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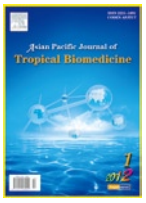

Document heading

Histological and physiochemical standardization of *Melia azedarach*. Linn bark

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ABSTRACT

Objective: To standardize *Melia azedarach* L. by detailed study of macorscopical, microscopical and physiochemical parameters. **Methods:** Bark sample and dried powder of *Melia azedarach* were studied macroscopically and microscopically. Preliminary phytochemical investigation, physiochemical parameters of the bark sample as per WHO recommended guidelines for standardizations were also performed. **Results:** The detail microscopy revealed the presence of collapsed phloem, non-collapsed phloem, sieve elements, sieve plates, sieve tubes, companion cells, crystals and starch grains, narrow and wide fibres. Physiochemical parameters such as percentage of foreign matters, ash values, loss on drying, swelling index extractive values were determined. Total phenolic and flavonoid content were also determined. Preliminary phytochemical screening showed the presence of carbohydrates, tannins, terpenoids, glycosides, flavonoids and phenolic compounds. **Conclusions:** The macroscopic, microscopic and physiochemical analysis is useful in standardization of the *Melia azedarach* bark.

1. Introduction

Melia azedarach Linn. (Family: Meliaceae) is a shrub or small evergreen, medium-sized deciduous tree, up to 5 feet girth and 45 feet high, branches are stout, with purplish bark dotted with buff-coloured lenticels. It grows in temperate and tropical countries like India, China, and Japan. It also known as pride of India and Persian lilac having a wide spectrum of pharmacological activities[1]. It is one kind of medicine in the Ayurvedic system[2]. The stem exudate is used to treat splenomegaly, bark decoction used in paroxysmal fever and skin diseases[3]. It has been useful in fever, thirst, nausea, vomiting, and skin diseases[4,5]. As prescribed by Sushruta fruits used internally to treat indigestion, colic and intestinal catarrh[6], as aphrodisiac, expectorant, anthelmintic and in rheumatism, oil is useful in skin diseases.

Melia azedarach has showed the analgesic activity mediated through opioid receptors, an aqueous extract of the leaf exhibited transient increase in packed red blood cell volume and haemoglobin concentration, an increase in neutrophil number and decrease in lymphocyte number[7]. The leaves also have the immunomodulatory activity, inhibits phagocytosis and respiratory burst triggered by post-receptor stimulus, phorbol 12-myristate 13-acetate in human monocytes[8].

Leaves and fruits showed antifeedant activity[9,10]. The stem extracts showed larval mortality[11] and insecticidal activity. Meliacine, a peptide isolated from leaves inhibited the multiplication of foot and mouth disease virus in BHK-21 cells[12] and showed antiviral activity against herpes simplex virus type [13].

The plant has also showed antifungal[14], antibacterial[15], cytotoxic[16], antimalarial[17], anthelmintic[18], antilithic[19] and antifertility activity[20].

Phytochemical studies have reported the presence of melianin, nimbinene, azaridine, meliacin, quercetin, rutin, kaempferol, rutin, margosine, lupeol, β-sitositosterol[21, 22]

2. Materials and methods

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2.1. Chemicals

Acetic acid, ethyl alcohol, formalin, paraffin (MP-58-60℃), safranin, tertiary butyl alcohol, toluidine blue and all other chemicals used in the study were of analytical grade.

2.2. Plant material

The plant specimen (bark) for the study was collected from Tumkur region, Karnataka, India. Care was taken to select healthy plants and identified by Prof. P. Jayaraman, Director, National Institute of Herbal Science, Chennai. India. A voucher specimen of the plant was preserved in the herbarium for reference (MCOPS/PHCOG/2009/PHD-02). The authenticated samples were processed and used for sectioning and photomicrographs.

