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***Research Article***

# COMPARATIVE PHARMACOGNOSTICAL STUDIES OF *TERMINALIA ARJUNA* USED IN AYURVEDIC DRUG "ARJUNA" WITH ITS ADULTERANT *KAVALAMA URENS*

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## ABSTRACT
"Arjuna" an ayurvedic drug used as cardiotonic is formulated with bark extracts of *Terminalia arjuna*. Because of number of factors the botanical specimens were adultered or contaminated and these adulterantions can potentially alter the results in clinicals and reports causing subtle variations to have effects on quality, efficacy of these botanical supplements. We focussed pharmacognostical studies of original specimen *Terminalia arjuna* which gets changed and substitueted with other taxon *Kavalama urens* in formulations of ayurvedic drugs. Comparative pharmacognostic evaluation of fine powdered barks of *T. arjuna* and *K. urens* for ash analysis, organoleptic characters, fluorescent analysis were undertaken. Comparative monograph of selected taxa depicted that showed larger variations in bark anatomy and pharmacognostical evaluation . Our investigations showed that it is vital that authenticity of botanical materials in ayurvedic drug market should be focussed.

***Key Words :*** *Terminalia arjuna, Kavalama urens, Bark anatomy.*

## INTRODUCTION
Medicinal plants research is an integral component focussing on isolation, extraction, formulation of natural metabolites which is a crucial development for pharmaceutical industries to yield its synthetic form and its analogues in the development of active drugs and compounds against different ailments. It is well known that in course of time ,drug materials get changed or substituted with other plant species. "*Arjuna*" an ayurvedic drug used as a cardio tonic is a best example in this context. In this paper we focussed on the comparative pharmacognostical studies of *Terminalia arjuna* the original plant used in ayurvedic drug "arjuna" with its adulterant *kavalama urens* which is a morphological fake.
The pharmacognostical study is the major and reliable criteria for identification of plant drugs. Accurate plant identification is the foundation of the safe use of plant based natural health products in pharmaceutical sciences. Without proper identification at a starting point, the safe use of quality products cannot be guaranteed. Dried products sold in the medicinal plant trade are generally difficult to identify, as many useful diagnostic characters are lost through desiccation. Many herbs are sold through brokers where the material can change hands several times. The originality of herbal drugs in terms of raw material extraction, preparation and marketability requires proper guide lines according to WHO standards (WHO, 2003). It is well known that in course of time, drug materials get changed to or substituted with other plant species (Muhammad Zafar, 2011). The overall objectives of the present paper is to use classical and modern techniques of chemotaxonomy to authenticate the original raw material of correct species marketed. The detailed and systematic pharmacognostic evaluation gives valuable information for future studies.

### *Botanical Description*
***Terminalia arjuna* (Roxb. ex DC.) Wight and Arn.** (genus *Terminalia*, family *Combretaceae*). *Pentaptera arjuna* Roxb. ex DC. is a synonym of *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn. The surface of the bark is smooth, pale-greenish grey or white and inner surface is red in colour. The bark peels off in irregular flakes or sheets, which are used for tanning. Arjuna (*Terminalia arjuna*) is a common medicinal plant used in the ayurvedic system of medicine to treat various ailments (Parakh, 2010).

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***Kavalama urens* (Roxb.) Raf.** ( genus *Kavalama*,family *Malvaceae*). *Sterculia urens* Roxb. is a synonym of *Kavalama urens* (Roxb.) Raf. It is commonly known as 'gum karaya tree' and it is valued for known as 'Indian tragacanth' .Tapping of the gum requires stripping of the bark. The surface of the bark is smooth, greenish grey or white and inner side of the bark is pale brown . The bark peels off flakes seasonally, which are used for its gum (Hussain T M ,2008).

