International Research Journal of Ayurveda & Yoga
Vol. 6 (7),13-20, July,2023
ISSN: 2581-785X: https://irjay.com/
DOI: **10.47223/IRJAY.2023.6702**

Microscopy of Ashoka Bark and Marketed Alternates.

C. S. Rana[1], P. P. Raturi[2],R. K. Rai.[3]
1-Principal Scientist at Pharmacognosy Lab, Bio-resources Department. Dabur Research & Development Centre, Dabur India Ltd. Ghaziabad, U.P.
2-Head Bio-resources Department, Dabur Research & Development Centre, Dabur India Ltd Ghaziabad, U.P.
3-Head Health Care Division of the Dabur Research, Dabur Research & Development Centre, Dabur India Ltd. Ghaziabad, U.P.

Article Info

Article history:
Received on: 28-05-2023
Accepted on: 10-07-2023
Available online: 31-07-2023

Corresponding author-
C. S. Rana, Dabur Research & Development Centre (DRDC), Dabur India Ltd. Plot No. 22, Site IV, Sahibabad-201010. Ghaziabad, U.P.

Email: - drcsir@gmail.com

ABSTRACT:

Introduction- Microscopic evaluation of herbal powder is the simplest method for the identification of raw materials or raw drugs. Today, many sophisticated modern research tools are available for the identification validation and authentication of plant based drugs. Ashoka (*Saraca asoca*) has been used traditionally in the Indian system of medicine. Internal structure (cells and tissues) of bark of the Asoka, Shal and Kastdaru have been studied through plant anatomy/histology.
Material & Methods-Raw drug sample powder of all species were dipped in water and centrifuged for five minutes to soften and sedimentation. Suspended material of the sample was transferred to a clean glass micro slide and mounted. Slides were observed under axiostar plus light microscope.
Results & Discussion- The Powder Microscopic method is still one of the inventive methods for validation and authentication of the raw drugs. It also helps to find out the impurities and helps in quality assessment and assurance of the drugs. Microscopic assessment of the herbal drug of *Asoka* (*Saraca asoca* Roxb. Willd) shows similarity and some dissimilarity with cells and tissues of the bark of *Shal* (*Shorea robusta* Gaertn.) and *Kastdaru* (*Polyalthia longifolia* Sonn. Thwaites) for the selection of possible alternates.

Keywords: *Ayurveda, Powder Microscopy, Ashoka, Alternative, Medicines*

INTRODUCTION

Plant Anatomy/histology deals with the study of internal structure of various organs of the plant species. It includes the structure of the cells, which makes the basic unit of all living organisms[1]. In the modern days, with the discovery of transmission electron microscope (TEM) and scanning electron microscope (SEM) many other interesting features have been discovered. TEM deals with valuable information on inner structure of the sample, such as crystal structure, morphology and stress state information, while SEM provides information on the sample's surface

and its composition. Histologically, there are two types of sclerenchyma; elongated fibre and variously shaped sclereids. Sclerenchyma is a supporting tissue, having thick wall lignified cells, whose main function is mechanical support. Collectively sclerenchyma cells make sclerenchyma tissue. Fibres are often organised into bundles; there are some surface, xylary and extraxylary fibres. Fibres are elongate and sclereid are isodiametric. Sclereids are packed together very densely; they are recognized as stone cells. Astrosclereids, Brachysclereids, Macrosclereids, Osteosclereids, Trichosclereids and filiform sclereids are quite common[2]. These cells are separately similar and dissimilar in their shape, size and texture, which may be key characteristics in the botanical identification[3]. Ashoka (*Saraca asoca*) has been traditionally used in the Indian system of medicine for treatment of uterine, genital, and other reproductive disorders in women, fever, pain, and inflammation[4]. Stem bark of S. asoca is reported to contain glycosides, flavonoids, tannins and saponins. It is also used as a spasmogenic, oxytocic, uterotonic, antibacterial and antidysentric agent. It has also been reported to possess anti progestational and anti oestrogenic activity against menorrhagia. *Kastdaru* (*P. longifolia*) shows effective antimicrobial activity, cytotoxic function, antiulcer activity, hypoglycemic activity, and hypotensive effect[5]. Its extract consists mostly of steroids, flavonoids, clerodane diterpenes, cleroda-oic acids and alkaloids. Shal (S. *robusta*) also has been used to treat diarrhoea, dysentery and vaginal discharges, wounds, ulcers, leprosy, gonorrhoea and earache[6]. It has the chemical constituents such as nor-triterpene, dammarenolic acid, asiatic acid, dipterocarpol, triterpenic acid, tannic acid and phenolic content and possesses antibacterial, analgesic and wound healing effect.

