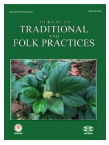
Volume 09 (01 & 02) December 2021, 158-172
ISSN 2278- 5906
http://jtfp.jntbgri.res.in



Journal of Traditional and Folk Practices

Pharmacognostic standardization, phytochemical investigation and antioxidant studies on *Phyllanthus reticulatus* Poir.

Syeda Nurunnesa Begum and Chowdhury Habibur Rahaman*

*Department of Botany (UGC-DRS-SAP & DST-FIST sponsored), Visva-Bharati, Santiniketan - 731235,West Bengal, India

*habibur_cr@rediffmail.com or habibur_cr@yahoo.co.in

Received: 30 September 2021 Accepted: 20 December 2021

Abstract

The present study describes the Pharmacognostic characters of *Phyllanthus reticulatus* Poir. (Family-Phyllanthaceae) for its precise identification and quality control of crude drugs procured from its leaf and stem bark through phytochemical screening along with analysis of antioxidant properties. *P. reticulatus* is an important plant that is used to treat oral diseases, diarrhea, muscular pain, asthma, conjunctivitis, and many other health problems. The macroscopic, microscopic and physicochemical evaluation of the crude drugs and other standardization methods recommended by WHO have been studied. Quantitative and qualitative analysis along with estimation of *in-vitro* antioxidant activity of therapeutically important selected phytochemical groups have also been performed. The Pharmacognostic study showed that the epidermal cells of abaxial and adaxial leaf surfaces are regular in shape and the cell walls are slightly wavy in outline. Stomata were anomocytic type with a stomatal index of 31.43±1.26% and palisade ratio of 10.83±0.12 and the leaves are of hypostomatic type. Only non-glandular, multicellular, and uniseriate trichomes were observed with trichome indices of 3.59±2.26% and 1.13±1.54% for both abaxial and adaxial leaf surfaces, respectively. Presence of glycosides, tannins, alkaloids, anthraquinones, saponins, etc. was noticed in the methanolic extracts of leaf and stem bark through microchemical colour reaction tests. The histochemical study revealed that the secondary phloem, cortex, cells of phelloderm and cork cells were the major localizing zones for different phytochemical groups. Total ash values of stem bark and leaf were 3.4% and 2.6%, respectively. Total phenolic content was found higher in stem brak (201.70±2.15mg GAE/g) than leaf (189.84±6.92mg GAE/g). Total tannin content in bark was 63.77±0.27mg of GAE /g and in leaf it was 41.55±0.57mg of GAE / g. The maximum content of alkaloids was measured in the leaf part (10.33±0.44 mg of PE/g) that is followed by stem bark (7.62±0.33 mg of PE/g). Total flavonoid contents of stem bark and leaf are 28.32±0.97mg of QE/g and 27.04±0.47 mg of QE/g, respectively. IC_{50} values of the methanolic extracts of leaf and stem bark in DPPH assay were estimated to be 41.27±0.48µg/ml and 33.54±0.54 µg/ml, respectively. IC_{50} values of the methanolic extracts of leaf and stem bark in ABTS radical scavenging assay were 37.02±0.033µg/ml and 31.27±0.51µg/ml, respectively. The Pharmacognostic including physicochemical profiles obtained through the present study may help in the identification, authentication, and establishment of a quality control standard of *P. reticulatus*. These findings also suggested that *P. reticulatus* is a good source of medicinally potent phytochemicals along with its promising antioxidant activity.

Keywords: Antioxidant study, *Phyllanthus reticulatus*, Pharmacognostic study, Phytochemical analysis

1. Introduction

Medicinal plants have been used in the traditional health-care system throughout human history**.** History evidenced the use of medicinal plants as a natural source of treatment and therapies for human health in many indigenous societies (Farombi, 2003). According to World Health Organization, about 80% of the world population rely mainly on plant-based medicines and the majority of traditional therapies apply either plant extracts or their active constituents (Farnsworth *et al.,* 1985). Now a days there has been a growing interest in drugs of plant origin instead of synthetic drugs because of their no harmful threat to humans and the

environment, low cost and easy availability as compared to synthetic drugs (Chanda, 2014). Tremendous exploitation of these herbal medicines leads to decrease in the population of medicinal plants from which herbal drugs are procured. To fulfil the rising demand, herbal drugs are often adulterated with low grade material that can be minimized through proper quality control study to ensure the superiority of crude drugs. There is a need for the appropriate methods of collection and identification of genuine plant materials, to avoid the misuse of herbal medicine and to ensure their standard quality. WHO also emphasizes the need to ensure quality control of medicinal plant's products by using modern techniques and suitable standards (Shinde *et al*., 2009). Pharmacognostic standardization includes morpho-anatomical characterization, physico–chemical parameters, phytochemical screening for successful identification, and authentication of the crude drugs obtained from the plant sources.

P.reticulatus is a roadside straggling shrub of the Phyllanthaceae family and commonly known as Black-Honey shrub. In the traditional system of medicine, this plant has a remarkable benefit as different parts of it have been reported to possess diverse medicinal properties. The stem, bark, leaf, root, fruits and the whole plants are used as crude drugs for curing health conditions such as oral problems, liver disorder, anemia, rheumatoid arthritis, conjunctivitis, muscular pain, skin diseases,etc.(Kirtikar and Basu,1935; Chopra *et al.,*1956; Unander *et al.,*1990; Ghani,2003;Shruthi and Ramachandra,2011;Sharma and Kumar, 2013).

In th past, scientists have carried out phytochemical investigations on this plant species, which demonstrated the presence of lupeol acetate, stigmasterol and lupeol, triterpenoids, diterpenoids, lignin, alkaloids, flavonoids, tannic acid, octacosanol, teraxerol acetate, friedeline, teraxerone, betulin, sitosterol, scopoletin and ellagic acids (Unander *et al.,*1990; Calixto *et al.,*1998;Lam *et al.,* 2007; Jamal *et al.,*2008; Shruthi and Ramachandra, 2011).The species has been validated by certain pharmacological assays for its anti-diabetic, anti-plasmodial, hypocholesterolemic,antimicrobial and cytotoxic, hepatoprotective, antinociceptive and antihyperglycemic, analgesic and anti-inflammatory activities (Omulokoli *et al*., 1997; Saha *et al.*, 2007; Kharat *et al.,* 2013; Sharma and Kumar,2013; Kumar *et al.,* 2014).

Irrespective of the phytochemistry and pharmacological research, till now very few pharmacognostic works have been carried out on this species. But no detailed studies on anato-pharmacgnostic, phytochemical and biological activity of the stem bark and leaf are available. However, stem bark and leaves of this medicinal plant are equally important in curing a wide range of diseases and ailments. In this context, the present study has been undertaken to evaluate the Pharmacognostic, phytochemical, and antioxidant properties of these two medicinally important parts i.e., stem bark and leaf of the plant *P. reticulatus*, which will provide a new insight to explore its wide application as herbal medicine with accurate quality assessment.

Scientific name - *Phyllanthus reticulatus* Poir.

Synonym - *Kirganelia reticulata* Poir.

Common name- Black-Honey shrub, black-berried feather foil, potato-bush, netted-leaved leaf-flower.

Local name- *Panjuli*

Botanical description- Small deciduous, straggling shrub or small tree, up to 5m tall, trunk up to 15cm in diameter; branches glabrous or pubescent (Fig. 1A). The external surface of bark looks dark brown in colour, rough. The thickness of the mature bark is 2-3 cm (Fig. 1B). Leaves alternate, simple, stipulate, petiolate; stipules 1-1.5mm long, linear to narrowly lanceolate, pale brown; petioles 1-2mm long, pubescent; lamina elliptical to ovate-oblong, entire, 1–5 cm × 0.5–3 cm, apex acute, glabrous, pinnately veined with 7–13 pairs of lateral veins. Inflorescence a fascicle on lateral shoots, with 1 female and several male flowers per fascicle, or female flowers solitary in upper leaf axils. Flowers green and purple, unisexual, campanulate, pedicellate; a male flower with 5-6 free stamens, pedicel 2-3mm long; female flowers with a superior subglobose ovary, 2-3 mm long. Fruits berry, 3–5 mm × 4–7mm, smooth, green, turning reddish purple or bluish black when ripe.

Habitat- Terrestrial, commonly found in the thickets and bushes on the roadside, forests, and grow as a hedge plant.

Flowering and Fruiting season- July to November.

Distribution- In the tropical areas of India, Bangladesh, China, and in the Malay Islands (Kirtikar and Basu, 1935; Ghani, 2003).

2.1. Medicinal importance

Leaves: The juice is beneficial in case of diarrhoea, as cooling medicine, young leaf paste used to relieve muscular pain. Leaf powder is administered in burn and skin diseases (Kirtikar and Basu, 1935; Ghani, 2003; Sharma and Kumar, 2013).

Bark: It is used as an astringent, diuretic, attenuant, and in urination disorder (Kirtikar and Basu, 1935).

Root: Root is administered to cure asthma. Decoction of root is advantageous in gonorrhoea, muscle spasms, purgative, dysmenorrhea, diarrhoea with anal bleeding, increase male fertility. Infusion of dried rootbark is very effective to control hookworm, promoting fertility

(Kirtikar and Basu, 1935; Chopra *et al.,* 1956; Unander *et al.,* 1990).