2.3. Macroscopic and microscopic analysis

The macroscopy and microscopy of the plant were studied according to the method of Brain *et al*[23]. For microscopic studies, transvers section of the processed bark was done with the help of Rotary microtome. Sections were stained with polychromatic stain toluidine blue. Photographs of different magnifications were taken with Nikon labphoto2 microscopic unit. Coarse powder was used to study powder characteristics of the bark[24, 25].

2.4. Physiochemical analysis

For standardization and quality assurance purpose authenticity, purity and assay[26] must be verified. In support of this, an attempt was made to standardize the *Melia azedarach* bark. Presence of foreign matter, loss on drying, swelling index, extractive value and ash value for stem bark were derived by employing WHO guidelines for quality standards of medicinal plants[27].

2.5. Preliminary Phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedure described by Kokate[28] and Harbone[29]. Total flavonoid content was also determined [30].

3. Results

3.1. Macroscopic characteristics

Melia azedarach bark was greyish brown to dark grey, surface had a shallow longitudinal fissures. Exfoliation was by fine powder. The cut surface of the bark showed thin dark surface layer (periderm) and pale white inner zone (Figure 1). Bark was fibrous in texture and soft, esaily breakable, had no specific odour. It was slightly bitter in taste.

3.2. Microscopic gross anatomy of the bark

3.2.1. Transverse section

The detailed transsectional view of the bark showed narrow dark, superficial zone of peiderm (outer bark) and wider, lightly stained secondary phloem (inner bark). The outer bark was compound structure and consisted of the light coloured, wavy tangential lines of phellam cells. These were about two or three layers of such undulate phellam cylinders. In between the phellam cylinders occured in encapsulated phloem tissue which became dead and crushed. In the alternating cyliders of phellem, dead phellem constituted a compound structure called rhylidome. This rhylidome was not well expressed (Figure 2, 3.1).

Table 1.
Physiochemical parameters.

Parameters	Values (%w/w)
Foreign matter	0.71
Total ash	8.91
Acid insoluble ash	0.56
Water soluble ash	2.14
Loss on drying	13.17
Swelling index	1.80 mL

Figure 1. Macroscopy of *Melia azedarach* bark.

Secondary phloem constituted the mojor portion of the inner bark. The secondary phloem could be distinguished into two zones namely outer wider zones of collapsed phloem and inner narrow zones of non collapse phloem (Figure 2).

Collapsed pholem: This portion of the bark was 3.7 mm

thick. It was charecterised by slightly wider and dialated phloem rays, dialated phloem parenchyma and crushed sieve elements (Figure 3.2).

The crushed (collapsed) sieve elements were seen in the form of thin, dark tangential lines. Phloem sclerenchyma was seen in the form of thin discountinous lines, running all aruond the noncollapsed phloem. The sclerenchyma cells occured in tangential oblong blocks.

