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ORIGINAL ARTICLE

Development of Internet Technology TIPHAM (Tool for identity of Powdered Herbals through Analytical Microscopy) for Microscopic Identification of Crude Herbal Drugs

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ABSTRACT

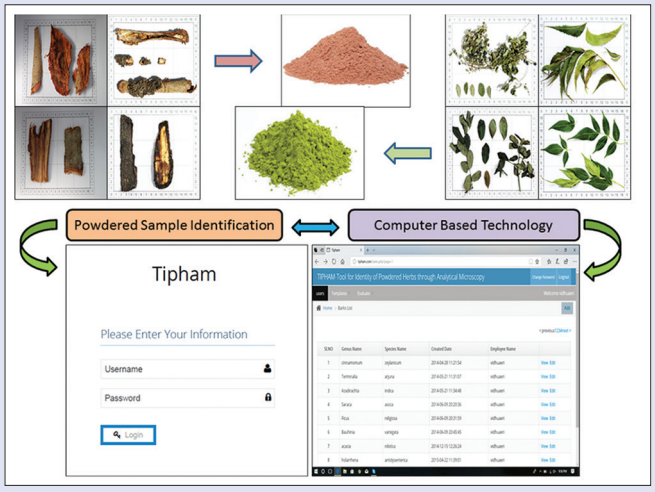
Background: Industrial usage of herbal plants has gone up, but techniques for verifying their botanical identity is still questionable. In the herbal industry, bulk consignments are received in powdered form as it is cumbersome to transport drugs in whole form. To ensure that the final product is safe and efficacious, the authenticity of the herbal plant should be established at the first stage. A proper methodology should be adopted in terms of computer technology to establish the correct botanical identity of the plant and to check the presence of substitutes and adulterants. **Objective:** To develop a software for identification of powdered samples of leaves and barks used in Ayurvedic Pharmacopoeia of India along with their substitutes and adulterants. **Materials and Methods:** Almost 100 plants have been selected from the Ayurvedic Pharmacopoeia of India comprising 54 barks and 46 leaves. Samples were self-collected and authenticated from the National Institute of Science Communication and Information Resources, Pusa, New Delhi. The selected crude herbal drugs were subjected to a detailed powdered microscopic identification and standard operating procedure for the preparation of slides was prepared. The features selected for identification of bark included14 specific characters - stone cells, calcium oxalate crystals, starch grains, medullary rays, fibers, sclereids, cork, isolated oil cells, tubular lactiferous canals, phloem parenchyma, masses, rhytidoma, parenchyma, and secretory canals. These characters are further differentiated into 75 features and 151 subfeatures, whereas for leaves, 13 specific characters were included, namely, epidermis, stomata, trichomes, calcium oxalate crystals, fibers, cell contents, cystoliths, lamina, starch grains, tracheids, lactiferous canals, and xylem vessels which are differentiated into 139 features. The details of all the features have been uploaded in the software under the name tool for identity of powdered herbals through analytical microscopy (www.tipham.com) with the database of 100 selected drugs. **Results:** A computer-based approach is developed which contains standard requirements for powdered plant parts, thus enabling identification of a bark or leaf powder in short time with minimum expertise. **Conclusion:** Computer-based technology would be a landmark in the field of pharmacognosy as proper identification of plant is the key to develop quality herbal products ensuring their safety and efficacy.

Key words: Bark, computer based, pharmacognosy, tool for identity of powdered herbals through analytical microscopy leaf powder microscopy

SUMMARY

Development of Internet Technology tool for identity of powdered herbals through analytical microscopy (TIPHAM) for microscopic identification of crude herbal drugs

- Samples of about 100 plants were self-collected from the National Institute of Science Communication and Information Resources. These samples were subjected to detailed powder microscopic evaluation with an aim to establish key diagnostic features to differentiate between powdered bark and leaf crude herbal drugs along with their substitutes and adulterants
- The features selected for identification of bark included 14 specific characters which are further differentiated into 75 features and 151 subfeatures, whereas for leaves, 13 specific characters which are classified into 139 features
- The details of all the features have been uploaded in the software under the name TIPHAM which contains database of 100 selected plants
- A computer-based approach is developed which will provide botanical authentication of powdered sample of bark or leaf in short time with minimum expertise.