Stem: The stem juice is operative for sore eyes and conjunctivitis (Kirtikar and Basu, 1935; Ghani, 2003).

Fruit: Fruits are convenient to cure inflammation, blood disease, fever, bowels (Kirtikar and Basu, 1935; Ghani,2003).

Whole plant: Oral intake of decoction prepared from the whole plant is able to improve liver disorder, intestinal hemorrhage, smallpox, syphilis, venereal sores, burn, anemia, constipation, inflammation, oral problems, rheumatism, and arthritis (Kirtikar and Basu, 1935; Shruthi and Ramachandra, 2011; Sharma and Kumar, 2013).

Tribal uses: Chewing of fresh leaves and twigs helps to treat mouth ulcers, bleeding gums, dryness in the tongue. Juices of leaf and fruits are helpful to prevent body tremors. Soup prepared from the leaves may reduce the pain and improve the movement of hands in case of carpel tunnel syndrome. Leaf paste is beneficial against diabetic foot ulcer. A decoction of whole plant can cure oral cavity, cancer, anaemia, liver disorder. Root extract improve the menstrual problem in females (Unander *et al.,* 1990; Manjula and Norman, 2017).

Economic uses: Dyeing of clothes by the extraction of fruits, leaves, root, and bark is a conventional approach. In Philippines, black ink is prepared from the ripe fruits. Wood is used as a roof binder, in west Africa. Twigs are used as chew sticks. Fruits are used as famine food in east Africa. Roots and fruits are sometimes given as fodder to the stock animals (Unander *et al.,* 1990).

2. Materials and methods

Plant parts: Leaf and stem bark of *P. reticulatus* were selected for the present investigation.

Collection of the plant sample: The fresh and matured plant specimens were collected from the roadside of Santiniketan, (GPS Coordinate is $23^0679570'$N-$87^0641264'$E), Birbhum, West Bengal, India in the month of June, 2020. After collection, the plant specimens have been preserved following standard herbarium techniques and kept in the Department of Botany, Visva-Bharati, Santiniketan, India for future references. Voucher specimen number: INDIA, West Bengal, Birbhum district: Santiniketan, 12.06.2020, *SN Begum* 07 (Visva-Bharati Herbarium, Santiniketan). The plant species has been identified with the help of different standard floras and it has been authenticated after consultation with the reputed taxonomists. The nomenclature of this plant species has been updated following the standard website like "The Plant List" (http://www.theplantlist.org/). The matured and full grown leaves and stem bark were collected in different days of the month of June in 2020 from the above mentioned location of Santiniketan and plant parts were then processed (presented in the Methods section) for its future use.

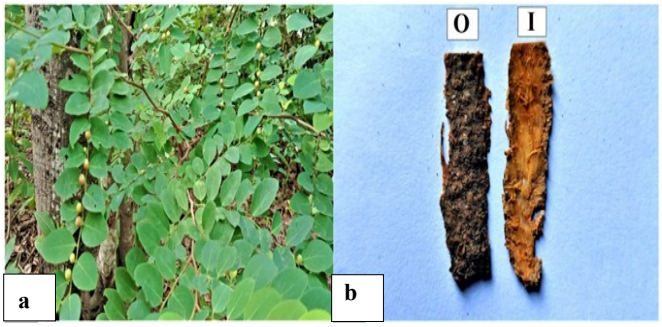


Fig. 1. a. A portion of the plant; b. The stem bark [outer (O) and inner (I) surfaces]

2.2. Collection and preparation of the plant sample

The collected stem bark and leaves were washed thoroughly under tap water, air dried separately, ground them into powdered, and the powered plant samples were kept separately in airtight containers for future analysis. The fresh plant materials were used for the macro and micro-morphological, anatomical, and xylem maceration studies. The powdered samples of bark and leaves were used for physiochemical, phytochemicals, and antioxidant activity studies.

2.3. Organoleptic study

This study of powdered crude drugs was done with the help of sensory organs following the standard methods, which includes colour, odour, taste, external markings, etc. of the crude drug (Ray and Rahaman, 2018).

2.4. Microscopic study

2.4.1. Study of foliar micromorphology: Leaf samples were cleared following Bokhari's method (Bokhari, 1970). The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin and observed under a compound light microscope (ZEISS, AXIOSTAR plus, 176045).

2.4.2. Vegetative anatomy (leaf and stem bark): For this study, free hand sections of the leaf and stem bark of the selected plant were made, then double stained following safranin-light green staining schedule (Johansen, 1940), and studied under compound light microscope (ZEISS, AXIOSTAR plus, 176045). Photographs of the suitable sections were taken with the help of a photographic system attached to the said microscope.

2.4.3. Xylem elements study: The stem pieces (1 cm) were macerated following the standard method (Johansen, 1940). Boiled wood samples were then washed in distilled water several times and observed under a compound light microscope.

2.4.4. Histochemical study: Transverse sections of the stem bark were laid out in several glass slides; one to two drops of different reagents (Wagner's, Dragendorff's, Mayer's, Lugol's, 1% lead acetate, Phloroglucinol, Ferric chloride, Millon's) were added to the sections and kept for few minutes. Then observed under a compound light microscope to detect different phytochemical groups localized in different tissue zones of the sections (Trease and Evans, 1983; Ghosh and Rahaman, 2015).

2.4.5. Powder study: A little quantity of powdered sample of respective plant part was taken in a test tube. 5 ml saturated chloral hydrate solution was added to it and boiled for 5 minutes. The boiled samples in test tubes were kept standing in the test tube rack for 3 days. Then a few drops of treated powder drug were placed on a glass slide, 1-2 drops of glycerine and a drop of safranin were added to it and mounted with a cover slip. It was then examined under a microscope. The characteristic structures and cell components of the drug samples were observed and their photographs were taken using photomicrography (ZEISS, AXIOSTAR plus, 176045) (Anonymous, 1985; Thitikornpong *et al*., 2011).

2.5. Physicochemical evaluation

Physicochemical parameters like moisture content, ash value (total ash, acid insoluble ash, water soluble ash, and sulphated ash and extractive value of the powdered plant samples were determined as per guidelines of Indian Pharmacopoeia (1985) and WHO (1998).

2.5.1. **Moisture content study:** About 5 gm of plant samples were weighed and air dried for a few days. Then the sample was incubated at 80°- 90°C temperature for one hour. The final weight of the sample was taken and calculated the percentage of moisture content by the following formula; (Eq.1).

$$\text{Moisture content (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100. \quad (1)$$

2.5.2. Determination of extractive value: 10 gm of a powdered sample of each plant part was extracted successively in a 100ml conical flask with the solvents like ethanol, ethyl acetate, chloroform, and hexane separately. The respective solvent extracts were then allowed to dry at room temperature. After drying, the weight of each solvent extract was noted and the extractive value was determined by the following formula (Eq.2) (Evans, 2008).

$$\text{Extractive value (\%)} = \frac{\text{Weight of the obtained residue}}{\text{Weight of the plant material}} \times 100 \ldots (2)$$

2.5.3. **Percentage of ash value (total ash, water soluble ash, and acid insoluble ash**): Fixed amount of precisely weighed powdered sample of leaf and bark has been taken in a pre-ignited crucible and ignited gradually increasing the temperature to 500-600°C until it became white indicating that the powdered sample is free from carbon. The crucible was cooled in a desiccator and weighed. The total ash was calculated as the percentage of ash with reference to the air-dried plant material. Water-soluble ash is the difference in weight between total ash and the residue obtained after adding 20ml of hot water to the total ash obtained from the plant sample. Acid-insoluble ash is the residue obtained by boiling the total ash with 25ml of diluted hydrochloric acid. It was determined according to the method of Indian Pharmacopoeia (Annonymous,1985).

2.6. Phytochemical study

2.6.1. **Preliminary phytochemical screening**: Ethanol, ethyl acetate, chloroform, and hexane extracts of leaf and stem bark powders were used for different chemical colour reaction tests with the help of different reagents to detect different phytochemical groups present in the powdered samples following standard methods (Swain and Hillis, 1959; Zhishen *et al*., 1999).

2.6.2. Estimation of total phenolic content: It was estimated by following the standard method (Swain and Hillis, 1959). The plant sample of 0.5g was homogenized in 5ml of 80% ethanol. Homogenates were centrifuged at 10,000rpm for 20min. The supernatant was collected and then dried. The residue was dissolved in 5 ml of distilled water. 0.5ml of aliquot, distilled water, and folin-ciocalteau reagent were mixed in a test tube. After 3 minutes, 20% sodium carbonate was added to the test tube and mixed it thoroughly. Test tubes were placed on a boiling water bath for 1min and they were cooled at room temperature. Then absorbance was measured at 650 nm wavelength against a blank.

2.6.3. Estimation of total flavonoid content: Total flavonoid content was estimated employing the aluminium chloride method (Zhishen *et al*., 1999). A stock solution of each plant part extract was prepared by dissolving 100 mg of extract in 5ml methanol and the volume was made to10ml with methanol. Then 0.5ml of sample extract was taken in a test tube, 1.5ml methanol, 0.1ml of 10% aluminium chloride solution, 0.1ml of 1M potassium acetate solution, and 2.8 ml distilled water were added to the test tube subsequently, and mixed it thoroughly. Absorbance was taken at 415 nm against the suitable blank using Shimadzu UV-1800 double beam spectrophotometer.