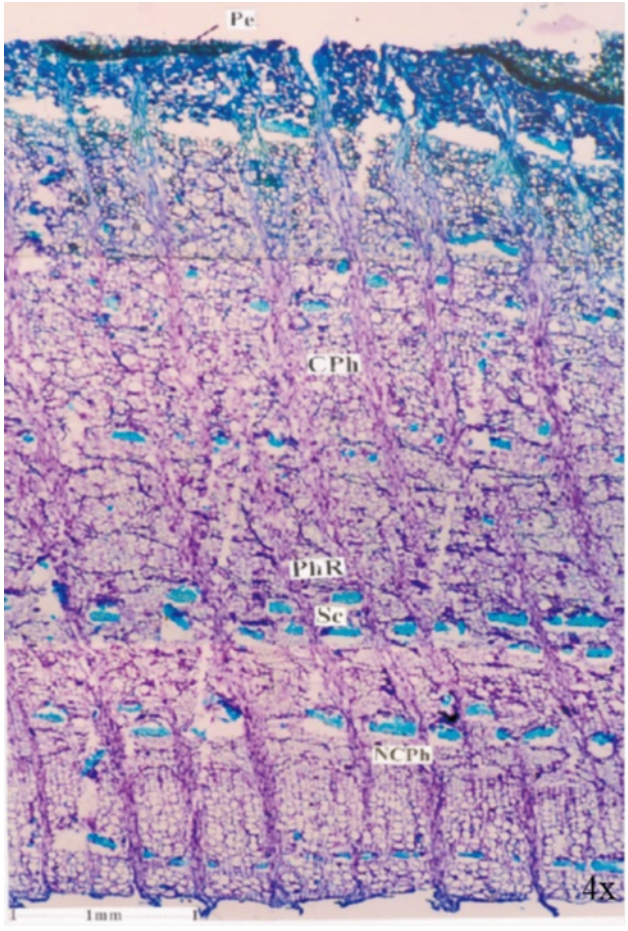

Figure 2. Microscopic gross anatomy of *Melia azedarach* bark. CPh: Collaped phloem; NCPh: Non collapsed phloem; PhR: Phloem ray; Pe: Periderm; Sc: Sclerenchyma.

Non collapsed phloem: The intact phloem was 700 μm wide. The demarcation between the collapsed and non collapsed phloem was distinct. The initial portion of the non collapsed phloem (the boundary between the collapsed and non collapsed phloem) consisted of regular radial and parellel files of rectangular cells. The inner portion included wide polygonal randomly oriented sieve elements and small phloem parenchyma cells. The rays in the non collapsed zone were narrow (Figure 3.3, 4.1, 4.3).

Sieve elements: The sieve elements (sieve tubes) were angular in outline with thick walls. The sieve plate was simple with numerous reticulate sieve pores. The companian cells were prominent, elliptical and densly cytoplasmic (Figure 4.2). The sieve tubes are 45 μm wide (Figure 4.1, 4.3).

Cells inclusions: Starch grains and calcium oxalate crystals were abundant in the phloem rays and phloem parenchyma. The crystals in the parenchyma cells were druses (sphaerocrystal). Those associated with the sclerenchyma elements were prismatic type. The strach grains were small and filled up the lumen of rays and parenchyma cells (Figure 4.3).

3.2.2. Tangential longitudinal section

The rays are non-storied *i.e.* the rays were arranged at different horizantal levels (Figure 5.1). The rays were multi-seriate, they were 3-6 cell layeres thick in the middle and one or two layers thick at the ends. They were homocellular, comprising only one type of cells. All the cells were polyhedral, thick walled and compact (Figure 5.2), ray freequency was 7 or 8/mm, ray height was 100-400 μm, thickness in the middle was 80 μm.

