples and also to distinguish them from adulterants or substitutes. In pharmacognostic investigations, standardization and authentication of natural drugs through phytochemical, physicochemical and morphological studies are usually conducted to ensure their identity. Such investigations are relevant to the pharmaceutical industries for quality control and in the field of pharmacological evaluation and development of formulations for various diseases. This will help in maintaining the quality of herbal products and their therapeutic efficacy.[1]

Grewia tiliifolia Vahl. is an ethnomedicinally important plant belonging to the family Tiliaceae. The plant has potential medicinal uses and β-sitosterol and stigmasterol were identified from the stem bark.[2,3] The mucilage and hot water extract of *G. tiliifolia* bark can be used as an antidote for opium poisoning.[4] γ-lactones of hepatoprotective properties have been isolated from the stem bark of *G. tiliifolia* through bioassay-directed fractionation and chromatography of methanol extract.[5] Study on analgesic and anti-pyretic activities of *G. tiliifolia* leaves proved that the aqueous extract has an effect comparable to that of paracetamol.[6] Lupeol isolated from *G. tiliifolia* can cause apoptosis in many cancer cells.[7] The presence of lupeol and betulin were reported in three species of *Grewia* viz *G. bicolor* and *G. tiliifolia*.[4,8] The bark of this plant is used as a source of herbal shampoo by the local populations in many areas of Kerala, India, after pressing and softening. Hence the present study has been undertaken that describes its pharmacognostic standardisation *via* macroscopic and microscopic characterization, physicochemical analysis and phytochemical studies with special emphasis on the stem bark.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh stem and bark of *G. tiliifolia* was collected from Muvattupuzha region of Ernakulam District, Kerala, India. The taxonomic identification was done at the Silviculture Department, Kerala Forest Research Institute (KFRI), Peechi and voucher specimen was deposited with accession No. 13053.

Processing of plant material

The present study focused on the stem and bark of *G. tiliifolia* owing to its use as a source of herbal shampoo. The stem and bark of *G. tiliifolia* was washed well in running tap water followed by rinsing in

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double distilled water. Fresh specimen as well as specimen fixed in FAA (Formalin – 10%, 70% Ethyl alcohol – 50%, Acetic acid – 5% + 35% water) were used. For dry specimen preparation, they were chopped and shade dried to complete dryness. It was powdered using an electric blender and passed through a sieve of 60 mesh size to get fine powder.

Pharmacognostic evaluation

Macroscopic evaluation

Morphological parameters are important taxonomic tools for the identification of plant species. The morphological characterization of whole plant, particularly leaves, flowers and stem were noted.

Microscopic evaluation

Anatomical studies - Both free hand sections and microtome sections were taken to study the diagnostic anatomical characters of the bark.

Hand sectioning - Fresh materials were used for free hand sections and single staining was done with safranin and toludene blue O and photomicrographs were taken.[9]

Microtome sectioning - The bark specimens fixed in FAA were dehydrated by passing through a graded series of tertiary butyl alcohol (TBA).[10] The sections were dewaxed with xylene, passed through ethyl alcohol series and then stained with safranin.[11]

Scanning electron microscopic studies

The cell inclusions were analysed using Scanning Electron Microscopy. A modified methodology proposed by Talbot and White was used for the preparation of specimens.[12] The photomicrographs were taken using TESCAN VEGA 3 SBH scanning electron microscope.

Histochemical localization studies

Temporary mounts of fresh plant sections were used for histochemical localization studies. Free hand sections of stem/bark were taken and treated with respective reagents to localize the components. Histochemical localization was carried out with various reagents viz. Lugol's iodine for starch [11] as well as for alkaloids,[13] Biuret reaction for total proteins,[14] Sudan III for lipids,[15] Sudan red for fixed oil,[16] Schiff's reagent for lignin,[17] Ruthenium red for mucilage,[16] Wagner's reagent for alkaloids,[18] Aqueous NaOH for flavonoids and Aqueous Ferric chloride for phenolic compounds[11] and Hydrochloric vanillin for tannins.[19]

Powder microscopy

Powder microscopic studies were conducted based on the methodology of Khandelwal.[20] The powdered sample was cleared with chloral hydrate solution. It was then treated with phloroglucinol and hydrochloric acid and mounted in glycerine.

Physicochemical analysis

Calibrated digital p^H meter (Eutech instruments, p^H 5-10) was used to measure the p^H of 1% and 10% aqueous extracts.[21] The determination of moisture content, ash values and extractive values were done based on the methodology of Ayurvedic Pharmacopoeia of India.[22]

Preliminary phytochemical screening

The preliminary phytochemical screening was done based on the standard protocol of Harborne to determine the presence of various phytoconstituents present in them.[23]

Preparation of extracts

10g of the plant powder was mixed with 50ml of different solvents (Petroleum ether, chloroform, ethyl acetate, acetone, methanol and distilled water) and kept for 48 hours with intermittent shaking. The extract was filtered and kept in water bath for 2 hours at 60°C and was used for further analysis.

Quantification of phytoconstituents

Total saponin content

The total saponin content was determined based on the methodology of Obadoni and Ochuko.[24] 20g of grounded sample was taken in a conical flask and 100ml of 20% aqueous ethanol was added. It was heated on a hot water bath for 4 hours by stirring continuously at a temperature of 55°c. This mixture was filtered and the residue was extracted with another 200ml of 20% ethanol. Both the extracts were mixed together and were reduced to 40ml by keeping in a hot water bath at 90°c. It was then transferred into a 250ml separating funnel. 20ml of diethyl ether was added and shaken thoroughly. The aqueous layer obtained was taken and ether layer was discarded. This purification process was repeated many times. 60ml of n-butanol was added and the combined extract of n-butanol was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation the samples were dried in an oven. The dried quantity was weighed and saponin content was calculated as percentage.