2.6.4. Estimation of total tannin content: Method of Afify *et al*. (2012) with slight modification was employed. The powdered plant sample of 500mg and 75ml distilled water were taken in a conical flask. It was then boiled for 30 minutes. After cooling, the boiled plant sample was centrifuged at 2000 rpm for 20 minutes. The supernatant was taken and its volume was adjusted to 100 ml with distilled water. Then the extract was used for estimation of tannins. One mL of the plant extract was taken in a volumetric flask containing 75mL distilled water. Then 5ml of Folin-Denis reagent and 10ml of a sodium carbonate solution were added to the flask and the volume adjusted to 100 ml with distilled water. Content in the flasks was thoroughly mixed and kept for 30 minutes and absorbance was measured at 700nm on Shimadzu UV-1800 double beam spectrophotometer. A blank was prepared with distilled water instead of the sample. Tannins were estimated and calculated with the help of a standard curve of gallic acid (0.1mg/mL) and expressed as mg of GAE/g.

2.6.5. Estimation of total alkaloid content: The total alkaloid contents in different parts of this plant sample were measured using the 1,10-phenanthroline method described by Singh *et al*. (2004) with slight modifications. Bark powder (100 mg) was extracted in 10ml of 80% ethanol. This was filtered through filter paper and centrifuged at 5000rpm for 10 min. The supernatant obtained was used for further estimation of total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M FeCl3 in 0.5M HCl and 1ml of 0.05M of 1,10- phenanthroline in ethanol. The mixture was incubated for 30 minutes in a hot water bath with a maintained temperature of 70±20ºC. The absorbance of the red coloured complex was measured at 510nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of a standard curve of pilocarpine (0.1mg/mL, 10mg dissolved in 10ml ethanol and diluted to 100mL with distilled water). The values were expressed as mg/g Pilocarpine equivalent.

2.7. Antioxidant activity study

2.7.1. DPPH radical scavenging activity: DPPH radical scavenging activity was determined following the standard method of Thaipong *et al.* (2006). The stock solution was prepared by dissolving 24 mg of DPPH in 100mL methanol. The working solution was then prepared by mixing a 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1± 0.02 units at 515 nm using the spectrophotometer. Plant extracts of 150 µL volume were allowed to react with 2850 µL of the DPPH solution for 24 h in the dark. Then absorbance was taken at 515 nm. The standard curve for ascorbic acid was prepared. Results were expressed in % of scavenging activity. The experiment was carried out in triplicate. The IC_{50} value was determined from the % inhibition vs. concentration of different plant extracts and ascorbic acid by comparing the absorbance values of control (Ao) and test compounds (At). Radical scavenging activity was determined by the following formula

$$\% \text{ Radical scavenging activity} = (A_o - \frac{At}{Ao}) \times 100$$

2.7.2. ABTS radical scavenging activity: ABTS radical scavenging activity was determined by the method described by Thaipong *et al.* (2006). The stock solution was prepared by adding of 7.4mM ABTS·+ and 2.6 mM potassium persulfate. The working solution was then prepared by mixing these two stock solutions in an equal ratio and stored in the darkroom temperature for 12-16 hours. Then the solution was diluted with methanol to obtain an absorbance of 1.1±0.02 units at 734 nm using the spectrophotometer. Each plant sample extract of 150 µl was allowed to react with 2850 µl of the ABTS solution for 2 hrs in a dark condition. Then absorbance was taken at 734nm using the UV-VIS spectrophotometer (UV1800). The standard curve was prepared with ascorbic acid. Results were expressed in % of scavenging activity. The experiment was carried out in triplicate. The IC_{50} value was determined from the % inhibition vs. concentration of different plant extracts and ascorbic acid by comparing the absorbance values of control (Ao) and test compounds (At). Radical scavenging activity was determined by the following formula

$$\% \text{ Radical scavenging activity} = (A_o - \frac{At}{Ao}) \times 100$$

3. Results and discussion

3.1. Organoleptic study

The colour, odour, taste and texture of the powdered stem bark and leaf (Fig. 2A, B) of investigated plant have been presented in the table below (Table 1).

Table 1. Organoleptic features of the powdered leaf and bark samples of the investigated plant

Organoleptic features	Leaf	Stem bark
Colour	Dark green	Yellowish brown
Odour	Aromatic	Aromatic
Taste	Bitter	Bitter
Texture	Powdery	Fibrous

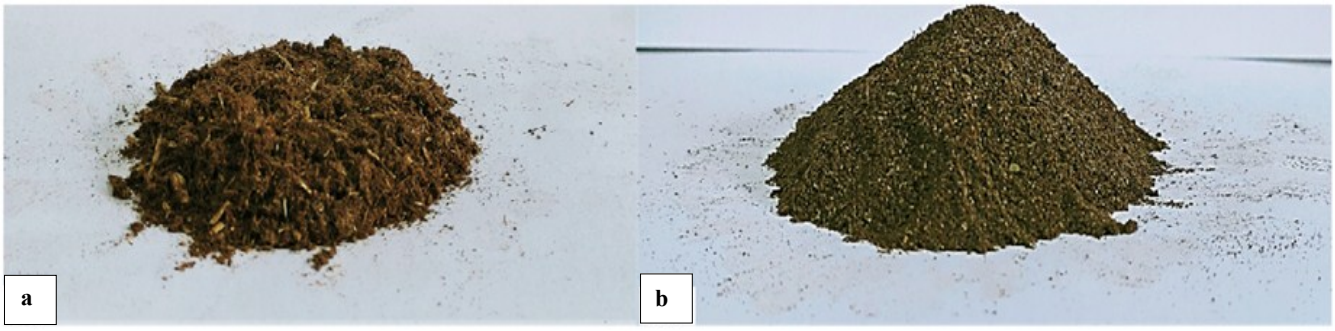


Fig.2. Powdered crude drugs: a. Stem bark; b. Leaf

3.2. Study of foliar micromorphology

General description of the leaf epidermal cells, stomata and trichomes along with their measurements are given below.

Epidermis*:* Epidermal cells of abaxial and adaxial leaf surfaces were regular in shape and the cell walls were slightly wavy in outline (Fig. 3A). The size of the epidermal cells on the upper surface is 56.96±4.24 µm ×25.82±3.13µm and it is 52.81±1.97µm× 24.95±4.20µm on the lower leaf surface. The frequency of the epidermal cells is 684.30/mm^2 on the upper surface and it is 796.66/mm^2 on the lower surface. Palisade ratio is of 10.83±0.12.

Stomata: Stomata were of anomocytic type (Fig. 3B) and distributed in lower surface of the leaf (hypostomatic). Stomatal index is 31.43±1.26%.

Trichomes*:* Only non-glandular, multicellular, and uniseriate trichomes are observed in both surfaces of the leaf (Fig. 3C). Trichome indices are 3.59±2.26% and 1.13±1.54% for abaxial and adaxial leaf surfaces, respectively.

3.3. Vegetative anatomy

3.3.1. Leaf anatomy: Lamina of the leaf is dorsiventral type and it is differentiated into vascular tissues, epidermis, and mesophyll tissues. Both abaxial and adaxial surfaces of the leaf are uniseriate. The epidermal cell is rectangular in shape compactly arranged and cuticularized. The mesophyll is differentiated into palisade and spongy parenchyma. The palisade consists of a single layer of regular, long, columnar chlorophyllous cells and it is present just beneath the upper epidermis. About 2-3 layers of thin walled spongy parenchyma cells are present in the lower part of lamina (Fig. 4A).

Midrib region consists of single layered upper and lower epidermises which are cuticularized, followed by 4-5 layers of collenchymatous cells present below the epidermis. In the middle of the midrib semi circular collateral vascular bundle is present. Parenchyma cells in5-6 layers are present throughout the midrib region just above the lower epidermis (Fig. 4B).

3.3.2. Stem anatomy: Cross-section of the stem was almost circular in outline. The epidermis was uniseriate, cuticularized (Fig 5A). Cortex was made of (i) 2 to 3 layers of thick collenchymatous hypodermis, and (ii) 7-10 layers of the parenchymatous zone. The vascular bundle is collateral, conjoint, and open type with phloem and xylem. Sclerenchymatous patches are present just above the phloem layer. At the centre massive, parenchymatous pith was noticed.

3.3.3. Bark anatomy The transverse section of bark showed the typical anatomical characteristics with an outer layer of periderm followed by cortex and secondary phloem region (Fig 5B). In the periderm, cork cells are rectangular in shape, arranged compactly in 7-8 layers and brown colour contents present in most

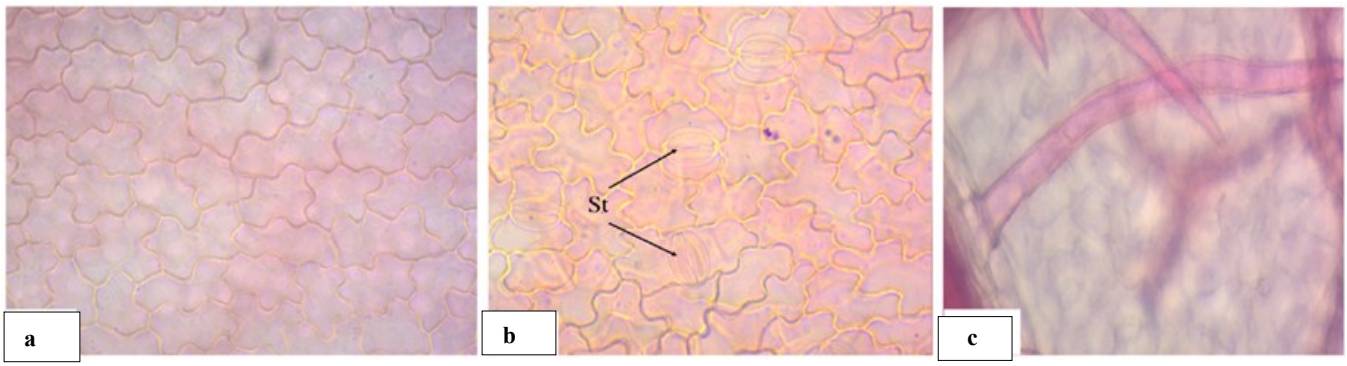


Fig.3. Foliar micromorphology: a. Epidermal cells of the upper surface of leaf; b. Lower epidermis with stomata; c. A non-glandular trichome.