By the names of Asoka bark there are mainly three species of raw drug available in the market like Sita Asoka (*Saraca asoca*), Kast Daru (*Polyalthia longifolia*) and Shal (*Shorea robusta*). Physical and organoleptic characters are more or less similar because bark as part used is same and all tree species have 10 to 15 years gestation periods for possible yields in the form of bark[7]. Common name, vernacular name or trade names and same plant part uses are the main reasons of misidentification, an unintentional substitution and adulteration of the raw materials or raw drugs. The commonest problem involving in medicinal plants stating raw material is the identification of genuine grantha based raw drugs and an intentional adulteration and substitution owing to multiple reasons like unavailability of raw material, higher cost of cultivation, long gestation period, high volume demand, unfair trade, fluctuating prices and sometimes illegal collection[8-16].

In addition to help of the available modern tools for identification, validation, authentication as well as quantification of the marker compounds, this powder microscopy study has been carried out to revamp the gap of identification, authentication and validation between actual species as per grantha and six Ayurvedic Pharmacopoeia of India (API) vs possible substitutes/alternative supported with studies based on histology, morphology, and phytochemicals[4-6] and therapeutic qualities to cope up the present and future demand and supply.

MATERIAL AND METHODS

Wild collection of the plant species developed into herbarium specimens and identified from the Botanical Survey of India (BSD), Dehradun. The usable parts of the plants have been kept in the museum as a standard for future references. Raw drug sample powder was dipped in water and centrifuged for five minutes to soften and sedimentation. Supernatant was discarded to obtain a cleaned pellet. Suspended material of the sample was transferred to a clean glass micro slide and mounted as per methods[17]. Slides were observed under axiostar plus microscope and captured images 10X and 40X magnifications using the camera AxioCam Icc5.

RESULTS

Microscopical evaluation was done for different sample images at different appearances. The sample appearances were matched with in-house standards of the concern species and compared with available literature (API, vol 1). Ashoka consists of dried bark of *Saraca asoca* (Fam. Caesalpinaceae), a small evergreen tree, found extensively in Central and Eastern Himalayas, Western Ghats and Deccan parts and Southern Pan India[18].

Description of Sita Asoka (*Saraca asoca*):

Powder microscopy of the bark represents as Sclereid with Brachysclereids stone cells, fibre cells, surface view of cork cells, Macrosclereids stone cells, Prismatic crystals of calcium oxalate, simple Starch Grains, Reddish and translucent tannin cells, Parenchymatous cells, Sclerenchymatous tissues, Tangential medullary rays and Crystalloid fibres (**Fig. 1**).

Kastdaru consists of dried bark of *Polyalthia longifolia* (Fam. Annonaceae), a long evergreen tree, found

extensively in parts of North-East and Central India and planted as an ornamental tree species[18].

Description of Kastdaru Asoka (*Polyalthea longifolia*):
Powder microscopy of the bark represents as surface view of Prismatic and rosette crystals of calcium oxalate, cork cells, Sclereid and large Brachysclereids stone cells with large lumen cells, Translucent tannin cells, Parenchymatous cells, Simple Septate Fibre, Schizogenous mucilage canal, Lysigenous mucilage cavity, Simple and compound starch grains and Radial medullary rays (**Fig. 2**). *Shal* consists of dried heartwood and bark of *Shorea robusta* Gaertn. (Fam. Dipterocarpaceae), a large sub-deciduous tree, found extensively in parts of North-East and Central India[18].