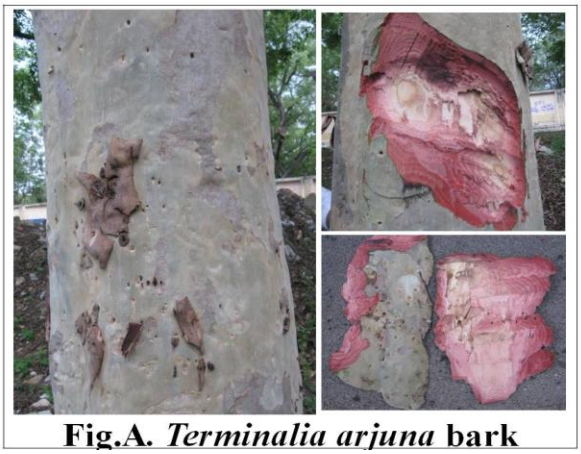


**Fig.A. *Terminalia arjuna* bark**

**Fig .B . *Kavalama urens* bark**

## MATERIALS AND METHODS

Plant specimens were collected during field visits. Crude bark raw materials collected from Srinivasa ayurvedic pharmacy and local medicinal plant vendours at herbal markets of Tirupati,Chittoor dt of Andhra Pradesh. Macroscopic, microscopic and chemomicroscopic studies (presence of lignin, tannin, oil and calcium oxalate crystals) on the fresh, powdered and anatomical sections of the stem bark were carried out for the purpose of identification and monograph preparation.

### *Microscopic Study*

Transverse sections of *T.arjuna* and *K.urens* were taken by using a microtome. Permanent mount of bark was prepared using saffranin fast green stain by double staining technique (Johansen DA 1940). The morphological characters were reconfirmed by using various Floras of Gamble (1957), Thammanna *et al.,* (1994) and Madhava chetty *et al* .,(2011). The Light micrographs of photographs were taken by means of an Images were obtained with a digital camera (DPx26, Olympus) attached to a light microscope (BX-50, Olympus). For the study of crystals, starch grains and lignified cells, polarized light was employed. Magnifications of the figures were indicated by the scale-bars.

### *Physicochemical Studies*

Physicochemical parameters were determined as per guidelines ofWHO. Total ash value, loss on drying, water soluble ash, acid insoluble ash alcohol oluble extractive value and water soluble extractive value were determined (Anonymous 1996 and 2009).

Fluorescence analysis of the whole plant powder drugs was carried out according to the methods followed by Chase and Pratt (1949). The fluorescence property of the powder is observed both in visible and ultra-violet for their fluorescence characters (short wave length 254 nm and long wave length 365 nm) after treatment with various chemical reagents.