% of saponin = Weight of saponins x 100 / Weight of sample

Total alkaloid content

The total alkaloid content was estimated based on the methodology of Khadabadi *et al*.[25] 5g of powdered sample was mixed with 50ml of 10% sodium hydroxide and sonicated for 10 minutes. 50ml chloroform was added and sonicated for 10 minutes. This was filtered and 50ml chloroform was added and sonicated. This was filtered and then taken in a separating funnel. The lower chloroform layer was taken and upper layer was discarded. 50ml of distilled water was added to the chloroform layer, shaken and the lower layer was collected. This process was repeated one more time. To the collected layer, 50ml of IN HCl was added and lower layer was collected. This was repeated again and to the collected layer, 30ml of 30% ammonia solution was added. The lower separated layer was collected in a pre-weighed beaker. It was kept in water bath and then in oven for drying. The weight of the residue obtained was noted. The percentage of total alkaloids was calculated using the formula

$$\% \text{ of alkaloid} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Total flavonoid content

The total flavonoid content was estimated using aluminium chloride colorimetric method proposed by Khadabadi *et al*.[25] 0.5g of sample powder was mixed with 25ml methanol and sonicated for about 15 minutes. It was filtered into 25ml standard flask and made up to 25 ml with methanol. 0.5ml of the sample was mixed with 0.1ml aluminium chloride (10 %), 0.1ml sodium acetate (1M), 1.5ml ethanol and 2.8ml distilled water. Similarly, 0.5ml of standard quercetin (40 ppm) was also mixed with the above reagents. These mixtures were allowed to stand for 30 minutes and absorbance was measured at 415 nm using spectrophotometer (Varian 50 Bio with CaryWin UV software). The total flavonoid content was expressed as percentage and calculated using the formula

$$\text{TFC} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Conc. of standard}}{\text{Conc. of sample}} \times \frac{\text{Purity of standard}}{100} \times 100$$

Total phenolic content

The total phenolic content was determined by Folin-Ciocalteu method.[25] 0.5g of the powder sample was mixed with 10ml of 90% methanol and centrifuged at 2800 rpm for 5 minutes. The supernatant was filtered into 25ml standard flask. The residue was again mixed with 10ml of 90% methanol and centrifuged at 2800 rpm. This was filtered into standard

flask and was made up to 25ml with 90% methanol. 0.1ml of this sample solution was mixed with 5ml of 10% Folin-Ciocalteu reagent, 4ml of 7.5% sodium carbonate and 0.9ml of distilled water. This mixture was incubated for 1 hour in dark and absorbance was measured at 765 nm using spectrophotometer (Varian 50 Bio with CaryWin UV software). Various concentrations of gallic acid were used (standard) for preparing the calibration curve. The total phenolic content was calculated from the calibration curve and the results were expressed as gallic acid equivalents.

Total tannin content

Total tannin was determined by titrimetric method[25]. 1g of powdered sample was mixed with 75ml distilled water and sonicated for 10 minutes. It was filtered and mixed with 25ml of indigosulfonic acid. This mixture was titrated with 0.1N potassium permanganate solution until the formation of golden yellow colour (end point) and the burette reading (Xml) was noted. Titration of blank (water) was also performed using the same procedure and blank reading (Yml) was noted. The percentage of tannin content was calculated using the following formula with reference to dry weight of substance taken.

$$\% \text{ of tannin } = \frac{(X - Y)\text{ml} \times 0.004157 \times \text{Normality of KMnO4} \times 100}{\text{Weight of sample taken} \times 0.1\ \text{N}}$$

Total carotenoid content

The carotenoid content of *G. tiliifolia* Vahl. bark was estimated based on the methodology of Harborne.[26] 100mg of fresh bark was taken along with 10ml of 80% acetone. It was ground well and centrifuged at 3000 rpm for 10 minutes. The supernatant was taken and made up to 10ml. The absorbance was measured at 480 nm using UV spectrophotometer. The total carotenoid content was expressed in mg/g and was calculated using the following formula

$$\text{Amount of carotenoids in 100mg plant tissue } = \frac{4 \times \text{OD value} \times \text{Total volume of sample}}{\text{Weight of fresh plant tissue}}$$

RESULTS

Macroscopic evaluation

G. tiliifolia is a `medium to large sized tree attaining a height of about 12-15m (Figure 1A). The bark is dark brown, rough, fibrous with red colouration inside (Figure 1B). Leaves are simple, alternately arranged and reddish when young (Figure 1C). The lamina is 13-16.5 x 8-11.5cm, large, broadly ovate, base obliquely cordate, margins serrate, glabrous above and pubescent beneath. Petiole is short and pubescent with a swollen tip. Stipules are leafy, veined and pubescent with a rounded lobe on the lower side (Figure 1D).

Flowers are small, yellow, bisexual and borne in axillary umbels (Figure 1E). Sepals 5 and pubescent outside. Petals 5, yellow coloured, become red at a later stage and half the length of sepals. Stamens are many, free with yellow filaments that turn red (Figure1F). They are inserted on a glandular torus. Ovary is superior with style longer than the stamens ending in a lobed stigma. Fruit is a drupe, globose or 2 lobed with 1 or 2 seeds, turning red when ripe (Figure 1G) and seeds are creamy white (Figure 1H).