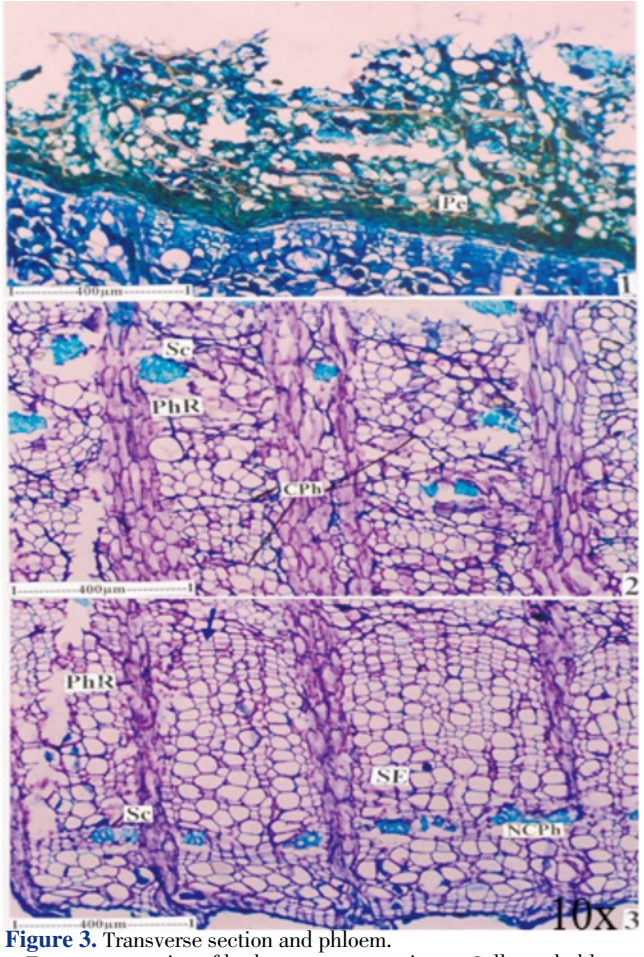

Figure 3. Transverse section and phloem.
1: Transverse section of bark, outermost portion; 2: Collapsed phloem; 3: Non-collapsed phloem.Pe: Periderm; Sc: Sclerenchyma; PhR: Phloem ray; CPh: Collaped phloem; SE: Sieve elements; NCPh: Non collapsed phloem.

Sieve tubes were fairly wide straight or slightly curved, 300-330 μm in height and 40 μm in wide. The sieve plate was simple and oblique (Figure 5.1, 5.3).

Phloem parenchyma cells were wide, vertically elongated and fairly wide, aligned one below the other forming verticle filamentous chain (Figure 5.2).

3.2.3. Radial longitudinal section

In radial longitudinal section view the phloem rays

appeared as horizantal wide ribbon. The phloem sclerenchyma and sieve tube were in verticle files (Figure 6.1). In radial section view, the raycells were horizantally elongated and were narrow, these cells were called procumbent cells.

3.3. Before the word powder microscopy

Powder microscopy: The bark powder exhibited the following elements.

Narrow fibres: Long, thin fibres with thick lignified walls were abundant their lumen is narrow; pits were not evident on the lateral wall. They appeared bright under polarized light (Figure 8.1). The ends were gradually tapering into pointed ends. The narrow fibres were 900 μm long and 10 μm wide (Figure 7.1, 8.2).

Wide fibres: Thin walled with wide lumen (Figure 8.2). They were 650-700 μm long and 20 μm wide. Their walls were also lignified.

Sclereids: Elongated fibre like wide cells or the fibre-sclereids were sporiadically seen. They had wide lumen and dense, wide canal like simple pits. The sclereids were 190 μ

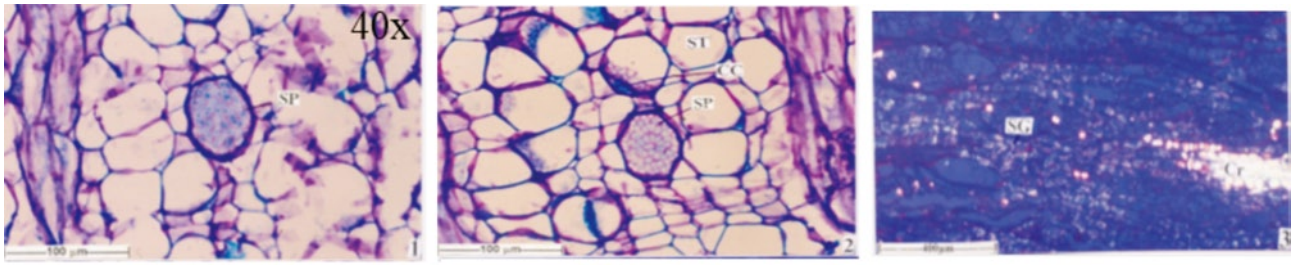

Figure 4. Sieve elements with sieve plate and starch grains and crystals.
1 & 2: Sieve elements with sieve plate; 3: Starch grains and crystals in the collapsed phloem.SP: Sieve plate; ST: Sieve tube; CC: Companion cell; SG: Starch grain; Cr: Crystals.