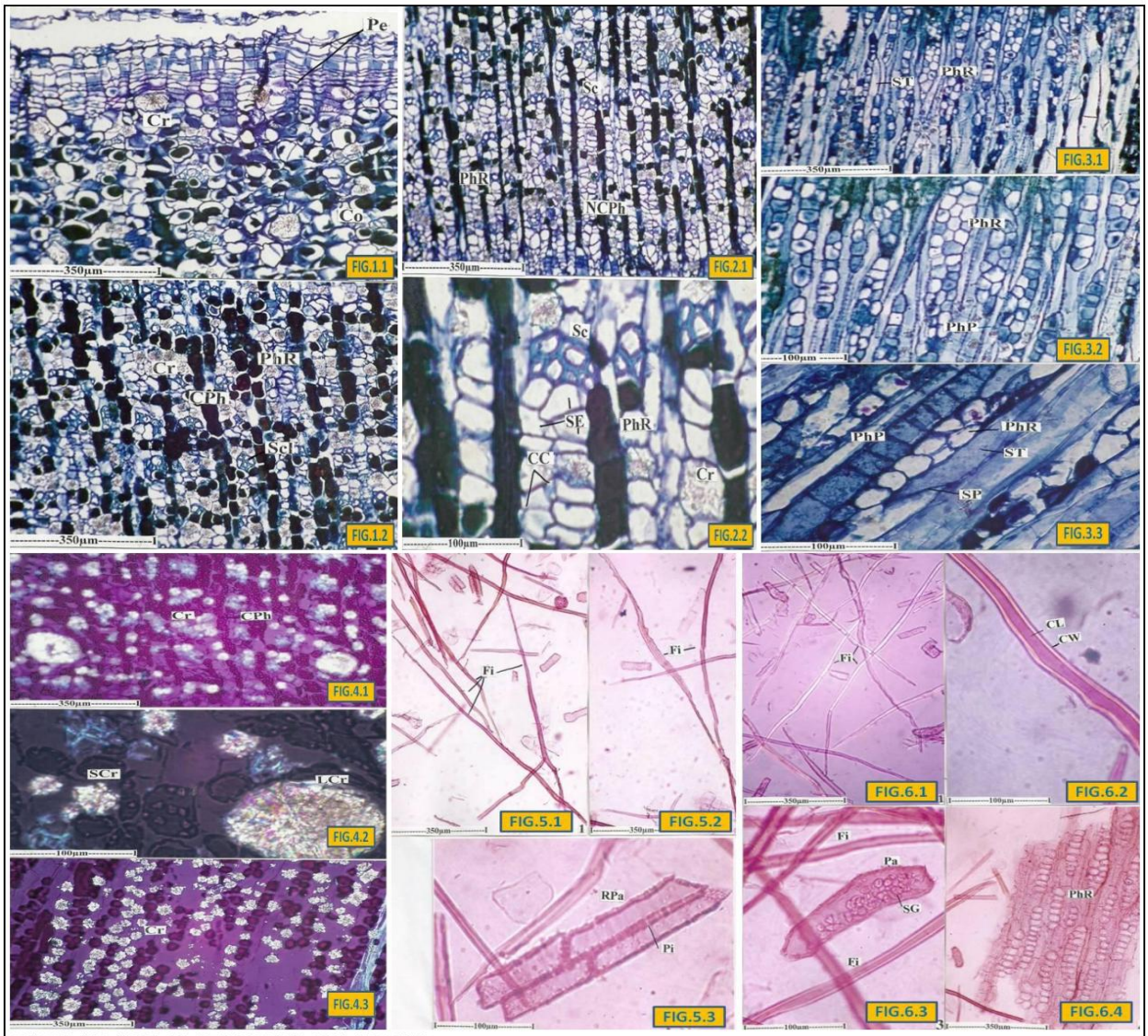
## RESULTS

## BARK ANATOMY OF *TERMINALIA ARJUNA*

*Microscopic features*

The periderm is superficial comprising uniformly thick phellem extending all around the trunk. The phellem is about 200 µm thick. It consists of alternating layers narrow tabular cells with wide squarish cells (Fig. 1.1). Both the narrow and wide phellem cells are thin walled and suberised.

Secondary phloem follows immediately next to the periderm. Secondary phloem is differentiated into outer wide collapsed phloem (Fig. 1.2) and inner non collapsed phloem (Fig.2.1). The collapsed phloem is characterized by several successive thin tangential bands of fibres, abundance of calcium oxalate druses and dense accumulation of tannin (Fig. 1.1& 4.1).




BARK ANATOMY OF *TERMINALIA ARJUNA*

**Fig.1.1: T.S of bark through periderm ;Fig.1.2: T.S of bark through collapsed phloem** (Co: Cortex,Cph: Collapsed phloem,Cr: Crystals,Pe: Periderm,PhR: Phloem ray,Scl: Sceleids) **;Fig. 2.1: T.S of bark through non collapsed phloem; Fig.2.2: Sieve – elements enlarged (** Cc: Companion cells,Cr: Crystals,NCph: Non collapsed phloem,Sc: Sclerenchyma,SE: Sieve elements; **Fig. 3.1: TLS of Phloem ;Fig.3.2: TLS – Phloem rays;Fig.3.3: Phloem elements – parenchyma, rays and sieve elements (**Php: Phloem parenchyma,PhR: Phloem ray,Sp: Sieve plate,ST: Sieve plate); **Fig.4: Crystal distribution as seen under polarized light ;Fig.4.1: T.S of bark with smaller larger druses ;Fig.4.2: Same as above enlarged ;Fig.4.3: Crystal distribution in TLS view** Cph: Collapsed phloem,Cr: Crystals,LCr: Large type of crystal ;**Fig.5.1: Libriform fibres; Fig.5.2: Wide and narrow fibres ;Fig. 5.3: Ray parenchyma cells** Fi: Fibre,Pi: Pits,Rpa: Ray parenchyma;**Fig.6.1: Fibres showing lignified walls; Fig.6.2: Lignified walls – magnified (under polarized light); Fig.6.3: Storage parenchyma with starch grains ;Fig.6.4: Phloem – rays – TLS view** CL: Cell lumen,CW: Cell wall,Fi: Fibre,Pa: Parenchyma,SG: Starch grains.