Microscopic evaluation

Anatomy of stem

The outer epidermis consisted of single layer of columnar cells covered by thick cuticle. It contained numerous stellate trichomes which were 5-7 armed. The hypodermis comprised of 3-5 layers of collenchyma in which few cells contained prismatic crystals (Figures 2A & 2B). The cortex was made of 5-6 layered thin walled parenchyma cells with mucilage cavities (Figure 2C). The parenchyma cells close to the pericycle also

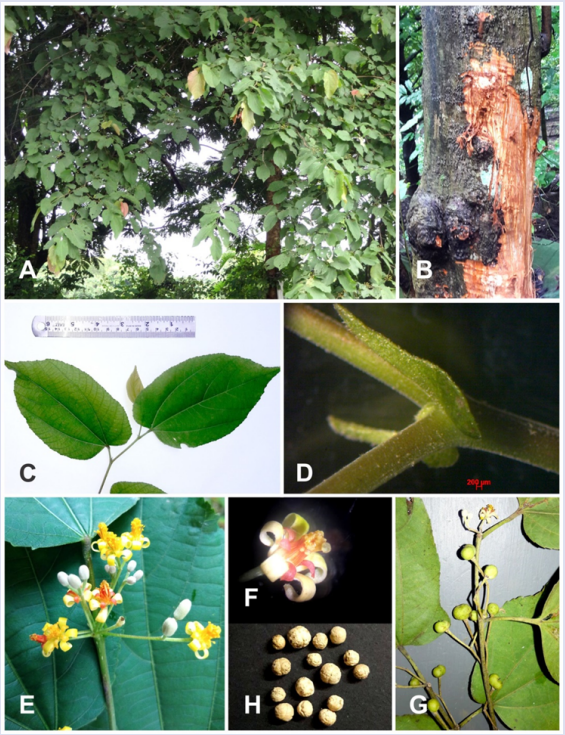

Figure 1: Morphological features of *G. tiliifolia*: (A) Whole plant, (B) Tree trunk with bark removed, (C) Leaves with oblique base, (D) Stipules, (E) Inflorescence, (F) Single Flower, (G) Twig with fruits, (H) Seeds.

contained prismatic crystals. Pericycle was 6-8 layered and made of thick walled cells (Figure 2D). This was followed by phloem which contained numerous calcium oxalate crystals. Next to phloem was cambium followed by xylem. There was a large central pith made of thin walled parenchyma cells in which starch grains were very prominent (Figure 2E & 2F). Mucilage cavities were also present in the pith region having an inner lining of secretory cells. Protein was histochemically localized in the cells of pith and cortex (Figure 2H). Lignin was localized in the pericycle region (Figure 2G) and mucilage was observed in the region of epidermis and vascular tissues (Figure 2I).

During secondary growth, periderm was formed in the outer most region. This includes the outer phellum (cork), middle phellogen (cork cambium) and inner phelloderm. Small raised openings called lenticels were formed in the bark for diffusion of gases (Figure 3F & 3G). Cork consisted of few layers of thick walled cells and cork cambium was made up of thin walled parenchymatous cells. Below the phelloderm was few layers of cortex. This was followed by secondary phloem which comprised of wide phloem rays and phloem fibers. Calcium oxalate crystals were found in the phloem rays and prismatic crystals were observed lying close to the phloem fibers (Figure 3B). The vascular cambial strip was present in between secondary phloem and secondary xylem. Large amounts of secondary xylem were produced towards the inner side and distinct growth ring formation was observed (Figure 3A). Secondary xylem was made up of large xylem vessels, tracheids and multiseriate xylem rays (Figure 3C). Primary xylem was seen near the parenchymatous pith. Mucilage cavities were embedded in the pith (Figure 3D).

Histochemical localization studies

Starch

Presence of starch was detected by treating the sections with Lugol's iodine. Bark gave a negative reaction for starch. But it gave a positive reaction for alkaloids by the formation of brown colouration in the phloem parenchyma (Figure 4A).

Protein

The presence of proteins was characterized by red colouration formed due to biuret reaction. In the bark, proteins were located in phellogen and phloem parenchyma (Figure 4B).

Lipids

Lipids were detected by the formation of reddish globules when treated with Sudan III. The lipid globules were present in the cells of phloem parenchyma (Figures 4C & 4D).

Fixed oil

Fixed oil was localized by treating bark with Sudan red solution. Oil droplets appeared as orange pink coloured globules in the phloem parenchyma cells (Figures 4E & 4F).