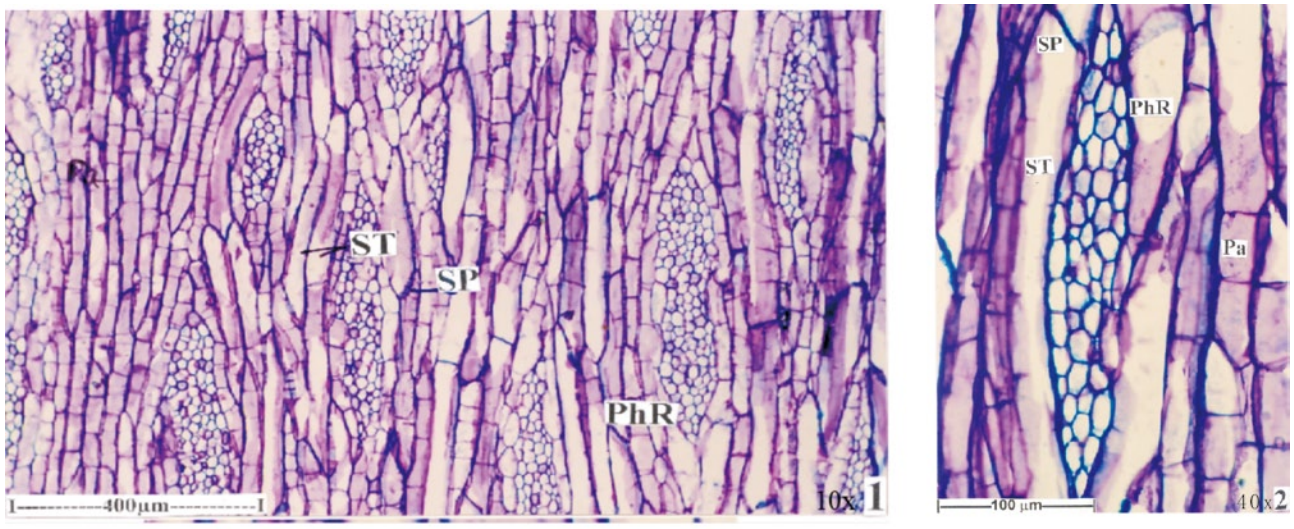

Figure 5. Tangential longitudinal section view.
1: Tangential longitudinal section view of phloem view of phloem; 2: Phloem ray and sieve plate enlarged. ST: Sieve tube; SP: Sieve plate; PhR: Phloem ray; Pa: Parenchyma.