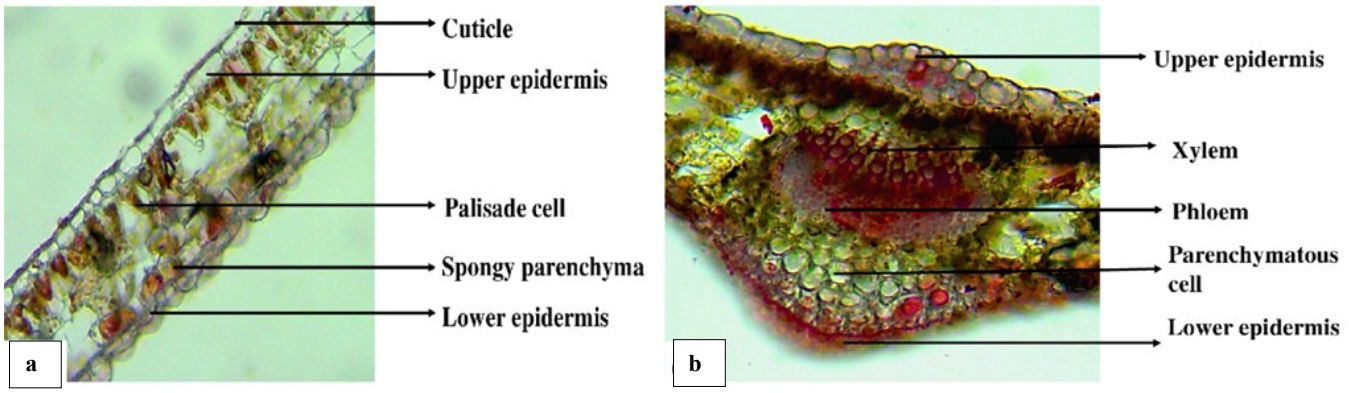


Fig.4. Leaf anatomy: a. T.S. of leaf lamina; b. T.S. of leaf midrib.

of the cork cells. Phellogen is compactly arranged in 1-2 layers, and phelloderm is not clearly distinguishable, somewhere below the phellogen, small patches of a few celled phelloderm observed. The cork originates in the sub-epidermal cells. In the cortex, some cells contain calcium oxalate crystal. Cortex region is followed by secondary phloem region. T.L.S. of the stem bark has shown ray structures and phloem fibers (Fig 5C). Ray structures are uniseriate to biseriate in nature (22-23 cells per ray structure). The frequency of the ray structure is 1.33±1.50/mm^2.The height and width of ray is 285.20±168.69µm and 32.89±23.28 µm, respectively. Here, fibres are comparatively thin walled. Some long, rectangular, thin walled phloem parenchyma cells are observed along with the fibres and ray structures.

3.3.4. Xylem elements study: General description along with measurements of the xylem elements of the stem has been presented below;

Vessel element: Vessel elements were simple, transverse, or obliquely placed perforation plates. Pits on the sidewall of the elements were simple and arranged in horizontal rows. Tails were absent. The size of the vessel element is 142.46µm × 21.8µm and the frequency is 18.24/mm² (Fig. 6A).

Tracheids: They were very long and with spiral side wall thickening. The diameter of the tracheid was 26.64µm and the frequency was 18.21/mm² (Fig. 6B).

Fibres: Fibres were typically libriform type with pointed ends. Septa and pits were totally absent. The size of fibre was 535.8µm × 16.01µm and the frequency was25/mm² (Fig. 6C).

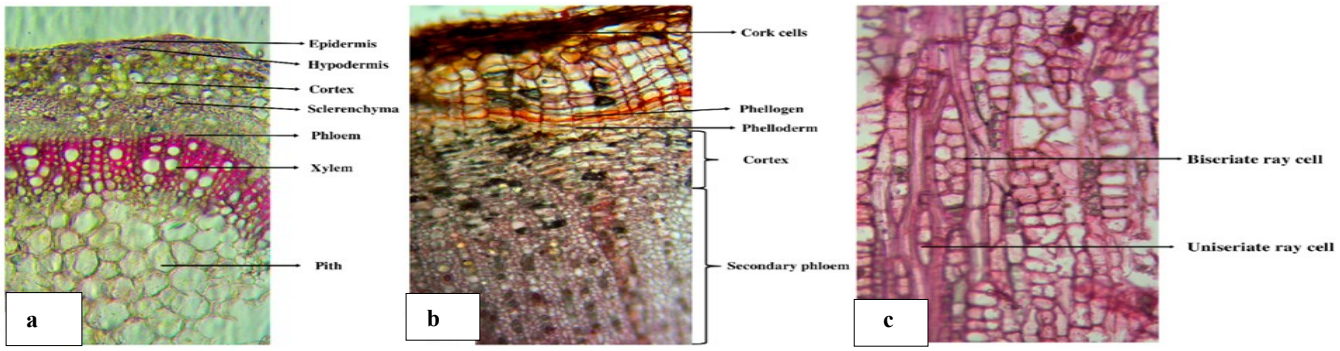


Fig. 5. Transverse sections: a. Stem; b. Stem bark; c. Transverse longitudinal section of stem bark

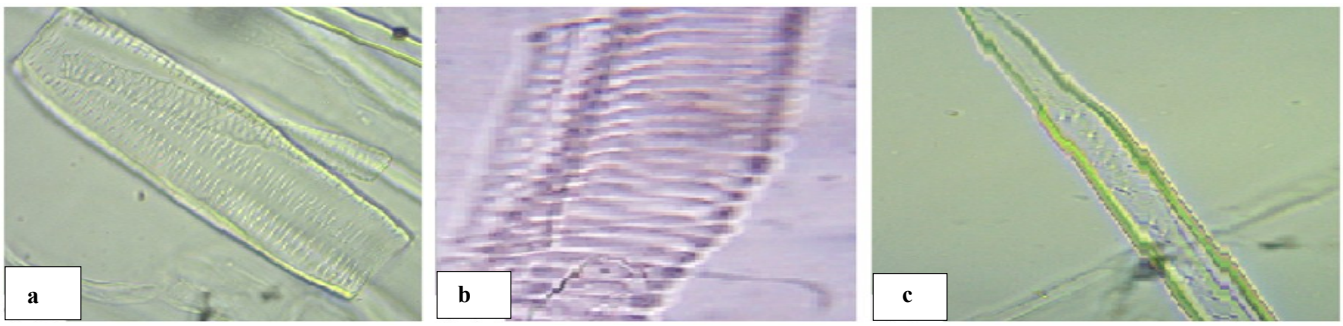


Fig. 6. Xylem elements: a. A vessel element; b. A portion of tracheid; c. Portion of a fibre

3.4. Histochemical study

Histochemical study of stem bark has shown the localization of different phytochemicals in various tissue zones. Phytochemical groups like alkaloids, flavonoids, tannins, proteins, saponins, etc. have been detected in this region. The secondary phloem, cortex, few cells of phelloderm and cork cell were the major localizing zones for different phytochemical groups (Table 2; Fig.7).

Table 2. Histochemical localization test of stem bark

TEST FOR	TEST/ REAGENT	BARK PART
ALKALOID	Dragendorff's reagent	Secondary phloem, Cortex, Phelloderm.
	Wagner's reagent	Secondary phloem, Cortex.
STARCH	1% Iodine solution	Secondary phloem, Cortex.
LIGNIN	Phloroglucinol+HCl	Cork cell
TANNIN	5%FeCL3	Secondary phloem, Cortex, Phelloderm.
	1% lead acetate solution	Secondary phloem , Cortex, Phelloderm

3.5. Powder study

Powder drug study of stem bark shows the following features.

Cork cell: Polygonal thick-walled cork cell has been observed (Fig. 8C); *Fibre:* Fibres were longitudinally present, thick walled narrow lumen and tapering ends (Fig. 8B); *Crystal:* Crystals were of different shape and size were found (Fig. 8A); Parenchymatous cell: Thin walled parenchymatous cell was noticed (Fig. 8D); Starch grain: Numerous globular shaped starch grains were observed (Fig. 8E).

3.6. Moisture content and Ash value

Moisture content, ash value of the leaf, and bark powder drugs are given in the tabular form (Table 3). The moisture content of leaf and stem bark drug is 9.7% and 7.4%, respectively. Ash value was 2.6% for leaf and it was 3.4% for stem bark powder. In leaf, the percentage of acid insoluble and water-soluble ash was1.02%and 1.5%, respectively. The percentage of acid insoluble and water-soluble ash in stem bark was 1.1% and 2.2%, respectively.