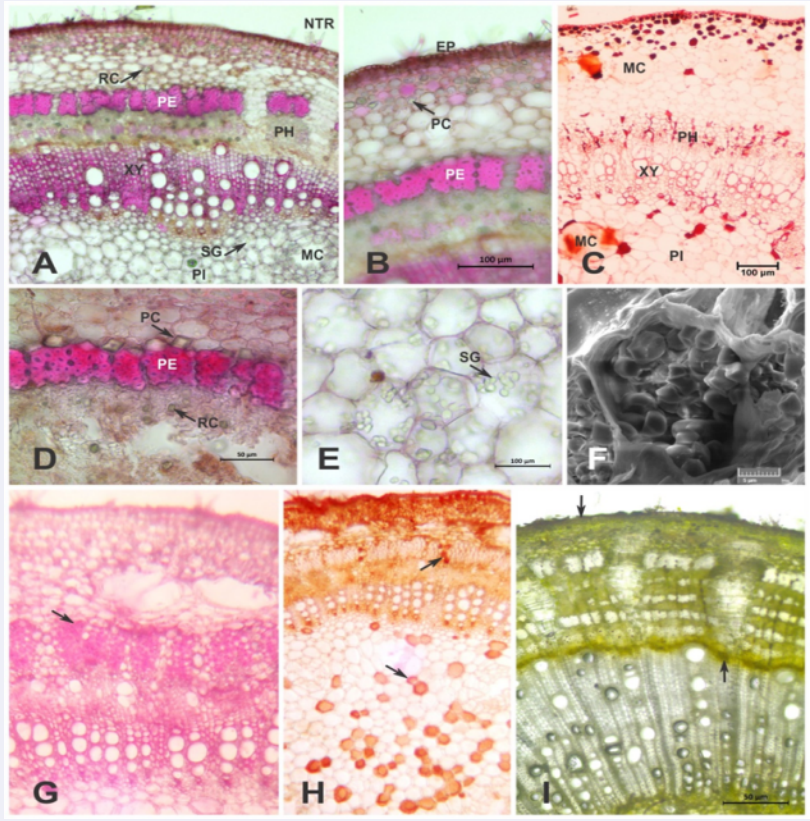

Figure 2: Anatomy and histochemistry of young stem (A) T.S of young stem stained in safranin (B) Portion of stem magnified (C) Microtome section of young stem (D) Enlarged view of stem showing rosette and prismatic crystals (E) Starch grains under light microscope (F) Starch grains under scanning electron microscope (G) Lignin stained magenta with Schiff's reagent (H) Protein stained by biuret reaction (I) Mucilage stained yellow with ruthenium red. NTR-Non glandular trichome, RC-Rosette crystal, PE-Pericycle, XY-Xylem, PH-Phloem, SG-Starch grains, MC-Mucilage cavity, PC-Prismatic crystal, EP-Epidermis, PI-Pith.