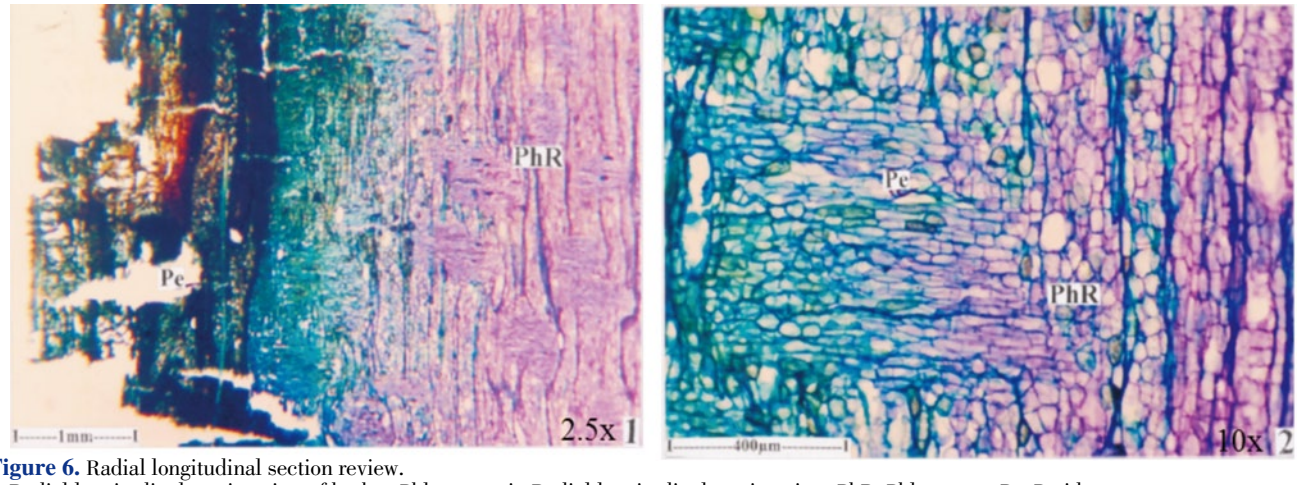

Figure 6. Radial longitudinal section review.
1: Radial longitudinal section view of bark; 2 Phloem ray in Radial longitudinal section view. PhR: Phloem ray; Pe: Periderm.

m long and 15 μm wide (Figure 7.2).

Crystal strand: Vertically elongated, sclerenchyma cells bearing prismatic crystals were common with powder. The crystals were polyhedral and cuboidal, they occured in a vertical, uniseriate row; cross walls were seen, separating the crystals one from the other. The crystals bearing cell was 350 μm long, 20 μm thick and crystals were 15 μm thick (Figure 8.3).

3.4. Physiochemical parameters

The physiochemical parameters such as foreign matter, total ash, acid insoluble ash, water soluble ash, loss on drying and swelling index were measured and results are shown in Table 1. Water soluble extractive value was 8.2% w/w, and alcohol soluble extractive value was 9.6% w/w.

3.5. Preliminary phytochemical screening

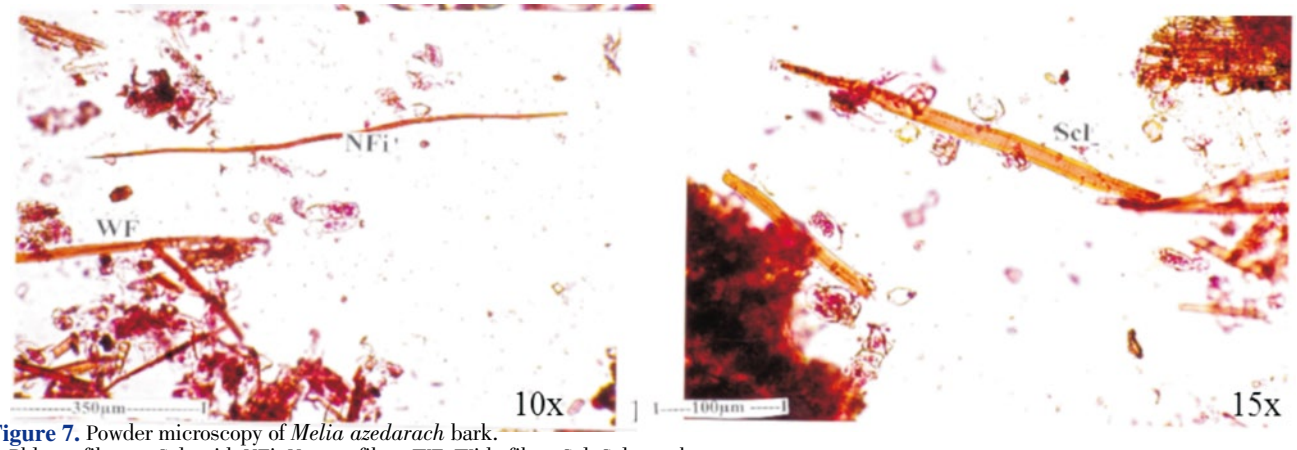

Figure 7. Powder microscopy of *Melia azedarach* bark.
1: Phloem fibre; 2: Sclereid. NFi: Narrow fibre; WF: Wide fibre; Scl: Sclerenchyma.