3.7. Extractive value

The polar solvent extract such as ethanolic extract (4.3% for leaf and 8.6% for stem bark) and Ethyl acetate extract (2.5% for leaf and 3.9% for stem bark) higher as compared to nonpolar extract such as ethanol and hexane extracts for all two parts of this plant (Table 4). Thus, ethanol and ethyl acetate are good solvent for extraction and it also give idea that stem bark and leaf of *P. reticulatus* contain more polar constituents in comparison to non-polar.

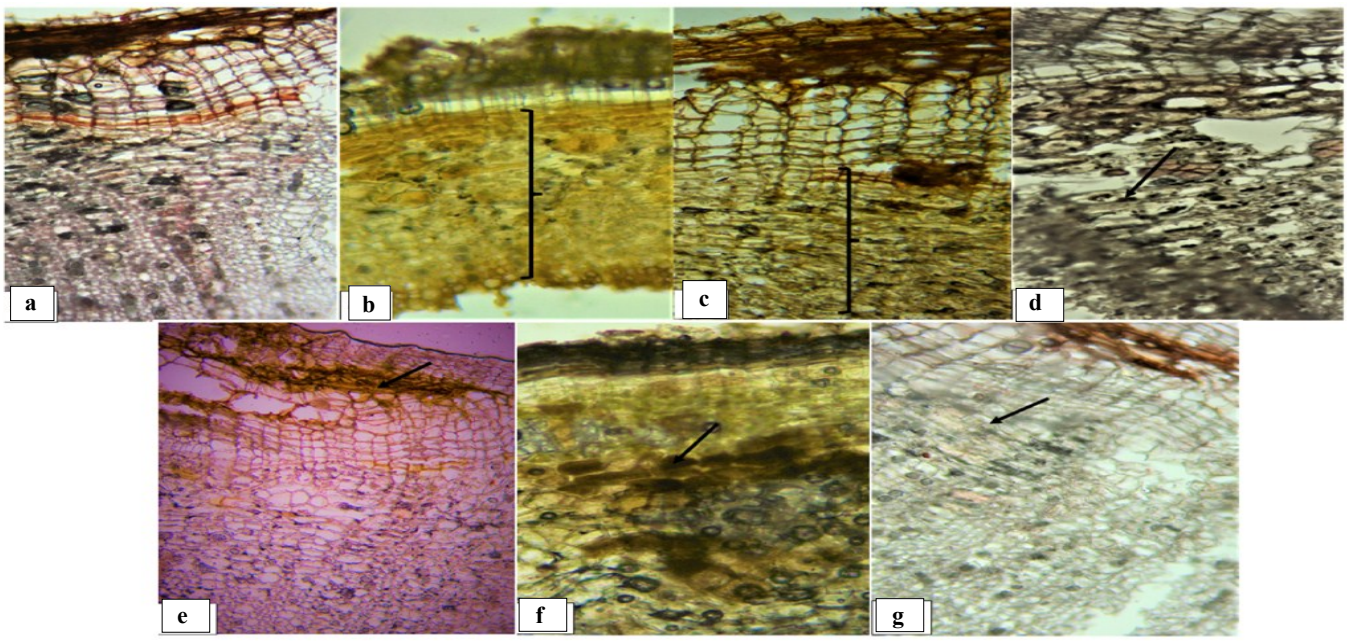


Fig. 7. Histochemical localization tests of stem bark: a. Untreated (control); b. Dragendorff's reagent; c. Wagner's reagent; d. 1% Iodine solution; e. Phloroglucinol +HCl; f. 5%FeCL3 reagent; g. 1% lead acetate solution.

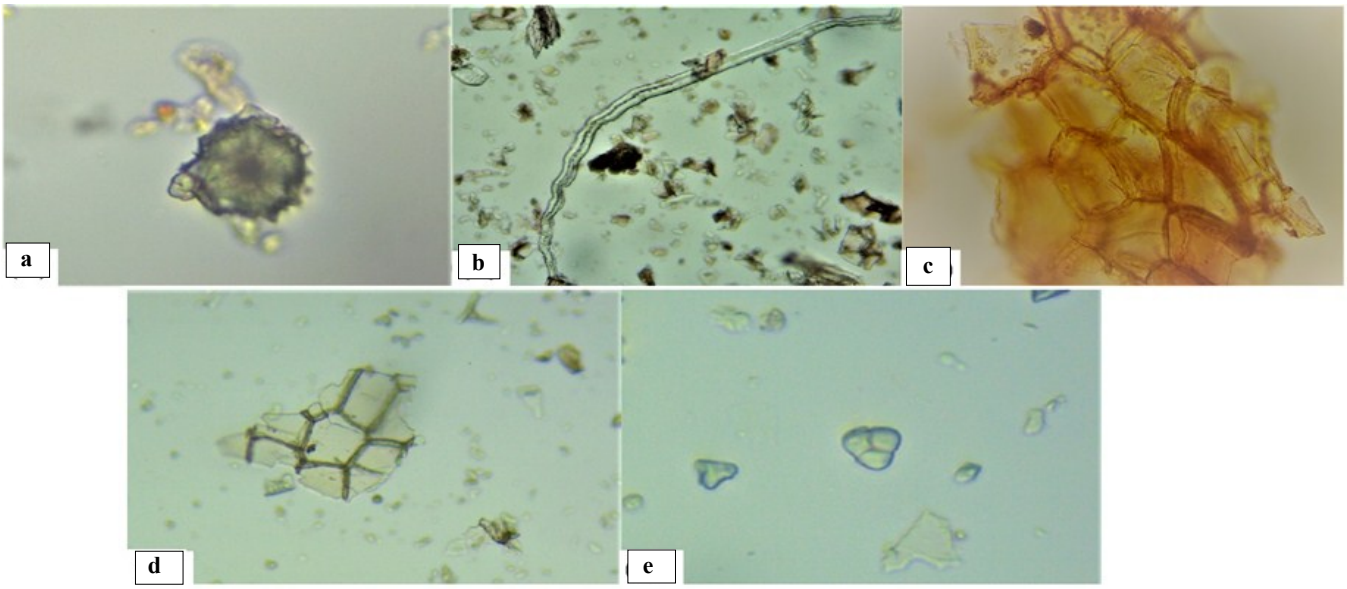


Fig. 8. Powder drug study: a.) Crystal; b. Fiber; c. Cork cells; d. Parenchyma cells; e. Starch grains

Table 3. Moisture content and ash value of crude drugs of leaf and stem bark

Plant parts	Moisture content (%)	Total ash (%)	Water soluble (%)	Acid insoluble (%)
Leaf	9.7%	2.6%	1.5%	1.02%
Stem bark	7.4%	3.4%	2.2%	1.1%

Table 4. Extractive values of different plant parts

Plant parts	Extractive value (%)			
	Ethanol	Ethyl acetate	Chloroform	Hexane
Leaf	4.3	2.5	0.7	1.3
Stem bark	8.6	3.9	1.6	1.1

3.8. Preliminary phytochemical screening

Phytochemical screening of different solvent extracts of leaf and stem bark (Fig. 9 A & B) of the investigated plant showed presence of different phytochemical groups in varying degrees (Table 5).

3.9. Estimation of total phenolic content

Phenolics are one of the major groups of antioxidant compounds reported to be involved in free radical scavenging activity. The total phenolic content in the leaf is 189.84±6.92mg of GAE/g tissue. The amount of total phenols in stem bark is 201.70±2.15mg of GAE/g tissue (Table 6; Fig.10). Here also the amount of total phenolic compounds is significantly higher in all the parts investigated.

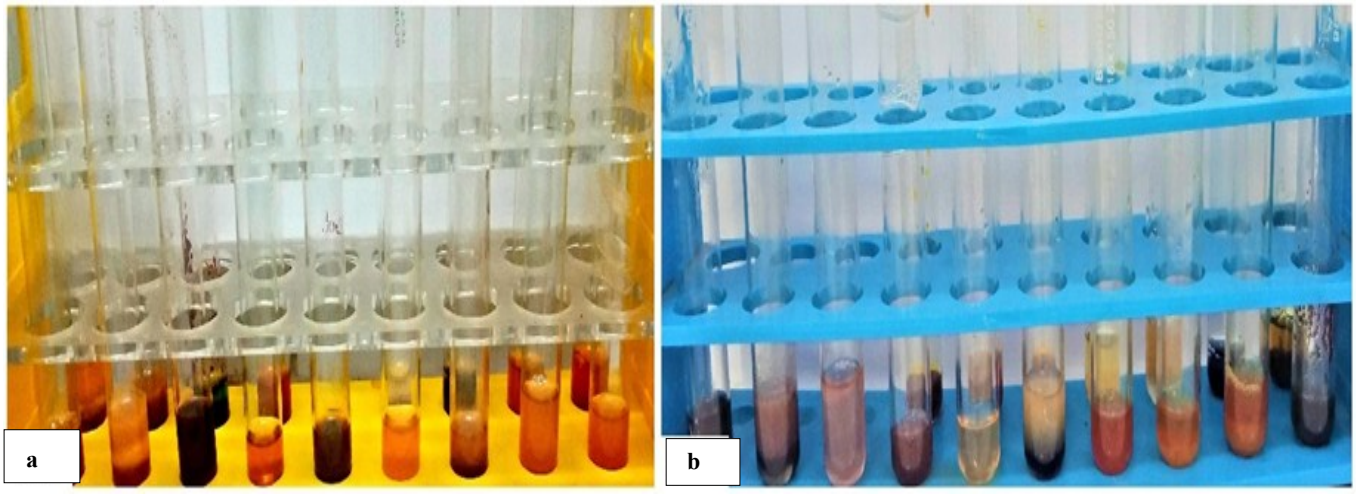


Fig. 9. Chemical colour reaction test for a. Ethanolic stem bark extract; b. Ethanolic leaf extract

Table 5. Micro chemical colour reaction tests of different solvent extracts of the investigated plant.