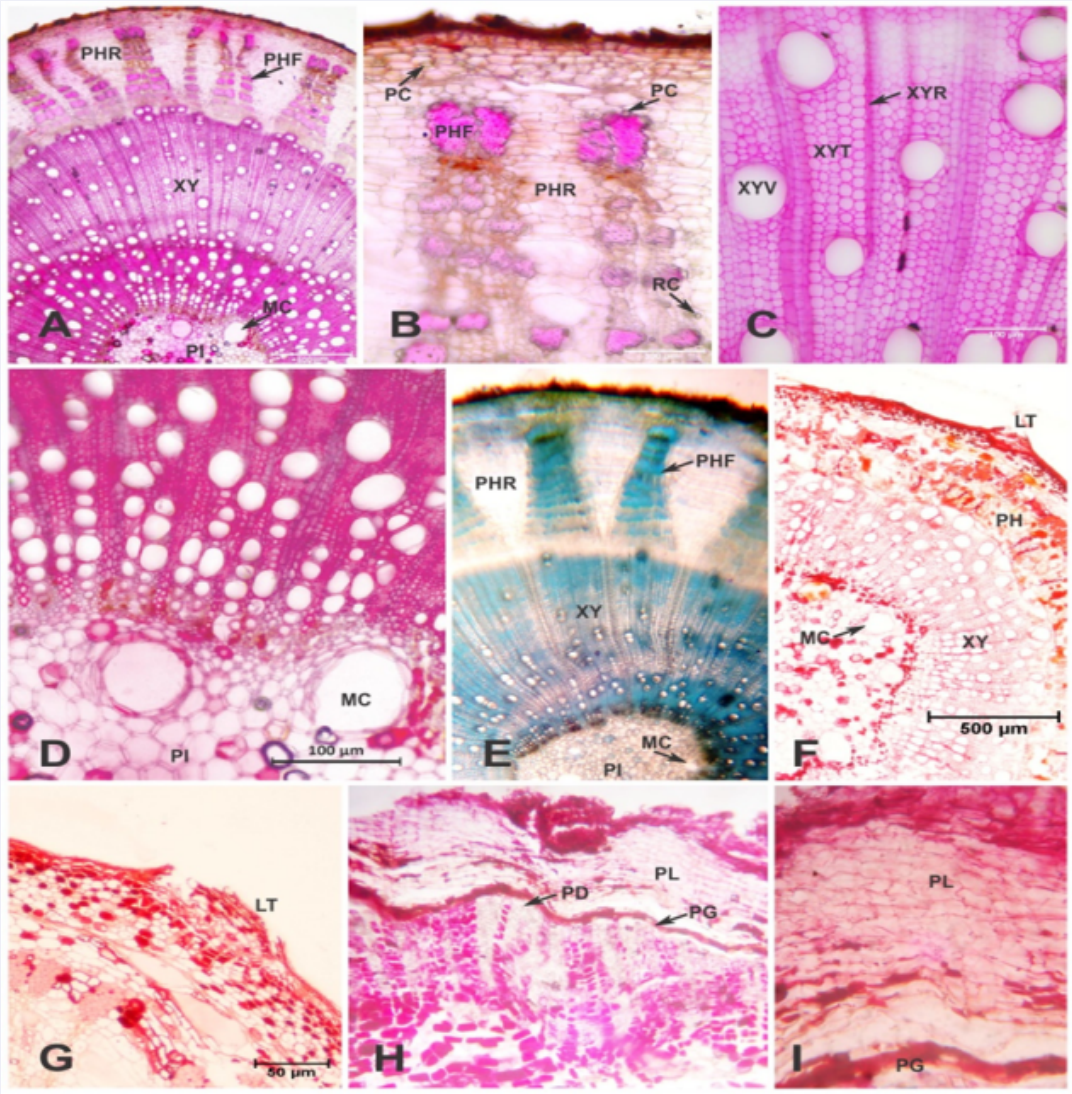

Figure 3 : Anatomy of mature stem and bark: (A) T.S of mature stem stained in safranin, (B,C&D) Portion of the stem magnified, (E) T.S of mature stem stained in toludene blue, (F &G) Microtome sections of mature stem with lenticel, (H) Microtome section of bark, (I) Enlarged view of periderm region. PHR-Phloem rays, PHF-Phloem fibre, XY-Xylem, MC-Mucilage cavity, PI-Pith, PC-Prismatic crystal, RC-Rosette crystal, XYV-Xylem vessel, XYT-Xylem tracheid, XYR-Xylem ray, LT-Lenticel, PH-Phloem, PL-Phellum, PD-Phelloderm, PG-Phellogen.

Lignin

Lignin can be localized histochemically by treating sections with Schiff's reagent. The presence of lignin was observed abundantly in the thick walled cells of bark (Figure 5F).

Mucilage

When treated with ruthenium red, the bark of *G. tiliifolia* stem showed the presence of pinkish red mucilage, whereas yellow mucilage was observed in the cortex, phloem and pith (Figures 5A & 5B).

Alkaloids

The reaction of fresh sections with Wagner's reagent revealed the presence of alkaloids in the bark. This was characterized by reddish brown colouration in the phloem parenchyma (Figure 5C).

Flavonoids

The presence of flavonoids in the bark was verified by the formation of vine red colouration, when treated with aqueous NaOH. Flavonoids were distributed in the regions of phellogen and phloem parenchyma (Figure 5D).

Phenolic compounds

Treatment of sections with aqueous ferric chloride revealed the presence of black coloured phenolic contents in the phloem parenchyma of the bark (Figure 5G).

Tannins

The presence of tannins was verified by red colouration when treated with vanillin-HCl in the cells of phloem parenchyma of the bark (Figure 5E).

Powder microscopy

The powder of *G. tiliifolia* bark was light brown in colour with a distinguishing odour. It was characterized by fragments of lignified parenchyma cells, starch grains, rosette crystal of calcium oxalate, parenchyma cells with starch grains, oil globules, fragments of cork cells, fragments of lignified parenchyma cells, pericyclic fibre with