**Figure A: Bark Anatomy of *Terminali Arjuna***

The collapsed phloem gradually transits to non collapsed phloem and the boundary between the collapsed and non collapsed zones is not evident. The non collapsed phloem has reduced fibre masses and the crystals are also gradually reduced (Fig. 2.1). The sieve elements are intact; they rectangular in outline thin walled and occur in parallel radial lines (Fig. 2.2). The companion cells are located along the corners of the sieve elements, the sieve tubes are 30 µm in height and 10 µm thick (Fig.2.2). The phloem rays both in the collapsed and non collapsed zones are densely filled with tannin. (Fig 1.2 & 2.1)

*TLS (Tangential longitudinal sections) of the phloem* (Fig. 3.1 and 3.2).

In TLS view, the phloem rays appear non-storied; the rays are at different levels. They are 1-3 seriate. The rays are heterocellular comprising marginal elongated upright cells and middle polygonal procumbent cells. The height of the rays range from 120 µm to 450 µm; the breadth is 20-50 µm; ray frequency is 13 / 1 mm.

The sieve tube members are straight and wide. The sieve plate is simple and oblique (Fig. 3.3). The axial parenchyma consists of vertical row of rectangular and squarish cells, the cells contain tannin. Tannin is also accumulated in the rays.

*Crystal distribution* (Fig. 4.1, 4.2 & 4.3)

Calcium oxalate crystals of druses or sphaero crystals are abundant in the phloem cells. As seen in transverse section the druses occur in regular transverse lines (Fig. 4.1). In TLS view, the druses are seen in several vertical parallel lines. The druses occur in ordinary, unmodified cells (Fig. 4.2). Occassionally, there are seen abnormally wider and circular mass of minute crystals (Fig. 4.3). The smaller druses are 40 µm wide; the larger crystals are 100-150 µm wide.

*Powder microscopy*

The bark powder exhibits the following inclusions when examined under the microscope.

i.Phloem fibres (Fig. 5.1, 2, 6.1, 2):

Liberiform fibres are abundant in the powder. Some of the fibres thin and gradually taper towards the ends (Fig.5.1). Others are wider in the middle and abruptly taper at both ends (Fig. 5.2). The fibre walls are thick and lignified (Fig. 6.2). Pits are not evident on their walls. The fibres are 850 µm to 1.2 mm long.

ii.Ray parenchyma (Fig. 5.3)

Narrow, rectangular cells with thick walls and dense simple pits are seen in horizontal position, at right angles to the fibres and parenchyma cells (Fig. 6.3); the ray parenchyma cells are also seen separated from ray system (Fig. 5.3). The cells are 100-180 µm long and 30 µm wide.

iii.Parenchyma of vertical system (Fig. 6.3, 4)

Rectangular, thick walled, simple pitted cells are seen in vertical orientation parallel to the fibres (Fig. 6.4). The vertical (axial) parenchyma cells are 100-170 µm long and 30 µm wide (Fig. 8.2).

***BARK ANATOMY OF KAVALAMA URENS***

The trunk bark of Kavalam *urens* is differentiated into two distinct regions as outer bark and inner bark.The outer bark consists of perioderm and dilating ray. There is no clear demarcation between the outer and inner bark.

a) *Out bark* (Fig. 7.1& 7.2)

The outer bark comprises periderm (Pe) and phloem sclereids (PhS). The periderm is 250 µm in thickness. By the activity of a single phellogen superficial in position a wide periderm is produced and rhytidome is not evident. Periderm consists of 4 to 5 layers of phellem cells which are thick walled, suberised, tabular and occur in radial series. Phelloderm cells are not clearly distinguishable. Phellem cells have dark colored cell contents .Inner to the periderm region is the collapsed secondary phloem which is characterized by the presence of irregular patches of phloem sclerenchyma cells and collapsed phloem cells having dark colored cell contents. The collapsed phloem region measures about 2 mm in thickness and extends upto the periderm zone. The outer bark constitutes the collapsed phloem region .Outer bark consists of patches of collapsed phloem alternating with discontinuous tangential blocks of

phloem slcereids in regular radial sequence, collapsed phloem and the phloem sclerenchyma (PhS) occur parallel with dilating phloem rays. These phloem rays (PhR) become wide and dilated towards the periphery and narrow in the non-collapsed phloem region (NCPh) (Fig 11). Phloem sclereids are thick walled and lignified and the collapsed phloem cells are crushed and obliterated. Crushed phloem is seen as dark streaks

b) *Inner bark*

Inner bark is characterized by non-collapsed secondary phloem region; the non-collapsed phloem region occur as discontinuous tangential bands, which alternate with phloem sclerenchyma the continuity of non-collapsed phloem bands alternate with the phloem ray and the non-collapsed phloem region is inner most region of the bark and it is the narrow zone lying next to the cambial zone. This region is 200 μm width.