Chemical group	Test	Nature of colour change	Parts used							
			Stem Bark				Leaf			
			Et	Ea	Ch	Hx	Et	Ea	Ch	Hx
Alkaloids	Mayer's reagent	White/ Cream ppt.	+	+	-	-	+	_	-	-
	Dragendorff's reagent	Orange brown ppt.	++	+	-	-	+	+	-	-
	Wagner's reagent	Orange brown ppt.	+	+	-	-	+	-	-	-
Reducing sugars	Fehling's reagent	Brick red ppt.	+	-	-	-	+	-	-	-
	Benedict's reagent	Brick red ppt.	+	-	-	-	+	-	-	-
Tannins	10% NH_4OH solution	Yellow	++	-	-	-	-	-	-	-
	10% lead acetate solution	Yellow ppt.	++	-	-	-	-	-	-	+
	5% $FeCl_3$ solution	Blackish-green colour	++	-	-	-	+	-	-	-
Flavonoids	Shinoda's test	Magenta colour	++	+	+	+	++	+	+	+
	10% NaOH solution	Yellow colour	++	-	-	-	++	-	-	-
Steroids	Salkowski test	Reddish-blue and green fluorescence	+	-	+	-	+	-	-	-
Anthraquinones	Bontrager's test	Pink colour	-	-	-	+	+	-	-	-
Proteins	Lugol's reagent	Faint yellow colour	-	-	-	-	-	-	-	-
	Millon's reagent	White ppt.	+	+	-	-	-	-	-	+
Saponins	1% Lead acetate solution	White ppt.	-	-	-	-	+	-	-	-
Lignin	Phloroglucinol + HCl	Red	++	+	-	-	+	+	-	-
Amino acids	Ninhydrin reagent	Purple colour	-	-	-	-	+	+	-	-

Table 6. Phytochemical profiles of different parts of the investigated plant.

Plant parts	Total phenolics (mg of GAE /g)	Total flavonoids (mg of QE/g)	Total tannins (mg of GAE /g)	Total alkaloids (mg of PE/g)
Leaf	189.84±6.92	28.32±0.97	41.55±0.57	10.33±0.44
Stem bark	201.70±2.15	27.04±0.47	63.77±0.27	7.62±0.33

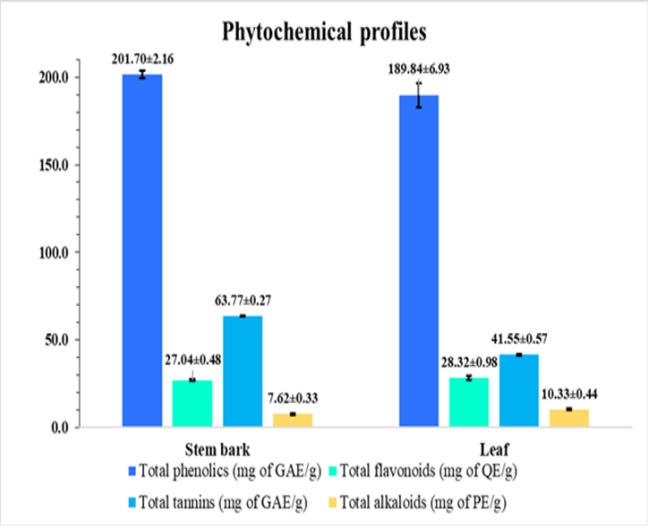


Fig. 10. Phytochemical profiles of different parts of the investigated plant

3.10. Estimation of total flavonoid content

Flavonoids are a very important group of phenolics that show a wide range of therapeutic properties. Total flavonoid contents in leaf and stem bark are 28.32±0.97mg of QE/g and 27.04±0.47mg of QE/g, respectively (Table 6; Fig. 10). The total flavonoid content of both the leaf and stem bark is more or less same.

3.11. Estimation of total tannin content

The high content of tannins was observed in the stem bark part (63.77±0.27mg of GAE /g) which is followed by the tannin contents of the leaf (41.55±0.57mg of GAE /g) (Table 6; Fig. 9).

3.12. Estimation of total alkaloid content

Here leaf part showed a slightly higher content of alkaloids (10.33±0.44mg of PE/g) than stem bark (7.62±0.33mg of PE/g) part of the plant (Table 6; Fig. 9).

3.13. Antioxidant study

3.13.1. DPPH radical scavenging activity: DPPH radical scavenging activity was significantly high for the ethanolic extract of stem bark of the investigated species. IC_{50} value of the stem bark extract was 33.54±0.27, and leaf 41.27±0.48 (Table 7). The stem bark part showed more antioxidant potential in respect of DPPH radical scavenging activity than the leaf part of this plant.

Table 7. Antioxidant properties of different parts of the investigated plant.

Antioxidant study	Stem bark	Leaf
IC_{50} Value of DPPH (µg/ml)	33.54±0.27	41.27±0.48
IC_{50} Value of ABTS (µg/ml)	31.27±0.51	37.02±0.03

3.13.2. ABTS radical scavenging activity: ABTS radical scavenging activity was significantly high for ethanolic extract of stem bark part of the investigated species. IC_{50} value of the stem bark extract was 31.27±0.51, and leaf 37.02±0.03 (Table 7). The stem bark part showed more antioxidant potential in respect of ABTS radical scavenging activity than the leaf part of this plant.

Discussion

The standardization of a crude drug through macroscopic, microscopic and physicochemical characters can help to gain knowledge about its proper quality, purity, and identification and to prevent its adulteration (Kumari and Kotecha, 2016). Pharmacognostic study on stem bark and leaf of *P. reticulatus* , a broadly used traditional medicinal plant for curing various diseases is not yet established with concrete evidences for its standardization in the modern history of medicine. Present study provides some basic Pharmacognostic data regarding morpho-anatomical, physicochemical and phytochemical properties of two medicinally important parts i.e., stem bark and leaf of the plant *P. reticulatus* for the identification and authentication of the respective parts of the plant in their fresh as well as dried form. World Health Organisation (WHO, 2011) recommends that the macroscopic and microscopic analysis such as an anatomical section of different parts of a plant, foliar micromorphology, etc, is to be the primary step for correct identification and quality control of any plant material, that may clearly distinguish specific diagnostic characteristics of the plant material (Kothari and Shah, 1975; Albert and Sharma, 2013).

In this study, it has been observed that epidermal cells of abaxial and adaxial leaf surfaces are regular in shape and the cell walls were slightly wavy in outline. Studies

of the stomatal index (SI) that have a great taxonomic as well as Pharmacognostic value in the proper identification of different plant taxa including medicinal plants (Rao and Ramayya, 1987;Choudhury *et al*., 2013; Saha and Rahaman, 2013), was found very distinct (31.43±1.26%) to the lower surface of this plant. Palisade ratio here is 10.83±0.12 and it gives a diagnostic character to the leaf part. Stomata were strictly of anomocytic type and found only on the lower leaf epidermal surface, which indicates a similar finding with previous work (Shruthi *et al*., 2010), and also exhibits dissimilarity with another study where anisocytic type of stomata was found (Ram *et al*., 2008). Trichrome is considered the most valuable taxonomic marker for proper identification of the plants (Rao and Ramayya, 1987; Saha and Rahaman, 2013). Epidermal trichomes of the investigated plant were non -glandular, multicellular and uniseriate type which conform the observation reported in earlier work (Ram *et al*., 2009). In present work, trichrome indices have been determined as 3.59±2.26% and 1.13±1.54% for abaxial and adaxial leaf surfaces, respectively. Like stomatal index, here trichome index also provides a diagnostic feature for identification of the studied leaf sample where two index values are found distinct in both upper and lower leaf surfaces.

The T.S. of fresh leaves showed presence of cuticle, epidermis, vascular and mesophyll tissues. The mesophyll was comprised of palisade and 2 -3 layered spongy parenchyma. Here palisade of the leaf was found single layered which is characteristic to the leaf anatomy of this medicinal taxon. The uniseriate palisade observed in present investigation has not been reported in previous works done on foliar anatomy (Ram *et al*., 2009; Shruthi *et al*., 2010). Midrib region was composed of epidermis followed by 4-5 layers of collenchyma cells and collateral vascular bundle, encircled by 5-6 layers of parenchyma. T.S. of stem showed circular outline, cuticularized epidermis, presence of cortex, sclerenchymatous patches, vascular bundle and a massive parenchymatous pith. Stem anatomy does not exhibit here any distinctive features . Moreover, stem bark T.S. showed periderm followed by cortex and secondary phloem region. Periderm was noticed with 7-8 layered rectangular, compactly arranged cork cells. Phellogen is 1-2 layered, compactly arranged and phelloderm was noticed. Calcium oxalate crystals were found in both the cortex region and secondary phloem region. T.L.S. of stem bark displayed uniseriate to biseriate ray structures with the frequency of 1.33±1.50/mm^2. Vessel elements were of simple, obliquely placed perforation plates having a frequency of 18.24/mm². Tracheids were long with thick spiral side wall and noticed in a frequency of 18.21/mm². Fibres were libriform type with pointed ends. All the above information could provide the basis for the future identification and authentication of *P. reticulatus*.