Description of Shal Ashoka (*Shorea robusta*):
Powder microscopy of the bark represents as Fibre cell, Group of simple and compound Starch Grains, Macrosclereids and group of Macrosclereids, Osteosclereids stone cells, Broad and large Lumen Cells, Group of sclereid and group of sclereid filled with reddish and translucent tannin cells, Tannin cells, Prismatic and Druse crystals of calcium oxalate, Crystalloid fibres, Brachysclereids, Cork cells and Heterogenous medullary rays (**Fig. 3**).

DISCUSSION

India harbors 17 mega biodiversity hotspots of the world and hosts 7-8% of recorded species of the world. About 1, 03, 258 of fauna and 55,048 species of flora have been documented in the country. India hosts 4 out of 35 globally identified biodiversity hotspots (Anonymous). Biodiversity broadly comprises animal and plant species and a forest comprises sedge, grasses, herbs, climbers, shrubs and trees. Sources of barks raw drugs are generally obtained from the all tree species which may be evergreen to deciduous and large to small trees. *Saraca asoca* (*Ashoka*) is red listed in vulnerable category as per IUCN and it has been assumed as an endangered species in India[7]. Annual demand of Asoka bark is more than 300 metric tons[19]. The bark of *S. asoca* is a rich source for many polyphenolic components[20]. The estimation of total phenolics, total flavonoids, and total catechin was assessed by Sulaiman, et al[4]. The bark of Shal (*Shorea robusta*) has also revealed phenolic content. Sulaiman, et al[4] has studied Phytochemical and pharmacological evaluation of *Saraca asoca*, *Polyalthia longifolia* and *Shorea robusta*. They have found that the bark of *Saraca asoca* and *Shorea robusta* both showed estrogenic activity. As per API, Asoka (*Saraca asoca*) is primarily used for estrogenic activity and management of female reproductive problems. Identification of scientifically validated substitutes for the bark of *Saraca asoca* is an urgent need of the Ayurvedic and herbal industry[21]. The available cultivation of the original Ashoka (*S. asoca*) never fulfilled commercial demands earlier nor can they do so in near future like commercial cultivation of the Kuth, Kutki and Pushkarmool[22]. Comparative powder microscopy of barks of all species has presented for the alternative source of genuine species (**Table 1**).

CONCLUSION

Trees are the main source of raw drugs/raw materials of marketed options of Ashoka barks either Sita Asoka (*Saraca asoca*), Kastdaru Asoka (*Polyalthia longifolia*) or Shal Asoka (*Shorea robusta*). Habit and habitats and population ecology of all plant species may be varied with the phytogeography. Indian traditional herbal system of therapy and Ayurveda both are majorly dependent on wild and partially on cultivated resources for their supply of raw materials for the herbal medicines. From the histological point of view all barks (available in market) are characterised with Parenchyma, Sclerenchyma, Brachysclereids stone cells, Tannin cells, Prismatic calcium oxalate and Starch grains. Ayurvedic points of view *Rasa*/taste, *Guna*/nature, *Veerya*/potency, *Vipaka*/results & *Prabhava*/action are also characterised by larger acceptability in the all species[16]. Only taxonomic points of view order, family, genus and species are varied but physical appearances of the barks are most of the time very similar and chemical composition and marker compounds are also more or less similiar[4-6]. Considering powder microscopical observation of the barks available in trade of all the tree species it can be concluded that powder microscopy is one of the tools to be used to evaluate the same. Observation of the microscopy of raw materials on similarity and dissimilarity along with some other parameters like phytochemistry, preclinical and clinical studies, will be helpful for the above evaluation.

The selected studied alternatives available in the market like Shal (*Shorea robusta*) and Kastdaru (*Polyalthia longifolia*) backed with other substantiation studies can be considered as an alternative source of Ashoka (*Saraca asoka*) for the current requirements. Simultaneously, the focus should be there to commercial cultivation and

propagation of grantha referred genuine API species to reverse the situation in future.