In the non-collapsed phloem region the cells are intact and occur in radial files. Sieve tube members , companion cells, phloem parenchyma are intact (Fig 11). The phloem rays are narrow and undilated. The sieve tube members are polygonal in cross sectional view and thick walled. The companion cells occur at the corners of the sieve tube members.

In tangential longitudinal section (TLS) the phloem rays appear non-storied and the sieve tube members and axial parenchyma are also non-storied.

The phloem rays are homocellular and have only procumbent type of cells. Phloem rays are broad, multi seriate and 350 μm in width and 1-1.5 μm in length, the sieve tube members and the axial parenchyma form the axial system and are non-storied; the sieve tube members are long, narrow, and have oblique sieve plate (Fig. 13).

In radial longitudinal section these are wide tangential bands of ray cells (Fig 14.1) . The phloem ray cells are homocellular and the phloem fibres are in thick longitudinal files, parallel and separated by the axial system. Some of the ray cells have dark colored tannin cell contents (Fig. 14.2).

Calcium oxalate crystals in the form of druses are abundant in the phloem parenchyma cells and ray cell, they appear bright under the polarized light microscope (Fig. 12). The druses are 40 μm thick.

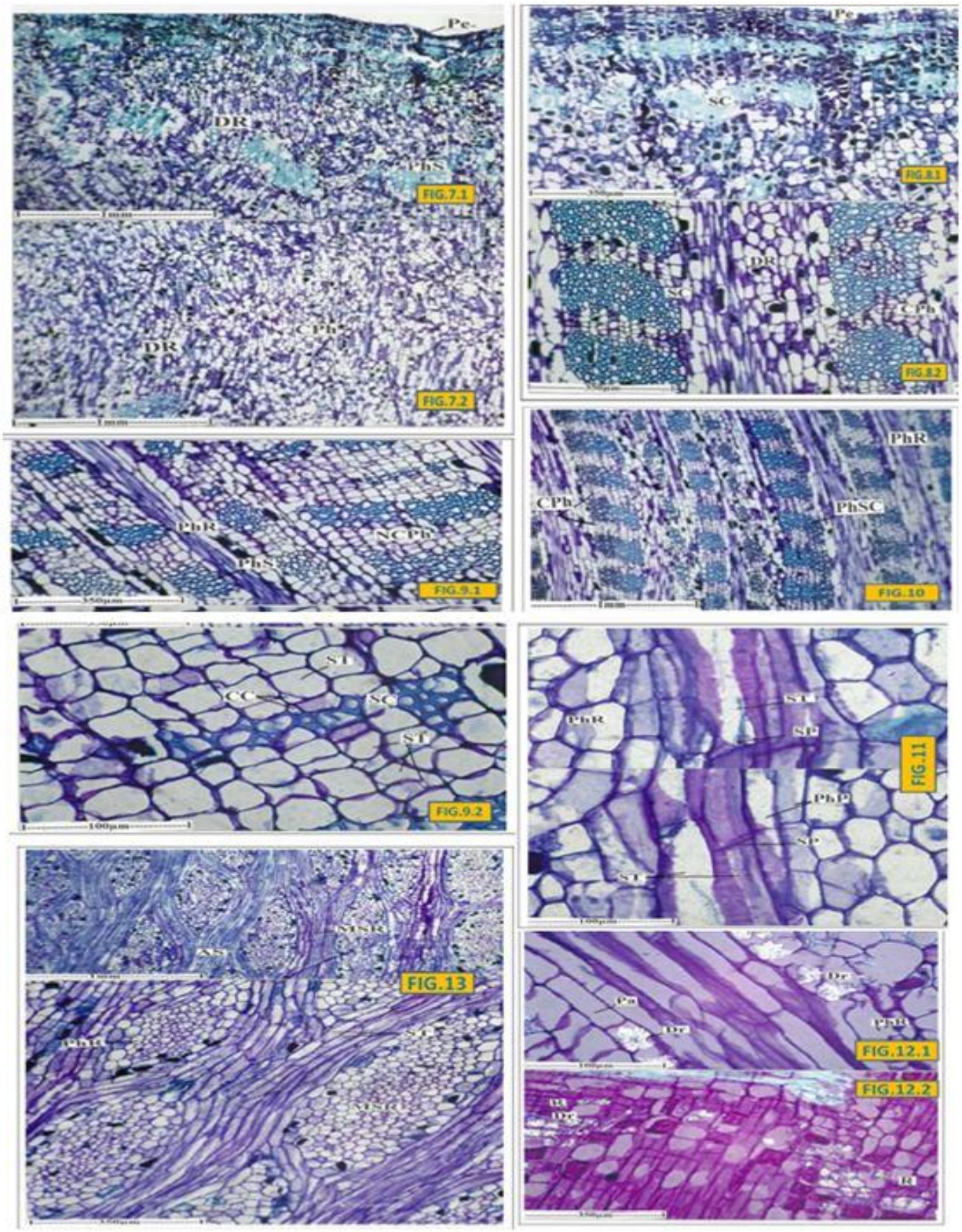
*Powder Microscopy*

When the bark cells are macerated, phloem fibres, periderm cells, parenchyma cells and slcereids are evident. The fibres are long, narrow, and slightly wide at the middle region and pointed at the tip, thick walled and lignified (Fig. 16.1). Fibres are 1.25 μm in length and 20 μm in width and parenchyma cells are rectangular to squarish, or brick like, thick walled (Fig. 15.1 & 15.2). The sclerenchyma cells are branchysclereids type Fig 16.2), thick walled and lignified.

**Table 1: Comparative studies on measurements of tissues and cells in bark**

| **Organ** | **Measurements (μ)** | |
|---|---|---|
| **BARK** | ***Terminalia arjuna*** | ***Kavalama urens*** |
| Thickness of periderm | 200 μm | 250 μm |
| Secondary phloem | | |
| Collapsed phloem | 1.1 mm | 2 mm |
| Non collapsed phloem | 700 μm | 800 μm |
| Sieve elements | | |
| Length | 30 μm | 1-1.5 μm |
| Width | 10 μm | 350 μm |
| Fibre length and thickness | | |
| Sclereid size | 850 μm to 1.2 mm length | 1.25 mm length 20 μm thickness |
| Length | 1.5 μm | 70 μm |
| Width | 10 μm | 50 μm |