Abbreviations used: µm: Micrometer; AHP: American Herbal Pharmacopoeia; DNA: Deoxyribonucleic acid; GMP: Good Manufacturing Practices; ICMR: Indian Council of Medical Research; Id: Identity Document; IT: Information Technology; MICROAID: Microaided Identification; MP: Megapixel; NA: Not Applicable; NISCAIR: National Institute of Science Communication and Information Resources; TIPHAM: Tool for Identity of Powdered Herbals through Analytical Microscopy; TLC: Thin-Layer Chromatography; UK: United Kingdom; WHO: World Health Organization.

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Figure 1: Samples procured from industry. Industrial grounded powdered samples

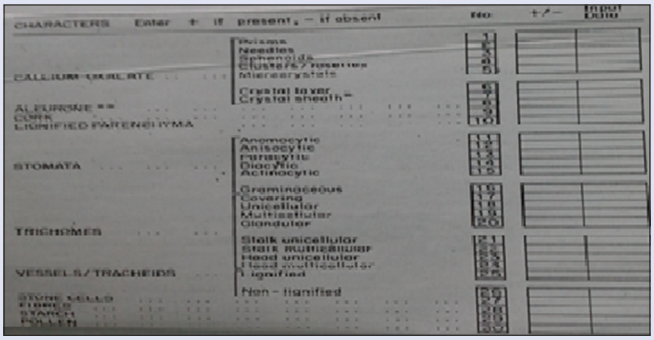

Figure 2: Program microaided identification available in the United Kingdom. It consists of characters of all plant parts irrespective of leaf, bark, fruit, or seed. For example, trichomes and stomata are present in leaves; stone cells, vessels/tracheids, lignified parenchyma in barks; aleurone in seeds; pollen in fruits, whereas calcium oxalate crystals and starch grains are present in almost every plant part

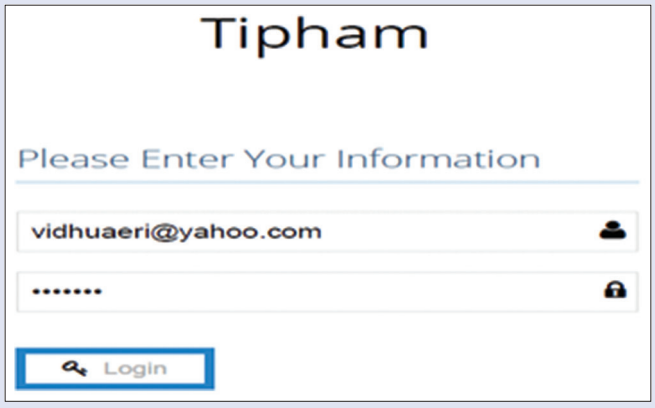

Figure 3: Login page for tool for identity of powdered herbals through analytical microscopy program. Enter the user identity document and password and get access into the program

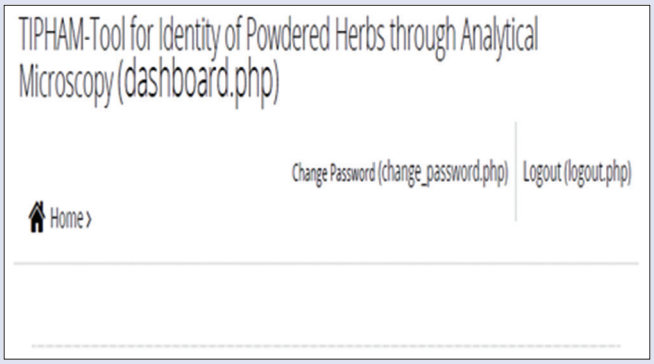

Figure 4: Homepage for tool for identity of powdered herbals through analytical microscopy program. Indicates logout option