Acknowledgment- Nil
Conflicts Of Interest- Nil
Source of finance & support – Nil

ORCID

C.S. Rana, https://orcid.org/0000-0002-7916-3667

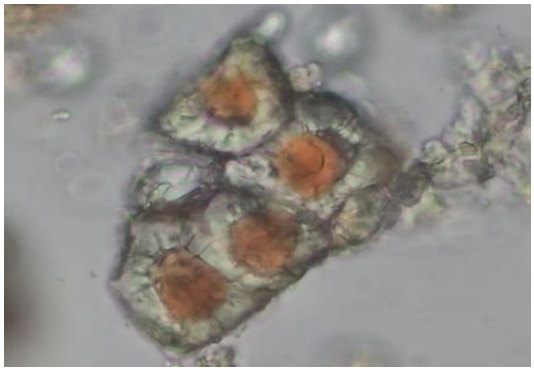
REFERENCES

1. Rana CS, Raturi PP, Rai RK. Microscopical identity of *Picrorhiza tungnathii* Pusalkar. Neelumbo. 2022; 64 (2): 276-281.
2. Rana CS, Kimothi GP, Rai RK, Narayan SB, Sastry JLN. Triphala churna myth and microscopic characterization. Ananls of Phytomedicine, 2018; 7(1): 158–164.
3. Rana CS, Kimothi GP, Rai RK, Narayan SB, Sastry JLN. Microscopical Identification of two piper spp., Piper longum and P, retrofractum. J. Res. Educ. Indian Med, 2020; 26(3-4).
4. Sulaiman CT, Jyothi CK, Jinu Krishnan Unnithan G, Prabhukumar KM, Balachandran I. Identification of validated substitute for Asoka (*Saraca asoca* (Roxb.) Willd.) by phytochemical and pharmacological evaluations. FJPS. 2020; 6:10; 1-11.
5. Jothy SL**,** Choong YS**,** Saravanan D**,** Deivanai S**,** Latha L Y**,** Vijayarathna S, Sasidharan S**.** Polyalthia longifolia Sonn: an Ancient Remedy to Explore for Novel Therapeutic Agents. RJPBCS. 2013; 4 (1); 714-730.
6. Bainsal N, Goyal P, Singh J. Shorea robusta Gaertn. F: a multi-therapeutic potential indigenous drug. Plant Archives, 2020; 20: 3313-3322.
7. Begum SN, Ravikumar K, Ved DK. Asoka - an important medicinal plant, its market scenario and conservation measures in India. Curr. Sci., 2014; 107: 26–28 6.
8. Joshi PR, Patel B, Shukla V. An overview of the causes practices in Pratinidhi Dravyas (substitution of drugs) in Ayurveda including newer techniques for their evaluation. Ayurveda. 2012; 33(4): 481-505.
9. Prakash O, Jyoti K, Kumar A, Kumar P, Kumar NM. Adulteration and Substitution in Indian Medicinal Plants: An Overview. JMPS, 2013; (1):4. 127-132.
10. Vaghela B, Soni H, Shukla L. A Concept of Herbal Pratinidhi Dravyas (substitute drugs) in Ayurveda. Pharmagene. 2013; 1(3):85-88.
11. Keshari, P, Pradeep. Controversy, Adulteration and Substitution - Burning Problems in Ayurveda Practices. IAMJ, 2017; 2501-2516.
12. Prachi A, Khaire, Prashant B, Nandwate. Substitutes for Ayurvedic medicinal herbs: A Review. Inter. J. Rec. Fut. Ayur. Sci. 2017; 2(1): 107-114.
13. Shinde A, Gahunge P, Rath S. The real concept of substitution in Ayurveda literature and adulteration the misleading concept of modern era. J. Ayur. Int. Med. Sci, 2018; 3:149-158.
14. Manisha SO, Garg NK. Relevance and impact of pratinidhi dravyas in chikitsa: a review article. World Journal of Pharmaceutical Research. 2021; 10 (12). 515-520.
15. Rathore P, Bhagat S. Substitution in Ayurveda W.S.R to Yogratnakar, a conceptual study. IRJAY. 2022; 5(9): 165-171.
16. Rana CS, Raturi PP, Rai RK. Notes on Ayurvedic alternates following plant Taxonomy. *J. Mount. Res.*, 2023; 18(1). 11-22.
17. Khandelwal KR. Practical pharmacognosy-techniques and experiments. Nirali prakashan Pune. 2012.
18. Mao AA, Dash SS. *Flowering plants of India, an annotated checklist*. Vols. 1-3. Botanical Survey of India, Kolkata. 2020.
19. Goraya GS, Ved DK. *Medicinal Plants in India: An Assessment of their Demand and Supply*. National Medicinal Plants Board, Ministry of AYUSH, Government of India, New Delhi and Indian Council of Forestry Research & Education, Dehradun. 2017.
20. Sharma PV. *Dravya-Guna Vigyan*. Varanasi: Chaukhambha Bharati Academy. 2014.
21. Singh S, Krishna TH, Kamalraj S, Kuriakose GC, Valayil JM, Jayabaskaran C. Phytomedicinal importance of Saraca asoca (Ashoka): an exciting past, an emerging present and a promising future. Curr Sci. 2015; 109:1790–1801.
22. Rana CS, Negi YS, Sawant LP, Raturi PP. Commercial cultivation and sustainability of Pushkarmool (*Inula racemosa*), a case study from Keylong, Lahaul & Spiti, Himachal Pradesh, India. J. Mount. Scie.2022; 17 (2); 25-33.