**Figure B: Bark Anatomy of *Kavalama urens***



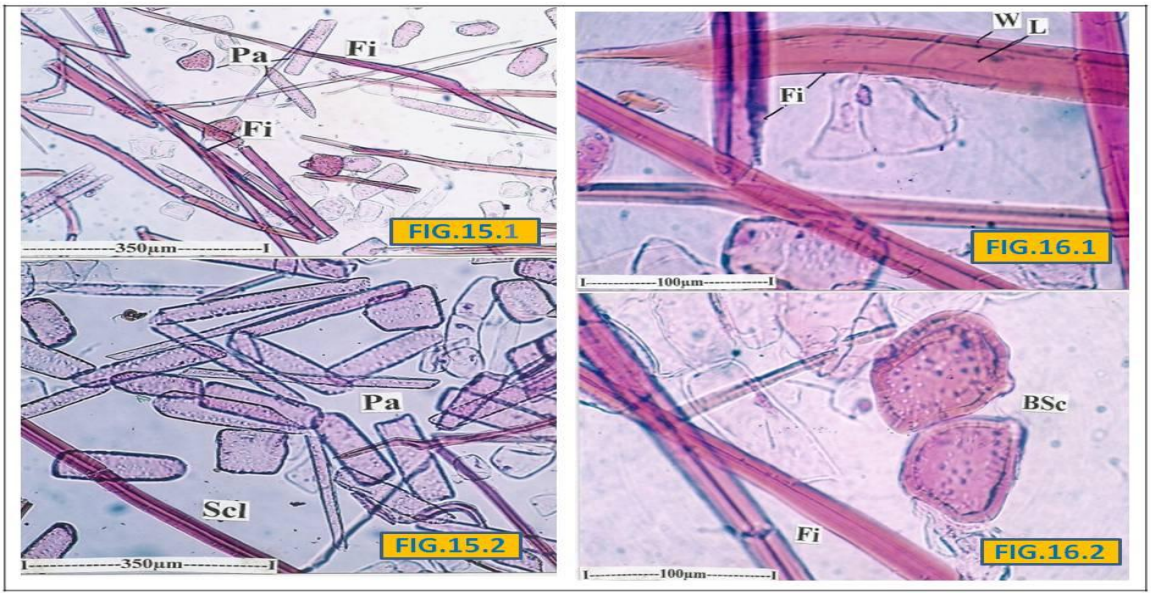



Fig.7.1& 7.2 : T.S of bark through periderm – outer periderm and inner phloem zones (Cph: Collapsed phloem,DR: Dilated ray cells,PhS: Phloem sclerenchyma) Fig.8.1&8.2: T.S of bark showing outer periderm (Pe) and inner sclerenchyma masses (SC) Fig.9.1 & 9.2: Collapsed phloem zone showing wide DR, thick radial bands of PhS alternating with tangential segments of collapsed phloem. Fig.10: Collapsed phloem region showing wide rays alternating with thick radial band collapsed sieve – elements – phloem sclerenchyma (PhSC) PhR: Phloem rays . Fig. 11: Sieve-elements enlarged CC: Companion cells, NCph: Non-collapsed phloem, ST: Sieve-tube .Fig.12.1 & 12.2: Phloem LS showing calcium oxalate druses in axial parenchyma and phloem rays Crystal distribution as seen under polarized light(All under polarized light microscope) PhR: Phloem ray . Fig.13: Rays and sieve tube enlarged (AS : Axial system of sieve tube and parenchyma cells MSR: Multiseriate ray) Fig. 14.1 & 14.2: Rays enlarged (AS: Axial system of fibres and sieve tubes ; PhF: Phloem fibres ) Fig. 15.1 & 15.2 : Phloem parenchyma and fibre – sclereids ,Fi: Fibres Pa: Parenchyma cells ,SCl: Fibre – Sclereid, Fig. 16.1: Fibres enlarged showing the walls and lumen (Fi: Fibres , L: Lumen, W: Walls) Fig. 16.2: Brachysclereids (BSc)

***Organoleptic and Powder Microscopy Comparison (Table 2 – 9)***

Comparitive organoleptic charecters revealed that in both the plants have similar properties since there is a possibility of adulterations.Table .2 gives the stated character. Powder analysis differed when the bark powder is treated with 5%aq.NaOH in colouration (Table 3).

**Table 2: Powder characteristics of the drug**

| Name of the Plant | Colour | Appearance | Odour | Taste |
|---|---|---|---|---|
| ***Terminalia arjuna*** | Pale brown | Powder | Characteristic | Astrindent |
| ***Kavalama urens*** | Pale brown | Powder | Characteristic | Astrindent |

Ash,Solubility values,Extractive values showed differences (Table 4,5,6). Fluorescence analysis of various extracts of the drug eluted various colours for both the plants which are described in Table 7. In Table 8& 9 Fluoroscence analysis of the drug powder of *Terminalia arjuna and Kavalama urens* bark powders are tabulated .These two plants showed variation under the florescence microscopy.

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**Table 3: Powder analysis of the drug**

| Treatment | Observation | |
|---|---|---|
| | ***Terminalia arjuna*** | ***Kavalama urens*** |
| Powder treated with water | Non-sticking | Non-sticking |
| Powder shaken with water | Foam like froth | Foam like froth |
| Powder treated with 5% aqueous NaOH | Brown | Dark brown |
| Powder treated with 60% aq.sulphuric acid | Pale brown | Pale brown |
| Powder pressed between filter paper for 24 hours | No oil stain | No oil stain |

**Table .4 Ash values of the drug**

| Name of the plant | **Total ash (% w/w)** | **Water soluble ash (% ww)** | **Akalinity of water soluble ash (ml)** | **Acid in soluble ash (% ww)** |
|---|---|---|---|---|
| ***Terminalia arjuna*** | 12.11 | 10.51 | 0.3 | 8.99 |
| ***Kavalama urens*** | 9.06 | 4.08 | 0.2 | 6.98 |