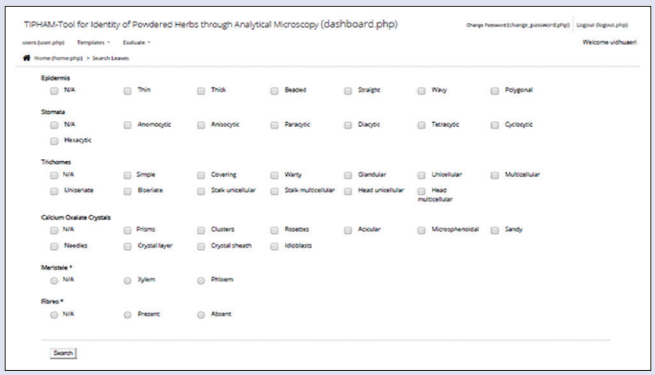

Figure 5: Template for identification of a leaf powder Go to the option "Evaluate." Select leaf template. Features for identification of a leaf powder will appear. Enter the desired features and click "Search"

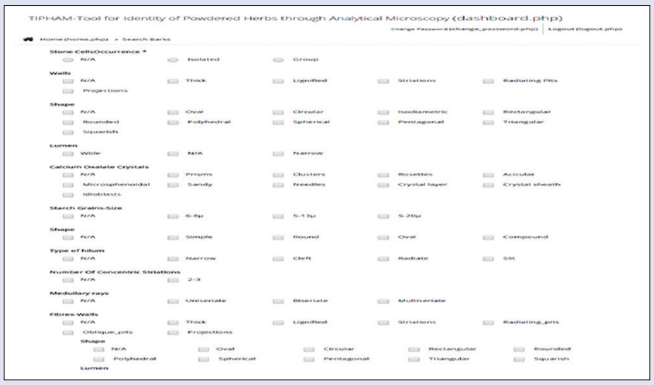

Figure 6: Template for identification of a bark powder. Go to the option "Evaluate." Select bark template. Features for identification of a bark powder will appear. Enter the desired features and click "Search"

INTRODUCTION

Herbal drugs have been used as dietary supplements, nutraceuticals, food additives, as ingredients in cosmetics, flavoring agents, and as fragrance. The industrial usage of plants and their parts, either as such or after processing has gone up, the technology for confirming their botanical identity is still questionable. The knowledge of microscopical features is not known in industry. Therefore, the use of microscopic technique is coming down. It is still a powerful tool to identify powdered crude drugs. The herbal industry is generally procuring crude drugs in the powdered form as a convenient form to whole drugs. The fear of substitution and adulteration of raw herbs is also on rise as most of

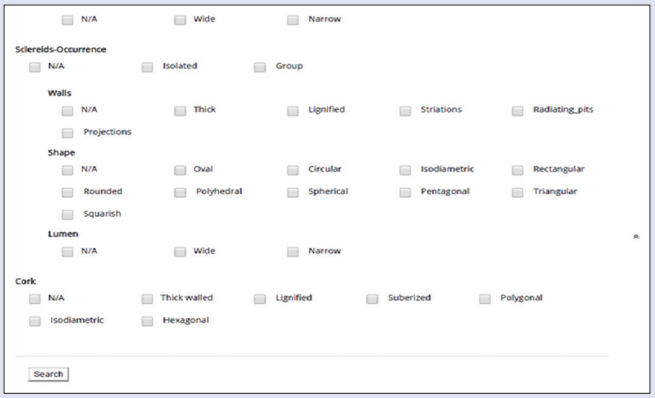

Figure 7: Template for identification of a bark powder. Go to the option "Evaluate." Select bark template. Features for identification of a bark powder will appear. Enter the desired features and click "Search"