How to cite this article: Rana C. S., Raturi P.P, Rai R.K "Microscopy of Ashoka Bark and Marketed Alternates." IRJAY. [online] 2023;6(7);13-20.
Available from: https://irjay.com.
DOI link- https://doi.org/**10.47223/IRJAY.2023.6702**

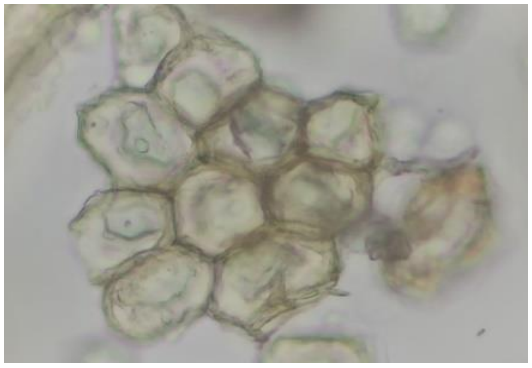
Fig 1: Microscopic images of bark powders of the Ashoka.

Brachysclereids cells without tannins

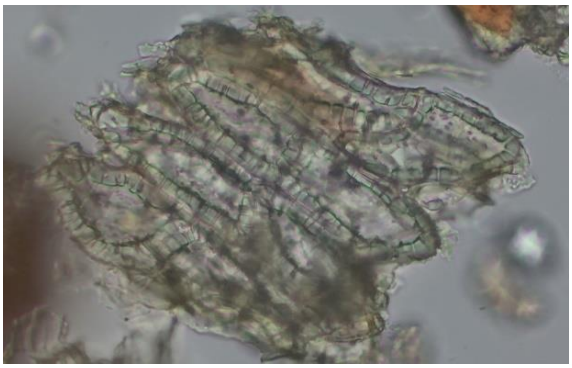


Brachysclereids cells with tannins