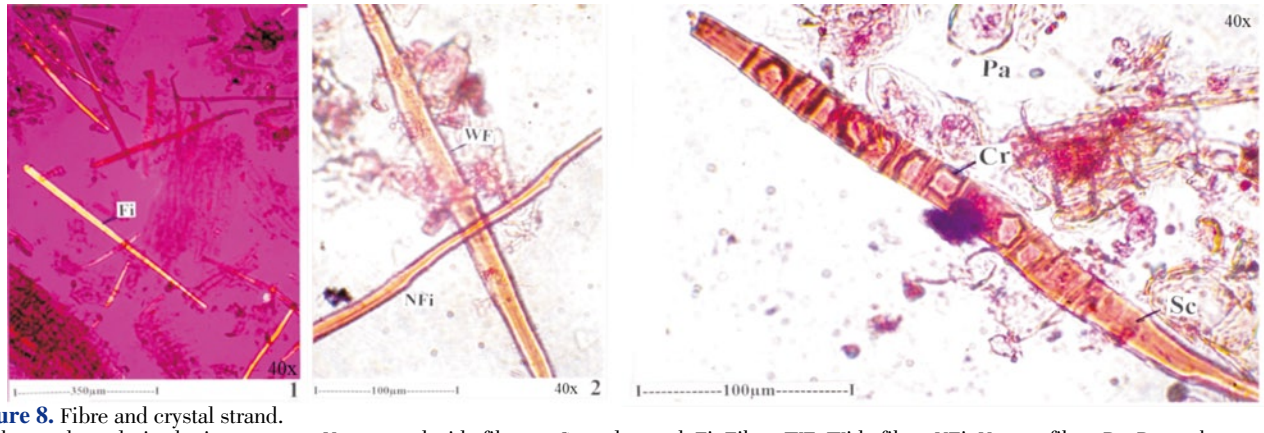

Figure 8. Fibre and crystal strand.
1: Fibre under polarized microscope; 2: Narrow and wide fibre; 3: Crystal strand. Fi: Fibre; WF: Wide fibre; NFi: Narrow fibre; Pa: Parenchyma; Cr: Crystals; Scl: Sclerenchyma.

The phytochemical profiling of the bark revealed the presence of carbohydrates, steroid, glycosides, triterpenoids and flavonoids. Tannin content of the bark was estimated by Folin–Denis reagent. Hydrolysable tannin content was (0.490±0.002)% w/w, while non– hydrolysable tannin content was (0.551±0.001) % w/w. Alcoholic total flavonoid content was (8.64±0.24) quercetin equivalent mg/g, aqueous total flavonoid content was (6.28±0.13) quercetin equivalent mg/g. Alcoholic total phenolic content was (82.68±0.03) gallic acid equivalent mg/g, aqueous total phenolic content was (54.94±0.20) gallic acid equivalent mg/g.

4. Discussion

For exploitation of medicinal plant bioresource, one of the major challenges is inadequacy of quality standards available for producing and maintaining quality and consistency of medicinal plant products. Macroscopy and microscopy of the raw material with special emphasis on diagnostic characteristics, organoleptic examination is one of the important tools to standardize the raw material. Standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostic parameters and standards must be established. Extractive values give us information about the nature of chemical constituents present in the plant, whereas the ash value tells us regarding contamination with sand and soil. Phytochemical evaluation revealed the presence of various secondary plant metabolites. Flavonoids and phenolic

compounds are responsible for various biological activities of the plant and hence its content determination becomes an integral part of the standardization process.

From the obtained results, it can be concluded that the pharmacognostic study of *Melia azedarach* bark have furnished a set of qualitative and quantita-tive parameters that can serve as an important source of information to ascertain the identity of plant material in future studies.

Conflict of interest statement

Authors declare that we have no conflict of interest.

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