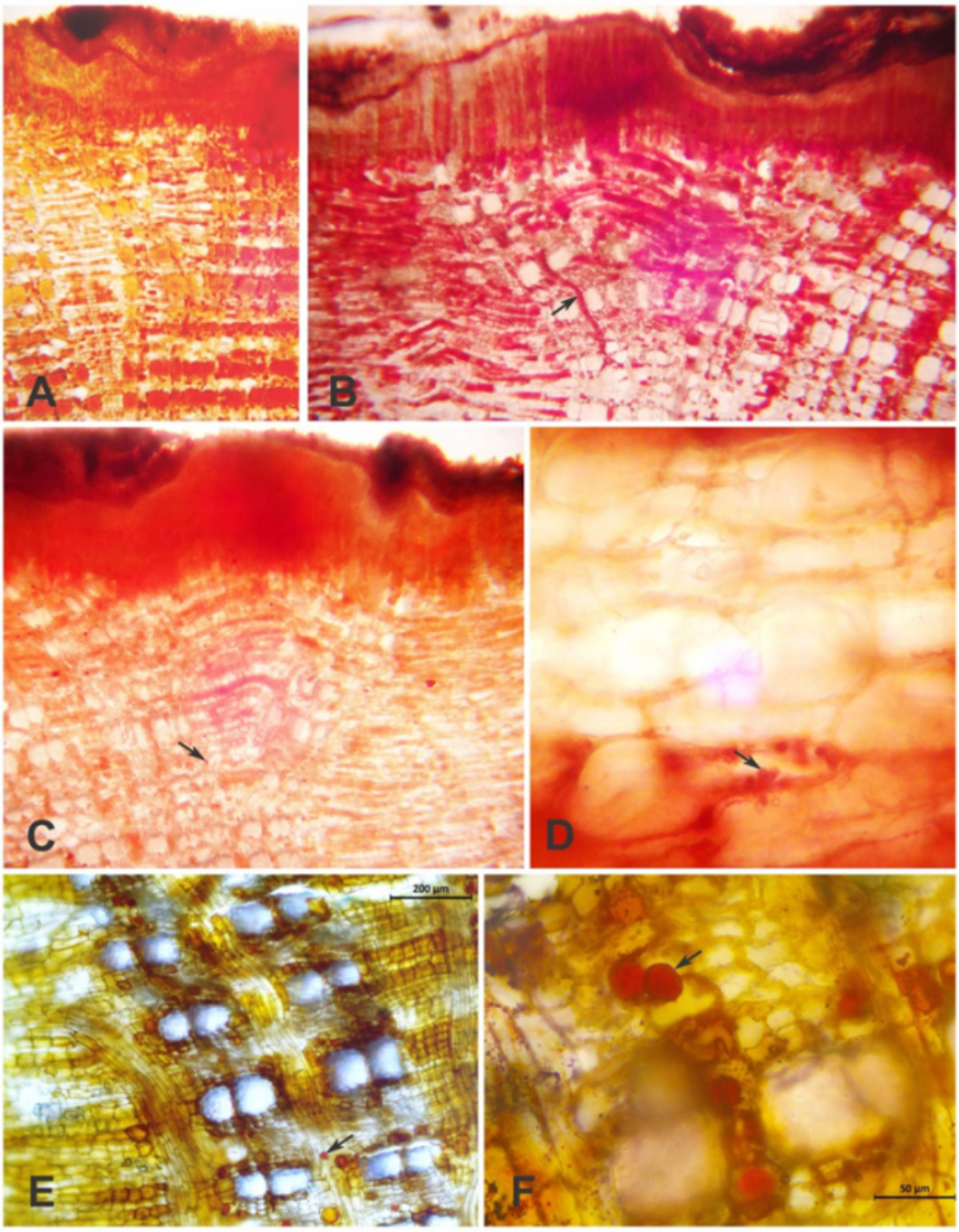

Figure 4: Histochemical localization in the bark tissues (A)Absence of starch when treated with Lugol's iodine (B) Protein stained red by biuret reaction (C&D) Lipid globules stained red with Sudan III (E&F) Fixed oil stained orange pink with Sudan red.

moderately wide lumen and tapered apices, prismatic crystals and fragments of lignified xylem vessel with scalariform thickening.

Physicochemical analysis

The p^H value was found in the acidic range. For 1% solution it was 5.40±0.01 and for 10% solution, p^H value was 5.62±0.01. Moisture content was recorded as 13.29±0.01%. Among the five solvents used for extraction, water was found to be the best extraction solvent because it separated out higher amount of phytoconstituents from the plant powder (10.03±0.04%). The next higher value was given by alcohol (9.31±0.07%). The lowest extractive value was shown by ethyl acetate (1.31±0.06%). This indicated that comparatively lesser number of phytoconstituents were leached out in ethyl acetate. *G. tiliifolia* bark powder exhibited a total ash value of 12.69±0.02%. The acid insoluble ash value was 2.44±0.03% whereas water soluble ash value was 3.50±0.02% (Table 1).

Phytochemical studies

Preliminary phytochemical screening of the different extracts revealed the presence of flavonoids, tannins, alkaloids, terpenoids, saponins, phenols and carbohydrates whereas coumarins, quinines, anthraquinones, resins, reducing sugars and proteins were absent. In the water extract all such compounds gave negative results (Table 2).

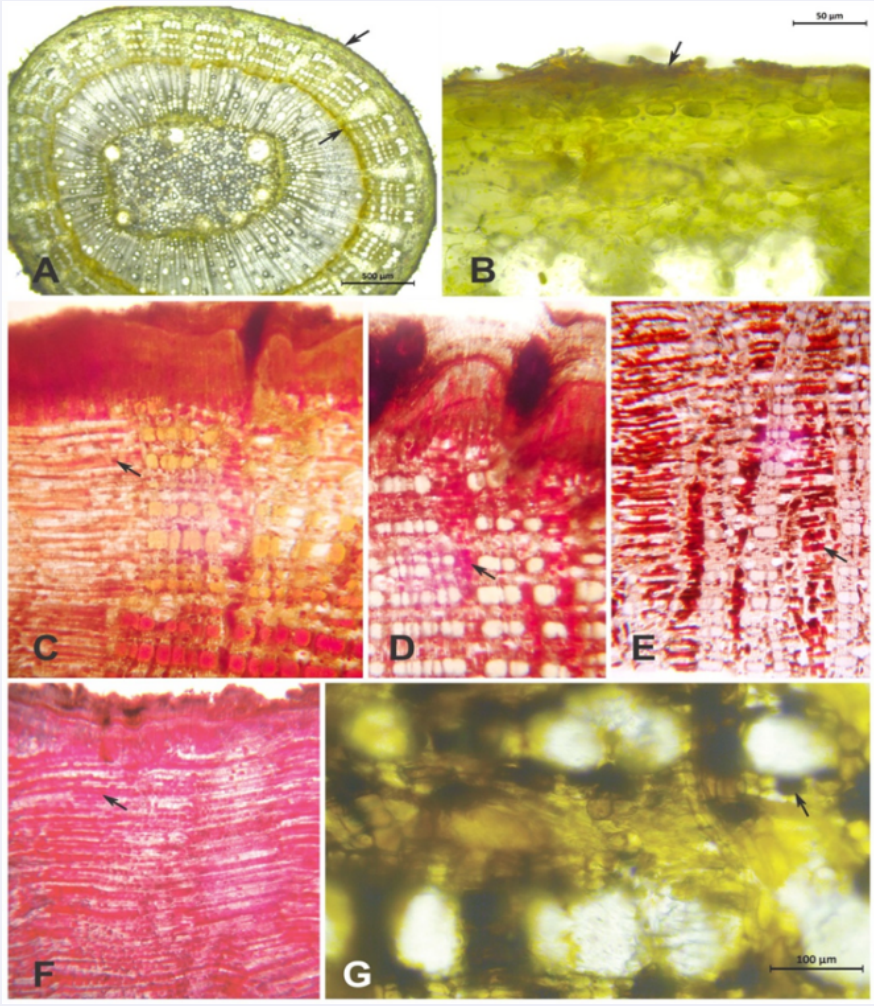

Figure 5: Histochemical localization in the bark tissues (A & B) Mucilage stained yellow and pinkish red with Ruthenium red (C) Alkaloids stained reddish brown with Wagner's reagent (D) Flavonoids stained wine red with aqueous NaOH (E) Tannins stained red with hydrochloric vanillin (F) Lignin stained magenta with Schiff's reagent (G) Phenols stained black with aqueous ferric chloride.