Fibre cells

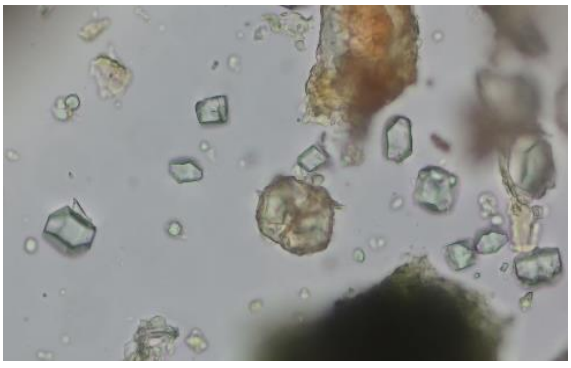


Cork cells

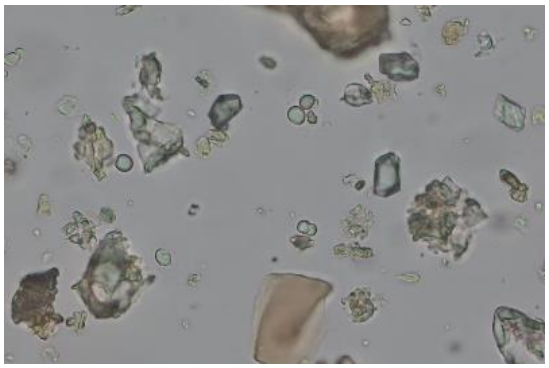


Macrosclereids stone cells

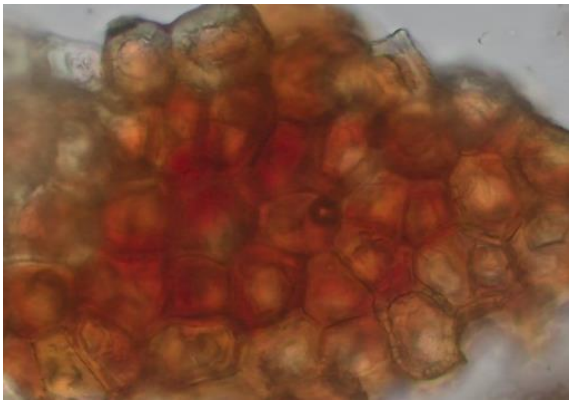
Macrosclereids stone cells



Prismatic calcium oxalate crystals



Starch grains

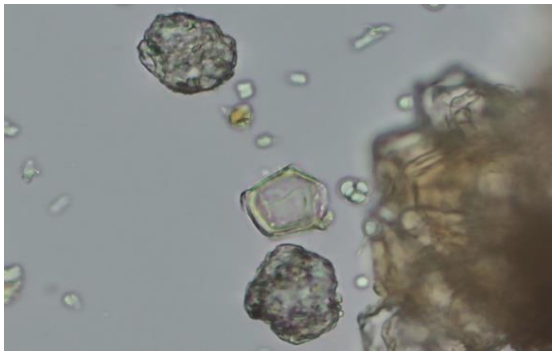


Translucent tannin cells

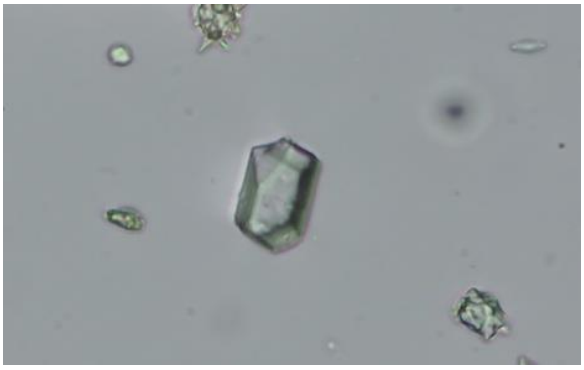
Tannin cell at 40X

Fig 2: Microscopic images of bark powders of the Kastdaru.