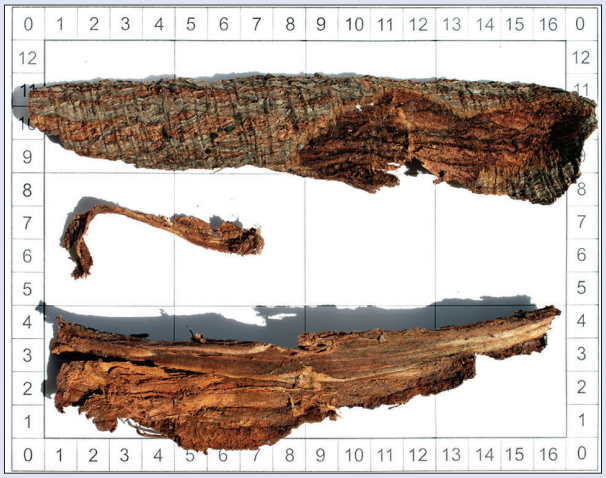

Figure 8: Macroscopic characters of *Ficus lacor* bark. Shape – Flat-to-curved pieces, Outer bark – Ash to whitish gray, numerous transversely arranged lenticels, Inner bark – Reddish brown, rough, fibrous, and longitudinally striated, Fracture – Fibrous, Odor – Characteristic

Figure 9: Macroscopic characters of *Ficus racemosa* bark. Shape – Flat, curved, or channeled, Outer bark – Rough, whitish papery flakes coming out of outer surface, Inner bark – Pale brown, uneven, longitudinally striated, Fracture – Fibrous, Odor – Nil

Figure 10: Macroscopic characters of *Ficus religiosa* bark. Shape – Lat or slightly curved pieces, Outer bark – Uneven surface light brown to ash colored, Inner bark – Smooth and brownish, Fracture – Fibrous, Odor – Indistinct

the times only plant parts are available in the industry: 50% roots, 15% seeds, 12% wood waste, 9% whole plants, 7% bark/stem, 4% leaves, and 3% flowers are used as raw material. Performing macroscopy and microscopy of each plant part in every consignment in industry is a tedious, time-consuming job and one does not see good documentation leaving doubts about the quality control of the tests being done. Nonpharmacognosists are being trained to identify drugs, but they are unable to identify herbal plants due to nonavailability of data on microscopical features of crude powdered drugs. The pharmacopeias have introduced mandatory thin-layer chromatography testing of the plant material under examination. This has improved the identity testing subject to availability of specific marker compounds but cannot replace the botanical identity testing. Therefore, a computer-based technology needs to be developed to establish the correct botanical identity of the plant and check the presence of adulterants and allied drugs. A computer-based approach within seconds will help to provide the fingerprint of any part of the plant: leaves, seeds, fruits, bark, root, and stem. Such a technology would be a landmark in the field of pharmacognosy as proper identification of plant part is the key to develop a formulation of utmost quality and safety.[1]

The manufacture, sale, and distribution of herbal products are regulated in India under Drugs and Cosmetics Act 1940 and Rules 1945. The World Health Organization and other organizations emphasize the need for quality and standardization of plants used in manufacturing of traditional medicines including Ayurveda where the first basic requirement is establishing the correct botanical identity of plant drug attributing it to a specific genus and species.[2-5] In India, Ayurvedic Pharmacopoeia, Indian Pharmacopoeia, Indian Herbal Pharmacopoeia, and ICMR monographs have standards for checking authenticity of botanicals used in herbal products. However, serious efforts are not made to resolve the issue of controversial nomenclature.[6-8] The long history of safe usage of herbal medicines can be extrapolated only when the botanical identity of the plant going into those medicines is established and standardized.[9]

Microscopic identification is the most commonly used method for authentication of herbal drugs. Microscopic techniques examine structural and cellular features of herbs to determine their botanical origin.[10] This method is useful for identifying species with similar