Table 1: Physicochemical analysis.

Sl. No.	Parameters		G. tiliifolia
1	p^H	1%	5.40 ± 0.01
		10%	5.62 ± 0.01
2	Moisture content		13.29 ± 0.01
3	Extractive values	Water soluble	10.03 ± 0.04
		Alcohol soluble	9.31 ± 0.07
		Acetone soluble	3.14 ± 0.02
		Ethyl acetate soluble	1.31 ± 0.06
		Chloroform soluble	2.05 ± 0.04
4	Ash values	Total ash	12.69 ± 0.02
		Acid insoluble ash	2.44 ± 0.03
		Water soluble ash	3.50 ± 0.02

The estimation revealed the presence of very low quantities of flavonoids and carotenoids when compared to alkaloids, phenols, tannins and saponins and the results are summarized in Table 3.

DISCUSSION

The morphological characterization of plants provides valuable information about their botanical identity and thus helps in differentiating the drug from its substituents. The oblique and heart shaped leaf base and characteristic flower helps to identify *G. tiliifolia*.

The presence of calcium oxalate crystals, their chemical composition, morphology and location within the plant can be a useful tool in the characterization of taxonomic groups and also in the identification and purity of plant drugs.[28-30] In *G. tiliifolia* prismatic crystals of calcium oxalate were present in addition to druses. In the creeping plant *Ipomoea asarifolia* the presence of crystals is a defense mechanism against attack by ruminant animals.[31] These crystals help to give protection from herbivores, to maintain ionic balance through storage of calcium and oxalate in idioblasts and support the plants.[32] Histochemical and morphoanatomical studies in the leaves of *Luehea divaricata* Mart. (Malvaceae) reported the presence of prismatic calcium oxalate crystals, druses and mucilage.[33]

Table 2: Preliminary phytochemical screening.

Sl.No.	Phytoconstituents	Petroleum Ether	Chloroform	Ethyl Acetate	Acetone	Methanol	Distilled water
1	Flavonoids	+	–	+	–	+	–
2	Coumarins	–	–	–	–	–	–
3	Tannins	–	+	–	+	+	–
4	Alkaloids(Mayer's)	+	–	+	–	+	–
5	Alkaloids(Wagner's)	–	–	–	–	–	–
6	Steroids/ Terpenoids	+	–	–	–	–	–
7	Saponins	–	+	–	–	–	+
8	Quinines	–	–	–	–	–	–
9	Anthraquinones	–	–	–	–	–	–
10	Phenol	–	+	+	+	+	–
11	Resin	–	–	–	–	–	–
12	Reducing sugar/ Glycoside	–	–	–	–	–	–
13	Protein	–	–	+	–	–	–
14	Carbohydrate	+	+	+	+	+	+

Table 3: Estimation of phytoconstituents.

Sl.No.	Phytoconstituent	Quantity (%)
1	Total alkaloid	0.92 ± 0.04
2	Total flavonoid	0.14 ± 0.03
3	Total phenol	3.90 ± 0.03
4	Total tannin	8.60 ± 0.05
5	Total saponin	3.40 ± 0.03
6	Total carotenoid	0.15 ± 0.01

In *G. tiliifolia* mucilage cavities were found embedded in the pith and cortical regions of the stem and were lined by small thin walled elongated cells. They undergo disintegration at the time of mucilage secretion. The presence of mucilage was also detected in the bark region. The occurrence of mucilage cavities in leaf was reported recently.[34] According to Bakker and Gerritsen the mucilage cells of *Hibiscus schizopetalus* do not possess suberized wall layer.[35] Here mucilage is deposited directly against the primary wall which later disintegrated to form mucilage cavities.

The combination of histochemistry and microscopy can provide substantial information about the cell wall composition, modifications, developmental stages and tissue differentiation. Histochemical localization studies allows a quick and cost effective method for the preliminary evaluation of plant species in search of new pharmaceuticals.[36-39] The tissues of phloem parenchyma was found to be the main localising region of various phytoconstituents in *G. tiliifolia* bark.

Powder microscopy is a cost effective, quick and reliable tool for detecting the adulterants and also to confirm the purity of crude drug. The analysis of *G. tiliifolia* bark powder revealed the presence of fragments of lignified parenchyma cells, starch grains, rosette crystal of calcium oxalate, prismatic crystals, parenchyma cells with starch grains, oil globules, cork cells, pericyclic fibres and xylem vessel with scalariform thickening. Oboh and Onwukaeme reported that the chemomicroscopical test conducted on the powder of *Sida acuta* leaves showed the presence of starch, calcium oxalate, mucilage and lignin.[40] Powder microscopy of *Pentace burmanica* (Tiliaceae) contained fragments of fibers, starch grains, calcium oxalate prismatic crystals, fragments of parenchyma, resin and tannin masses in the bark powder.[41]

The evaluation of physicochemical parameters helps in the setting of pharmacopoeial standards. Ash value is one of the common parameters which are used to determine the identity and purity of a drug.[42] The values of total ash, acid insoluble ash and water soluble ash were 12.69±0.02%, 2.44±0.03% and 3.50±0.02% respectively in *G. tiliifolia* bark. The value of extractable matter varies according to the purity of crude drug and the polarity of solvent used. The determination of extractive values will help to assess the nature of chemical composition of a crude drug and to estimate the specific constituents soluble in a particular solvent.[43] Water was the best extractive solvent for *G. tiliifolia* bark and the lowest extractive value was shown by ethyl acetate. Similar results were reported in *Bombax ceiba* bark also.[21] The moisture content is related to the stability and quality of crude drugs because if the water content is high the drug will get deteriorated thereby spoiling the biomass and active principles present in it.[44] *G. tiliifolia* bark powder has 13.29±0.01% moisture content.