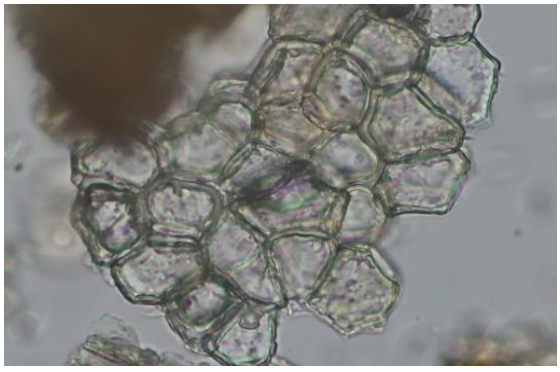
Rossete crystal of Calcium oxalate



Simple crystal of calcium oxalate

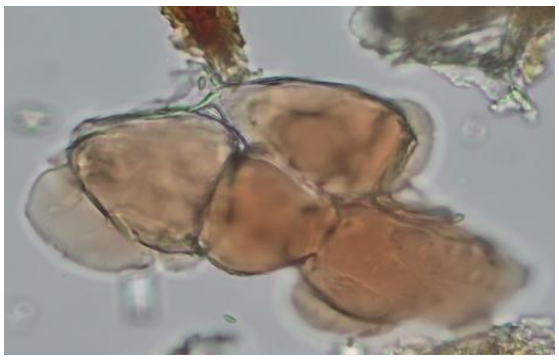


Prismatic crystal of calcium oxalate

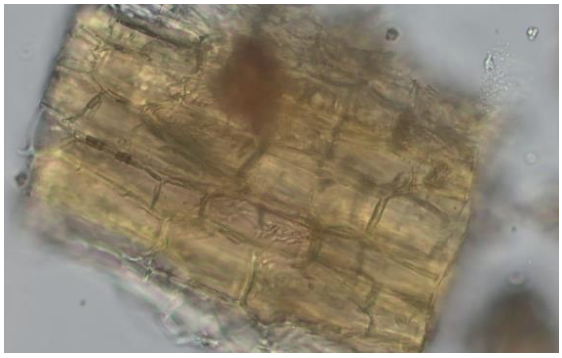


Cork cells

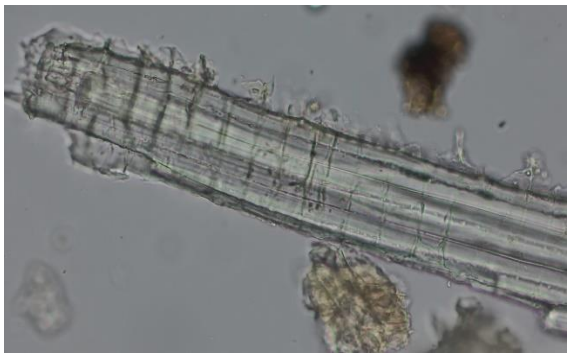
Scleroid cell



Translucent tannin cells



Parenchymatous cells



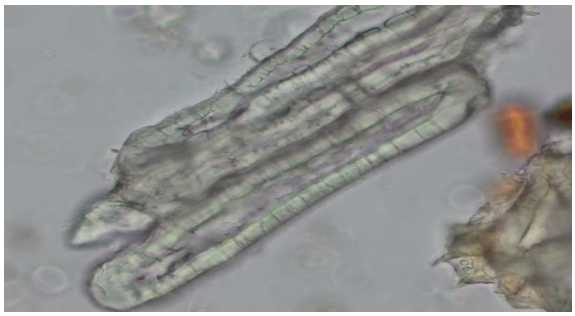
Fibre cells

Fig 3: Microscopic images of bark powders of the Shal.