The microscopic powder analysis of stem bark highlighted presence of some distinctive microscopic characteristics such as polygonal thick-walled cork cell, thick walled fibre with narrow lumen and tapering ends, spheroidal crystals, parenchymatous cells and starch grain, which will serve as specific anatomical markers for identification of the bark sample of investigated taxon.

In Pharmacognosy, physicochemical characters help in setting a standard for a crude drug and are successfully employed in detection of adulterants and improper handling of the crude drug (Anonymous, 1985). Total ash value is constant for a genuine or pure drug and any deviation of it gives an indication regarding presence of any foreign matter like soil, non-volatile inorganic impurities, other contaminants in the drug or about the drug improperly prepared (De *et al*., 2020). The total ash values were 2.6% for leaf and 3.4% for stem bark powder in this study. The acid insoluble ash usually contains silica and the water-soluble ash indicates the quantity of inorganic elements in the sample. Acid insoluble ash values of leaf and stem bark were 1.02% and 1.1%. Water-soluble ash value of leaf and stem bark was 1.5% and 2.2%, respectively. All the ash values will be considered as the standard in case of ensuring the authenticity of the bark as well as leaf drug of this studied species and determining adulteration, if any.

The moisture content, which is an indication of storage life, was estimated through drying method to determine the water content of the crude drug sample. Since, high moisture content hydrolyses the moisture sensitive active ingredients and encourages growth of microorganism in the drug (Blainski *et al*., 2017) which finally lead to the poor quality and inferiority of the drug. The moisture contents of leaf and stem bark drug are 9.7% and 7.4% w/w, which indicate the standard value of moisture content for these two drug parts and ensure their storage by checking the microbial growth to certain extent.

Extractive value is defined as the weight of crude extract to be obtained from the drug sample through extraction with different solvents. It has a practicable

importance in determining the adulterated or exhausted drug. High extractive value gives the indication regarding better extractability of phytochemicals from the plant material. Extractive values also aid in selection of the best solvent that will further assist in having maximum yield and also gives an idea about the nature of the chemical constituents present in the plant (Baidoo *et al*., 2019; Ghosh and Rahaman, 2015). In present study, polar solvent extracts such as ethanolic extract (4.3% for leaf and 8.6% for stem bark) and ethyl acetate extract (2.5% for leaf and 3.9% for stem bark) exhibited maximum value as compared to the nonpolar solvents such as chloroform and hexane extracts of *P. reticulatus*. Thus, these are a good choice as a solvent for greater amount of extraction and it also suggests that these two plant parts contain more polar constituents in comparison to non-polar ones.

Secondary metabolites of the plants such as alkaloids, phenolics, glycosides, tannins, flavonoids, etc. are responsible for the therapeutic efficacy of the crude drugs. These groups of secondary metabolites have been proved very effective in antibiotic, antifungal, anticancer and many other biological activities (Eleazu *et al*., 2012). The results of preliminary screening of leaf and stem bark of *P.reticulatus* showed presence of some therapeutically potent chemical groups such as alkaloids, phenolics, saponins, tannins, anthraquinones, glycosides, etc. The presence of such important phytochemical groups in two different parts of this medicinal plant clearly indicates its therapeutic properties and also validates its wide range of ethnomedicinal uses to some extent (Pal and Jain, 1998; Pakrashi and Mukhopadhyay, 2004; Khare, 2007).

Phytochemicals present in medicinal plants play a very important role in curing diseases and these biologically active compounds are responsible for exhibiting diverse pharmacological activities. Chemical analysis and biological assay are considered very important aspects of the pharmacognostic studies of medicinal plants (Harborne and Williams, 1994; Zheng *et al*., 2013; Sudhakar and Reddy, 2017). Histochemical localization tests of stem bark showed that secondary phloem, secondary cortex, few cells of phelloderm and cork cells contain alkaloid, lignin, tannin and starch grains.

The most important secondary metabolites explored in plants are phenolics, flavonoids, and tannins which have antioxidants activity along with other biological activities such as antimicrobial, anti-inflammatory, antiallergic and anticancer action (Ahmad *et al*., 2015; Mireku *et al*.,2017). It has been reported that the phenolic has redox potential, hydrogen donating and singlet oxygen quenching properties, therefore it exhibits antioxidant activity (Rao *et al*., 2010). From this study, the investigated plant parts have been identified as a good source of phenolics, where the total phenolic content of the leaf part is 189.84±6.92mg of GAE/g tissue and for stem bark it is 201.70±2.15mg of GAE/g tissue. As stem bark contains a larger amount of phenolic compounds than the leaf, thus antioxidant activity of bark is quite higher than the leaf part of the studied plant.

Total flavonoids content of leaf was 28.32±0.97mg of QE/g followed by stem bark 27.04±0.47mg of QE/g. The higher amount of tannin content was found in the stem bark part (63.77±0.27mg of GAE /g) than the leaf (41.55±0.57mg of GAE /g). Here the leaf part was observed as the richest source of alkaloids (10.33±0.44mg of PE/g) than stem bark (7.62±0.33mg of PE/g) part of the investigated plant.

Antioxidant activities of the methanolic extracts of selected plant parts were evaluated using the DPPH, ABTS radical scavenging activity. IC_{50} values are irreversibly related to the antioxidant activity of the crude drug, therefore the plant extract rich with phenolic and other antioxidant phytochemicals exhibited significantly low IC_{50} values. In present study, DPPH radical scavenging assay of stem bark extract shows a lower IC_{50} value (33.54±0.27) than the leaf part (41.27±0.48). A similar trend is followed in ABTS radical scavenging activity study where IC_{50} value of stem bark is lower (37.02±0.03) than the leaf (41.27±0.48). From our results, it was observed that methanolic extracts of stem bark exhibited greater antioxidant activity than the leaf extract of same solvent.

This higher antioxidant activity of stem bark is correlated with its higher contents of total phenolics. Different phenolic groups have hydroxyl groups which are responsible for their radical scavenging activity. Among the leaf and stem bark it was found that stem bark is more potent in respect of its certain phytochemical contents (phenolic and flavonoids) as well as antioxidant activity.

In this current investigation, pharmacognostic characters obtained, that will be helpful in identification of crude drugs obtained from leaf and stem bark of *P. reticulatus* and will ensure the authentication of this plant material, by detecting the adulterant or admixture, if present .

Pharmacognostic characters of the investigated plant:

- Foliar epidermal cells of abaxial and adaxial leaf surfaces are regular in shape and the cell wall is slightly wavy in outline.

- Hypostomatic leaf; stomata- strictly anomocytic type; stomatal index-31.43±1.26%.Non-glandular, multicellular, and uniseriate trichomes; trichome indices-3.59±2.26% (lower surface) and 1.13±1.54% (upper surface).
- In leaf part: Moisture content-7.4%, total ash content- 2.6%, water soluble ash- 1.5% and acid insoluble ash- 1.02%.
- In stem bark: Moisture content-9.4%, total ash content- 3.4%, water soluble ash- 2.2% and acid insoluble ash- 1.1%.

4. Conclusion

The diagnostic characters obtained from this pharmacognostic study will be very useful in proper identification of the crude drugs procured from leaf and stem bark of *Phyllanthus reticulatus* and they will also be instrumental in quality assurance of these two studied vegetable samples. From the qualitative and quantitative phytochemical analysis, it was found that few important subgroups of phenolics are predominant among other medicinally potent phytochemical classes detected in the investigated plant. The presence of such phytochemicals in this medicinal plant clearly indicates its therapeutic properties and also put the rationale regarding various traditional uses of this medicinal plant. Further scientific investigations are needed on this ethnomedicinally important plant to fulfill the lacunae of standardizing toxicity and evaluation of bioactive potential of the chemicals from different parts of this species of *Phyllanthus*.

Acknowledgements

We are thankful to the Head, Department of Botany, Visva-Bharati, for providing the necessary laboratory facilities. Thanks are also due to the UGC for financial assistance sanctioned in the form of Maulana Azad National Fellowship.

Conflict of interest

The authors have no conflict of interest

References

Afify A M, El-Beltagi H S, El-Salam S M and Omran A A 2012. Biochemical changes in phenols, flavonoids, tannins, Vitamin E, beta carotene and antioxidant activity during soaking of three white Sorghum varieties. Asian Pac. J. Trop. Biomed. 2(3): 203-209.

Ahmad A, Husain A, Mujeeb M, Khan S A, Alhadrami H A A and Bhandari A 2015. Quantification of total phenol, flavonoid content and pharmacognostical evaluation including HPTLC fingerprinting for the standardization of *Piper nigrum* Linn. fruits. Asian Pac. J. Trop. Biomed. 5(2): 101-107.

Albert S and Sharma B 2013. Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species. Turkish J. Bot. 37: 276-281.