Phytochemical screening can help in detecting the bioactive compounds present in medicinal plants and thus lead to the discovery and development of drugs.[45] The presence of flavonoids, tannins, alkaloids, steroids/ terpenoids, saponins and phenol were confirmed by the preliminary phytochemical screening of *G. tiliifolia* bark. Phytoconstituents which gave negative results were coumarins, quinines, anthraquinones, resin and reducing sugar/glycosides. The presence of alkaloids, flavonoids, terpenoids, tannins and saponins in the stem bark of *G. optiva* reported.[46]

In *G. tiliifolia* bark tannin showed the highest content (8.60±0.05%) and flavonoid showed the lowest content (0.14±0.03%). The aqueous fraction of the stem bark of *G. optiva* was reported to contain the highest content of phenol and flavonoid.[46] Ejikeme *et al.* reported 4.8% of alkaloids and 4.4% of saponins in the wood of *Glyphea brevis* (Tiliaceae).[47] The red colour exhibited by *G. tiliifolia* bark extract can be attributed to the presence of carotenoid pigments. The total carotenoid content was 0.15±0.01% which was comparable with that of many known plants. Carotenoids help in providing protection against cancer, macular degeneration and cardiovascular diseases.[48]

The pharmacognostical evaluation can thus give valuable information regarding its morphology, microscopic features and physicochemical characteristics which will help in the exact identification as well as assessment of purity of crude drugs.

CONCLUSION

The results obtained from this study can be used as a pharmacognostic standard for the exact identification and evaluation of the raw bark of *G. tiliifolia.*

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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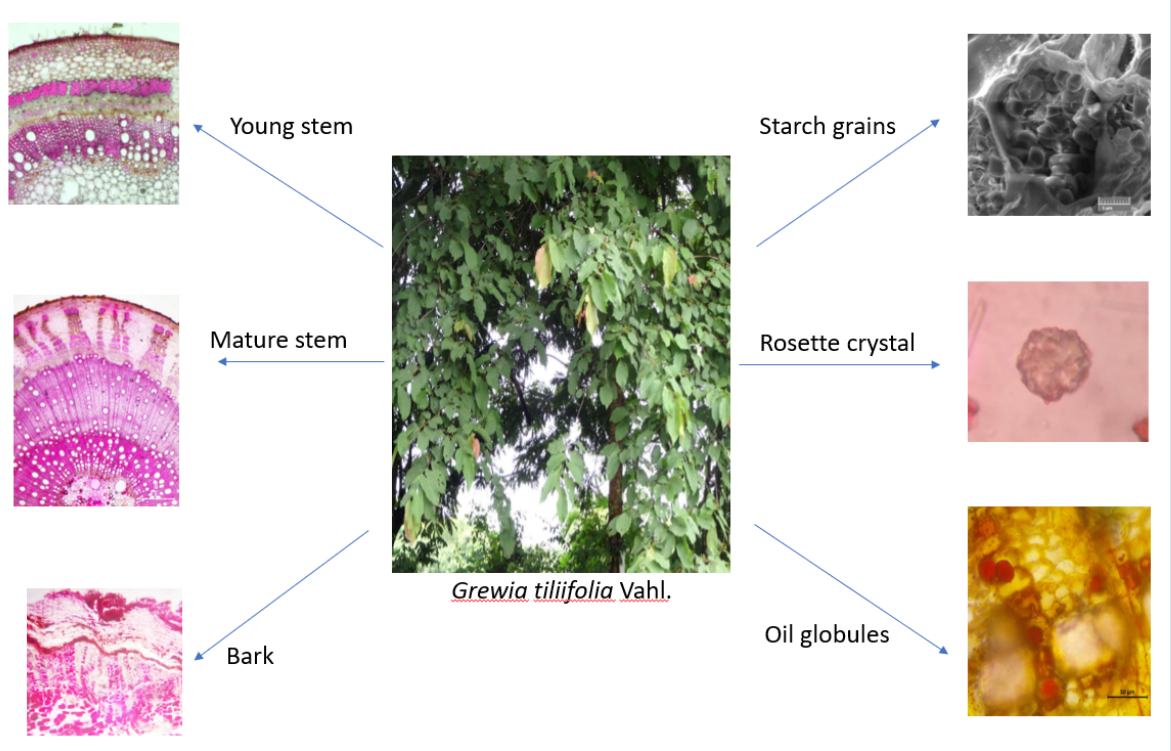
GRAPHICAL ABSTRACT



SUMMARY

- Anatomical studies revealed the presence of prismatic crystals of calcium oxalate, druses, starch grains and mucilage cavities.
- Histochemical studies helped in identifying the tissues of phloem parenchyma as the main localising region of various phytoconstituents.
- Powder microscopic studies can be utilized to confirm the purity of crude drug.
- The physicochemical examinations helped in setting the pharmacopoeial standards.
- The estimation of alkaloids, flavonoids, phenols, tannins, saponins and carotenoids were carried out.

ABOUT AUTHORS

Jaya Kuruvilla
She is an Assistant Professor in Botany at St. Xavier's College for Women, Aluva, Kerala.

M. Anilkumar
He is an Assistant Professor and Research Guide in Botany at Union Christian College, Aluva, Kerala. His areas of research include Pharmacognosy, Phytochemistry, Pharmacology and Endophyte Biology.

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