Fibre cell

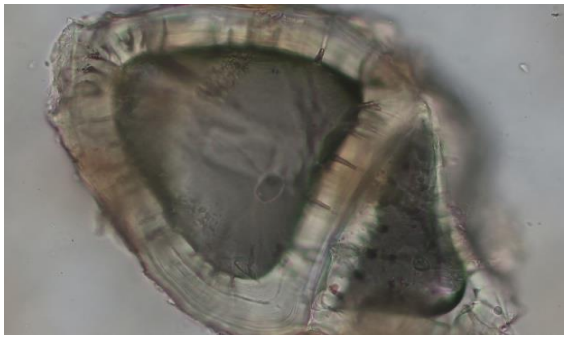
Group of Starch grains

Macrosclereids

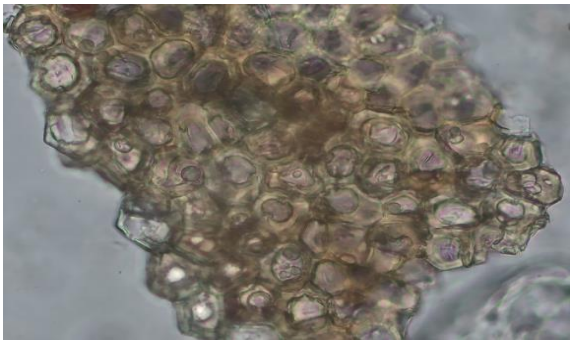


Group of Macrosclereids

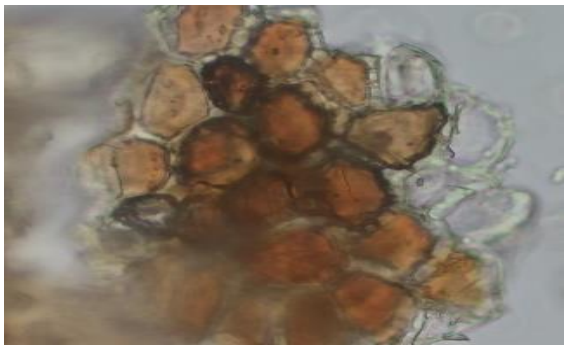
Osteosclereids



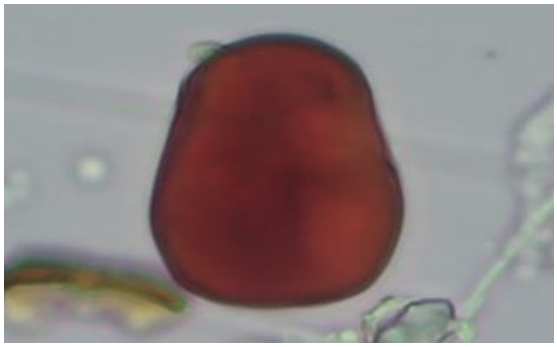
Large lumen cells



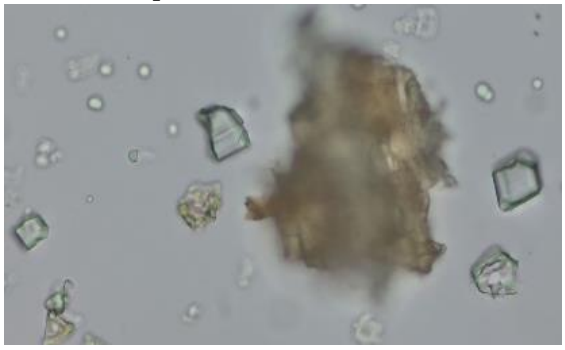
Group of sclereid cells



Group of Sclereid with tannin cells



Tannin cell at 40X oxalate



Prismatic crystal of calcium

Table 1: Comparative powder microscopy of all tree barks.

Plants	Description (Cells and Tissues)	Similarities	Dissimilarity
Asoka (*S. asoca*)	Cork cells, Parenchymatous cells, Reddish and translucent tannin cells, Sclerenchyma tissues with Brachysclereids stone cells, Tangential medullary rays, Prismatic and rosette crystals of calcium oxalate, Crystalloid fibres and starch grains.	Cork cells, Parenchyma, Sclerenchyma tissues, Radish and translucent tannin cells, Brachysclereids stone cells, prismatic calcium oxalate and starch grains.	Brachysclereids types of stone cells.
Kastdaru (*P. longifolia*)	Cork cells, Schizogenous mucilage canal, Lysigenous mucilage cavity, Brachysclereids stone cells with large lumen cells, Parenchymatous cells, Simple and compound starch grains, Prismatic and rossete crystals of calcium oxalate, Translucent tannin cells, Radial medullary rays and simple Septate Fibre.		Brachysclereids with large lumen cells and rossete crystals of calcium oxalates.
Shal (*S. robusta*)	Cork cells, Parenchymatous cells filled with reddish and translucent tannin cells, Brachysclereids, Macrosclereids and Osteosclereids stone cells, Broad and large Lumen Cells, Tannin cells, Translucent Tannin cells, Heterogeneous medullary rays, Prismatic and Druse crystals of calcium oxalate, Crystalloid fibres, simple and compound starch grains.		Osteosclereids types of stone cells and Druse crystals of calcium oxalates.