Table 1: Microscopic characters bark powder

Stone cells				Sclereids			
Occurrence	**Walls**	**Shape**	**Lumen**	**Occurrence**	**Walls**	**Shape**	**Lumen**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Isolated	Thick walled	Oval	Wide	Isolated	Thick walled	Oval	Wide
Group	Lignified	Circular	Narrow	Group	Lignified	Circular	Narrow
Abundant	Striations	Isodiametric	Pitted	Abundant	Striations	Isodiametric	Pitted
Few	Radiating pits	Rectangular	Slit like	Few	Radiating pits	Rectangular	
	Projections	Rounded			Projections	Rounded	
	Crenate margin	Polyhedral			Peg-like extension	Polyhedral	
	Beaded	Spherical			Beaded	Spherical	
	Pitted	Pentagonal			Branched	Pentagonal	
	Transversely pitted	Triangular			Three-sided thickening	Triangular	
	Branching pits	Squarish			Distinct pits	Cylindrical	
	Distinctly pitted	Irregular			Simple pits	Elongated	
	Devoid of pits	Oblong			Pitted walls	Fiber like	
	Thin walled	Angular			Thin walled	Very long	
						Blunt end	
						Pointed ends	
						Oblong	
						Cone shaped	

Fibers				Starch grains			
Occurrence	**Walls**	**Shape**	**Lumen**	**Occurrence**	**Shape**	**Type of hilum**	**Number of concentric striations**
N/A	N/A	N/A	N/A	Abundant	N/A	N/A	N/A
Isolated	Thick-walled	Oval	Wide	Few	Simple	Narrow	2-3
Numerous	Lignified	Circular	Narrow		Round	Cleft	Distinct striations
Group	Striations	Rectangular	Uneven		Oval	Radiate	
Few	Radiating pits	Rounded	Highly pitted wide lumen		Compound	Slit	
	Oblique pits	Polyhedral	Slit like		Spherical	Eccentric	
	Projections	Spherical			Triangular	Centrally located hilum	
	Frequently broken	Pentagonal			Small		
	Occasionally twisted	Triangular					
	Beaded	Squarish					
	Nonlignified	Irregular margin					
	Septate	Very long					
	Crenate margin	Pointed apex					
	Smoothly/finely striated	Bent					
	Dentate margin						
	Faint transverse septa						
	Thin walled						
	Irregular swellings						
	Crystal fibers						
	Transverse pits						
	Bifurcating ends						
	Distinct pits						
	Slit like						

Calcium oxalate crystals	**Medullary rays**	**Cork**	**Isolated oil cells**	**Tubular lactiferous canals**	**Phloem parenchyma**	**Massess**	**Rhytidoma**
N/A	N/A	N/A	Ovoid	N/A	N/A	N/A	N/A
Prismatic	Uniseriate	Thick walled	N/A	Groups	Spherical	Granular masses	Irregular
Clusters	Biseriate	Lignified		Oval	Oval	Dark brown cells containing tannins	Rectangular
Rosettes	Multiseriate	Suberized		Spherical	Pitted	Brown gummy	

Contd...

Table 1: Contd...

Calcium oxalate crystals	Medullary rays	Cork	Isolated oil cells	Tubular lactiferous canals	Phloem parenchyma	Massess	Rhytidoma
Acicular Microsphenoidal Sandy Needles Crystal layer Crystal sheath Idioblasts Rod shaped	Tangentially cut Radially-cut Longitudinally-cut	Polygonal Isodiametric Hexagonal Rectangular Pentagonal Beaded walls Pitted lumen Oval Elongated Thin walled Multilayered Radially arranged Irregular		Isolated	Lignified	Circular Large oval	