Anonymous 1985. Pharmacopoeia of India. Controller of Publications: Ministry of Health, Govt. of India, New Delhi, India. 2(3): pp.65-66.

Baidoo M F, Asante-Kwatia E, Mensah A Y, Sam G H and Amponsah I K 2019. Pharmacognostic characterization and development of standardization parameters for the quality control of *Entada africana* Guill. & Perr. J. Appl. Res. Med. and Aromat. Plants 12: 36-42.

Blainski A, Antonelli-Ushirobira T M, Godoy G, Leite-Mello E V and Mello J C 2017. Pharmacognostic evaluation, and development and validation of a HPLC-DAD technique for gallocatechin and epigallocatechin in rhizomes from Limonium. Brazilian J. Pharmacogn. 27(2): 162-169.

Bokhari M H 1970. Morphology and taxonomic significance of foliar sclereids in Limonium. Notes Roy. Bot. Gard. 30: 43-53.

Calixto J B, Santos A R, Filho V C and Yunes R A 1998. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. Med. Res. Rev. 18(4): 225 -258.

Chanda S 2014. Importance of pharmacognostic study of medicinal plants: An overview. J. Pharmacogn. and Phytochem. 2(5):69-73.

Chopra R N, Nayar S L and Chopra I C 1956. Glossary of Indian Medicinal Plants. CSIR, New Delhi, India. 1: pp. 138-139.

Choudhury S, Rahaman CH and Mandal S 2013. Pharmacognostic studies of Ampelocissus latifolia (Roxb.) Planch - An important ethnomedicinal plant. Int. J. Curr. Res. 5(3): 643-648.

De Alencar Filho J M T, Sampaio P A, De Carvalho I S, Guimarães A L, E Amariz I A, Pereira E C V, Rolim-Neto P J, Rolim L A and Da Cruz Araújo E C 2020. Flavonoid enriched extract of *Alternanthera brasiliana* with photoprotective effect: Formulation development and evaluation of quality. Ind. Crops. Prod. 149: 112371.

Eleazu CO, Eleazu KC, Awa E and Chukwuma S C 2012. Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. J. Biotech. and Pharm. Res. 3(2): 42-46.

Evans W C 2008. Pharmacognosy. 15_{th} Edn, Saunders Comp. Ltd. (Elsevier), Singapore. pp. 515-538.

Farnsworth NR, Akerele AS, Soejarto DD and Guo Z 1985. Medicinal plants in therapy. Bulletin of the WHO. 63(6): 965.

Farombi EO 2003. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. Afr. J. Biotechnol. 2 (12): 662-671.

Ghani A 2003. Medicinal Plants of Bangladesh: Chemical constituents and uses. J. Asiat. Soc. Bangladesh Sci. 2: 345.

Ghosh P and Rahaman C H 2015. Pharmacognostic, Phytochemical and Antioxidant Studies of *Adenanthera pavonina* L. Int. J. Pharmacogn. and Phytochem. Res. 7(1): 30-37.

Harborne J B and Williams C A 1994. Recent advances in the chemosystematics of the monocotyledons. Phytochem. 37(1): 3-18.

Jamal A K, Yaacob W A and Din L B 2008. A chemical study on *Phyllanthus reticulatus*. J. Phys. Sci. 19(2): 45-50.

Johansen D A 1940. Plant Microtechnique. McGraw-Hill, New York. pp.523-538.

Kharat 2013. Review on *Kirganelia reticulata*. J. Drug Deliv. and Therap. 3(4): 208-212.

Khare C P 2007. Indian Medicinal Plants-An Illustrated Dictionary. Springer, Berlin. pp.699-700.

Kirtikar K R and Basu B D 1935. Indian Medicinal Plants. Periodical Experts Book Agency, New Delhi. 2(III): pp.2219-20.

Kothari M J and Shah G L 1975. Epidermal structure and ontogeny of stomata in the Papilionaceae. Bot. Gaz. 136: 372-379.

Kumar S, Sharma S, Kumar D, Kumar K and Arya R 2014. Immunostimulant activity of *Phyllanthus reticulatus* Poir: a useful plant for infectious tropical diseases. Asian Pac. J. Trop. Dis. 4: S491-S495.

Kumari R and Kotecha M 2016. A review on the standardization of herbal medicines. Int. J. Pharma. Sci. and Res. 7(2): 97-106.

Lam S H, Wang C Y, Chen C K and Lee S S 2007. Chemical investigation of *Phyllanthus reticulatus* by HPLC SPE NMR and conventional methods. Phytochem. Anal. 18(3): 251-255.

Manjula V and Norman T S J 2017. *Phyllanthus reticulatus* for oral health. J. Med. Plants. Stud. 5: 117-119.

Mireku E A, Mensah A Y, Mensah M L K, Amponsah I K and Mintah D 2017. Phytochemical constituents and anti-oxidative properties of *Landolphia heudelotti* roots. Int. J. Pharm. Sci. and Res. 8: 2862–2866.

Omulokoli E, Khan B and Chhabra S C 1997. Antiplasmodial activity of four Kenyan medicinal plants. J. Ethnopharmacol. 56(2): 133-137.

Pakrashi S C and Mukhopadhyay S 2004. Medicinal and Aromatic Plants of Red Laterite Region of West Bengal. West Bengal Academy of Science and Technology, Government of W.B., Kolkata, West Bengal & Department of Biotechnology, Government of India, New Delhi, India. pp.508.

Pal D C and Jain S K 1998. Tribal Medicine. Naya Prakash, Calcutta. pp.125-128.

Ram H A, Shreedhara C S, Gajera F P and Zanwar S B 2009. Phamacognostical evaluation of *Phyllanthus reticulatus* Poir. Pharmacogn. Mag. 5(18): 176.

Rao A S, Reddy S G, Babu P P and Reddy A R 2010. The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. BMC Complement and Altern. Med. 10: 1-9.

Rao R S and Ramayya N 1987. Trichome types and their taxonomic importance in the Tiliaceae. Indian J. Bot. 10: 65-73.

Ray A S and Rahaman C H 2018. Pharmacognostic standardization and phytochemical investigation of *Cajanus scarabaeoides* (L.) Thouars. J. Pharmacogn. and Phytochem.10(1): 120-131.

Saha A, Masud M A, Bachar S C, Kundu J K, Datta B K, Nahar L and Sarker S D 2007. The Analgesic and Anti-Inflammatory activities of the extracts of *Phyllanthus reticulatus* in Mice Model. Pharm. Biol. 45(5): 355-359.

Saha S and Rahaman C H 2013. Pharmacognostic and anatomical studies of *Antigonon leptopus* Hook. and Arn.: A promising medicinal climber. Int J. Ayurveda. and Pharm. 4(2): 186-191.

Sharma S and Kumar S 2013. *Phyllanthus reticulatus* Poir. -an important medicinal plant: a review of its phytochemistry, traditional uses and pharmacological properties. Int. J. Pharm. Sci. and Res. 4(7): 2528-2534.

Shinde V M, Dhalwal K, Potdar M and Mahadik, K R, 2009. Application of quality control principles to herbal drugs. Int. J. Phytomedicine. 1(1): 4-8.

Shruthi S D and Ramachandra Y L 2011. RBP-J as a therapeutic target to rheumatoid arthritis -an in silico study. Int. J. Preclin. and Pharm. Res. 2(1): 38-44.

Shruthi S D, Ramachandra Y L, Rai S P and Jha P K 2010. Pharmacognostic evaluation of the leaves of *Kirganelia reticulata* Baill.(Euphorbiaceae). Asian and Australas. J. Plant Sci. and Biotechnol. 4(1): 62-65.

Singh DK, Srivastva B and Sahu A 2004. Spectrophotometric determination of Rauwolfia alkaloids, estimation of Reserpine in pharmaceuticals. Anal. Sci. 20: 571-573.

Sudhakar P and Reddy R 2017. Pharmacognostic and HPTLC Finger printing of *Gardenia gummifera* L.f. Res. J. Pharmacogn. and Phytochem. 9(4): 235-240.

Swain T and Hillis W E 1959. The phenolic constituents of *Prunus domestica*. I.-The quantitative analysis of phenolic constituents. J. Sci. Food and Agric. 10(1): 63-68.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne D H 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. and Anal. 19: 669–675.

Thitikornpong W, Phadungcharoen T and Sukrong S 2011. Pharmacognostic evaluations of *Lagerstroemia speciosa* leaves. J. Med. Plant Res. 5(8):1330-1337.

Trease G E and Evans W C 1983. Pharmacognosy. 12th Edn, English Language Book Society/Bailliere Tindall, Eastbourne. pp. 725-733.

Unander D W, Webster G L and Blumberg B S 1990. Records of usage or assays in Phyllanthus (Euphorbiaceae) I. subgenera Isocladus, Kirganelia, Cicca and Emblica. J. Ethnopharmacol. 30 (3): 233-264.

WHO 1998. Quality control methods for medicinal plant materials. World Health Organization, Geneva. http://www.who.int/iris/handle/10665/41986.

Zheng X, Wang W, Piao H, Xu W, Shi H and Zhao C 2013. The Genus *Gnaphalium* L. (Compositae): Phytochemical and Pharmacological characteristics. Molecules. 18: 8298-8318.

Zhishen J, Mengcheng T and Jianming W 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64 (4): 555–559.