**Table 5: Solubility values of the drug**

| Name of the plant | Ethanol (% w/w) | Water aq.(% ww) | Methanol (% ww) |
|---|---|---|---|
| *Terminalia arjuna* | 59.33 | 22.25 | 68.45 |
| *Kavalama urens* | 55.06 | 20.01 | 67.05 |

**Table 6: Extractive values of the drug**

| Name of the plant | Ethanol soluble Extract (%w/w) | Watersoluble Extract (% w/w) | Hexane soluble extract (ml) | Chloroform soluble extract |
|---|---|---|---|---|
| *Terminalia arjuna* | 49.53 | 29.99 | 6.56 | 5.9400 |
| *Kavalama urens* | 35.99 | 26.78 | 5.96 | 2.0579 |

**Table 7: Fluorescence analysis of various extracts of the drug**

| Extract | Treatment | Observation | |
|---|---|---|---|
| | | ***Terminalia arjuna*** | ***Kavalama urens*** |
| **Ethanol** | Daylight | Pale brown | Pale brown |
| | Short UV | Pale brown | Pale brown |
| | Long UV | Pale brown | Pale brown |
| **Water** | Daylight | Pale brown | Pale brown |
| | Short UV | Pale green | Colourless |
| | Long UV | Pale green | Colourless |
| **Hexane** | Daylight | Pale green | Colourless |
| | Short UV | Colourless | Colourless |
| | Long UV | Colourless | Colourless |
| **Chloroform** | Daylight | Green | Colourless |
| | Short UV | Green | Colourless |
| | Long UV | Green | Colourless |

**Table 8: Fluoroscence analysis of the drug powder of *Terminalia arjuna***

| Experiments | Visible / Day light | UV light | |
|---|---|---|---|
| | | **254 nm** | **365 nm** |
| Drug powder | Pale brown | Pale brown | Pale brown |
| Drug powder + 1 N NaOH (aq.) | Pale brown | Pale brown | Colourless |
| Drug powder + 1 N NaOH (alc.) | Pale brown | Pale brown | Colourless |

| | | | |
|---|---|---|---|
| Drug powder + 1 N HCl | Pale brown | Green | Pale brown |
| Drug powder + 50% $H_2SO_4$ | Brown | Green | Brown |
| Drug powder + 50% $HNO_3$ | Brown | Green | Pale brown |
| Drug powder + Picric acid | Reddish brown | Green | Brown |
| Drug powder + Acetic acid | Pale brown | Green | Colourless |
| Drug powder + Ferric chloride | Red | Dark green | Colourless |
| Drug powder + $HNO_3$ + $NH_3$ | Red | Dark green | Colourless |

**Table 9: Fluoroscence analysis of the drug powder of *Kavalama urens***

| **Experiments** | **Visible / Day light** | **UV light** | |
|---|---|---|---|
| | | **254 nm** | **365 nm** |
| Drug powder | Brown | Green | Pale brown |
| Drug powder + 1 N NaOH (aq.) | Brown | Pale green | Pale brown |
| Drug powder + 1 N NaOH (alc.) | Pale brown | Colourless | Pale blue |
| Drug powder + 1 N HCl | Pale brown | Green | Pale brown |
| Drug powder + 50% $H_2SO_4$ | Pale brown | Green | Pale brown |
| Drug powder + 50% $HNO_3$ | Brown | Green | Pale brown |
| Drug powder + Picric acid | Yellow | Green | Brown |
| Drug powder + Acetic acid | Pale brown | Pale green | Colourless |
| Drug powder + Ferric chloride | Dark red | Green | Colourless |
| Drug powder + $HNO_3$ + $NH_3$ | Red | Green | Colourless |

## DISCUSSION

The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of the crude drug. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. These comparitive studies provide referential information for correct identification and selection of the drug from various adulterations

The ash values are criteria to judge the identity of purity and quality of the crude drug. Ash value is an important characteristic of a drug to trace adulteration and to check the quality and purity of the drugs. The water soluble ash is used to estimate the amount of inorganic elements which indication of the presence of exhausted material substituted for the genuine drug. The acid soluble ash content is determined and recommended for certain drugs which may be coated with dirt and sand. The solubility values also indicate the nature of the constituents present in a crude drug. Fluorescence studies help in the identification of drugs which may be more or less difficult to distinguish. Our investigations provided the genuinity of the original Ayurvedic drug Arjuna with complete medicinal monograph in Indian herbal pharmacopoeia .

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