NA: Not available

Table 2: Microscopic characters leaf powder

Epidermis	Stomata	Trichomes	Calcium oxalate crystals	Fibers	Stone cells and sclereids	Cell contents
N/A Thick Thin Beaded Straight Wavy Polygonal Anticlinical walls Striated cuticle Devoid of stomata Slightly sinous walled Hexagonal Lignified wall Dome-shaped papillae Papillose cells Lignified pith cells Rectangular	N/A Anomocytic Anisocytic Paracytic Diacytic Tetracytic Cyclocytic Hexacytic Actinocytic Sunken	Abundant Few N/A Simple Branched Covering Warty Glandular Unicellular Thick walled Sessile Multicellular Uniseriate Biseriate Short stalk unicellular Stalk multicellular Head unicellular Oval head multicellular Shaggy wooly trichomes Straight Irregularly bent Striated walls Pedestial base Stellate Spear shaped Twisted Narrow neck Oval inflated apical cell Thread-like terminals Oval bicellular head Conical Cystolithic Bicellular cylindrical stalk 8-12 celled head Cup-shaped head Globular head Pointed apex Short Curved Cicatrix Bent Long	N/A Prismatic Clusters Rosettes Acicular Microsphenoidal Sandy Needles Crystal layer Crystal sheath Idioblasts Rod shaped Diamond-shaped idioblasts	Isolated Group N/A Thick walled Thin walled Longitudinally cut Narrow lumen Wide lumen Slit-like pits	Lignified Non lignified Wide lumen Narrow lumen Rectangular Oval Triangular Fibrous	Volatile oil Mucilage Tannin cell Yellowish pigment Fatty oil globule Resin ducts Brownish matter

Contd...

Table 2: Contd...

Epidermis	Stomata	Trichomes	Calcium oxalate crystals	Fibers	Stone cells and sclereids	Cell contents
		Hook shaped Swollen bases Sickle shaped				
Cystoliths	**Lamina**	**Starch grains**	**Tracheids**	**Lactiferous canals**	**Xylem vessels**	
Cigar shaped	Thick-walled cells	Few	Spiral	Elongated	Annular	
Longitudinally cut	Multilayered epidermis	Abundant	Annular pitted	Tubular	Spiral	
Warty	Palisade cells	Simple	Bordered pitted	Yellowish granular latex	Pitted	
Cylindrical	Spongy parenchyma	Spherical		Long septate fragments	Reticulate	
		Compound Oval Pear shaped			Longitudinally cut Crystal fibers	

NA: Not available

Table 3: Represents specific distinguishing characters of barks of four species of *Ficus*

Features	*Ficus lacor*	*Ficus racemosa*	*Ficus religiosa*	*Ficus benghalensis*
Common name	Pakar[25]	Gular[26]	Pipal[27]	Bargad[28]
Stone cells				
Occurrence	Abundant	Isolated Groups	Few	Isolated Groups
Walls	Striated Distinctly pitted	Beaded	Thick walled Pitted Striations	Thick walled Radiating pits
Shape	Isodiametric Squarish Oblong Oval	Polygonal Spherical Squarish	Triangular Oval	Circular Oval
Lumen	Narrow Wide Pitted	Wide Pitted	Narrow	Narrow Radiating pits
Size	15-45 µm [Figure 18 and 19]	>60 µm [Figure 20 and 21]	18-73 µm [Figure 22]	<50 µm [Figure 23]
Sclereids				
Occurrence	NA	NA	Abundant Isolated Group	Few
Walls	NA	NA	Striated Peg-like extensions	Beaded
Shape	NA	NA	Rectangular Irregular	Rectangular Oval Very long
Lumen	NA	NA	Narrow Wide	Wide Pitted
Size	NA	NA	<100 µm [Figure 24]	>100 µm [Figure 25]

NA: Not available

morphological characters. Today, there are a variety of methods available to authenticate herbal drugs, ranging from simple morphological examination to physical and chemical analysis and DNA molecular biology. Owing to cost, powder microscopy is still the most practical method for primary authentication.[11,12] The National and International Pharmacopoeia, namely, Chinese Materia Medica,[13] European Pharmacopoeia,[14] British Pharmacopoeia,[15] United States Pharmacopeia,[16] Japanese Pharmacopoeia,[17] Ayurvedic Pharmacopoeia of India,[18] and Vietnamese Pharmacopoeia[19] emphatically provide the powder microscopic characters as one of the most identifying features of the crude herbal drug. Botanical microscopy is a unique, valuable, rapid, and cost-effective assessment tool. It continues to play an important role in the authentication and assessment of medicinal plants. American Herbal Pharmacopoeia gives special emphasis on the powder microscopy of the herbal plants.[20]

The aspect of identification and study of pharmacognostic properties of raw herbs appears to have not got the attention it deserves. Powdered drugs are used in herbal industry in formulations. To ensure that final product is safe and efficacious, its quality should be checked at first stage. Therefore, a databank should be developed which contain standard features of powdered plant parts. Individuals with minimal botanical and microscopical training can successfully identify powdered materials using this aid. This databank may also be modified to meet individual and industrial needs. The computer-based technology may reduce time and labor required to identify individual samples. It is known that computers are free from personal bias and will act according to

Figure 11: Macroscopic characters of *Ficus benghalensis* bark. Shape – Flat or somewhat curved, Outer bark – Ashy white color, transversely and longitudinally furrowed and cracked, Inner bark – Light brown in color, Fracture – Outer granular, inner hard, fibrous, and pinkish in color, Odor – Not characteristic

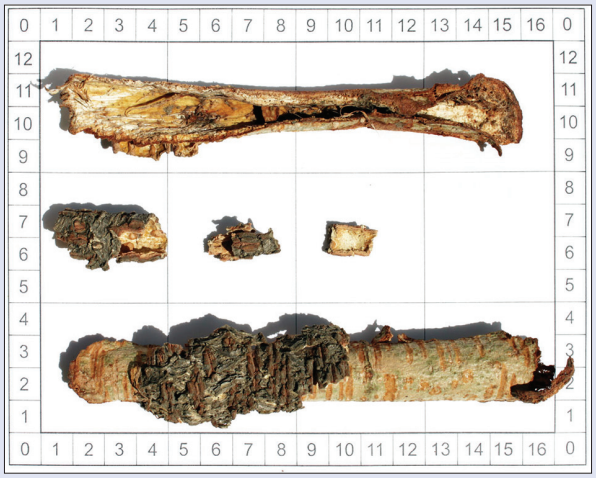

Figure 12: Macroscopic characters of *Albizia lebbeck* bark. Shape – Thick and flat pieces, Outer bark – Dark grayish brown, rough due to longitudinal and transverse cracks, Inner bark – Light yellow to gray and fibrous. Fracture – Laminated in outer region and fibrous in inner region, Odor – Characteristic

Figure 13: Macroscopic characters of *Albizia odoratissima* bark. Shape – Flat pieces, Outer bark – Dark blackish gray, Inner bark – Light yellowish brown and longitudinally striated, Fracture – Short and Fibrous, Odor – Characteristic

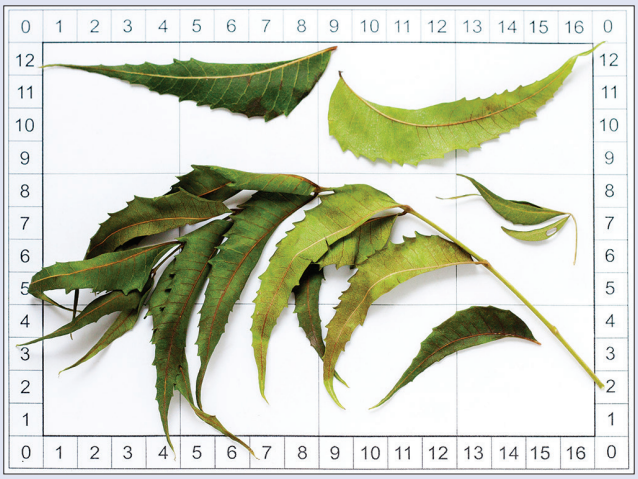

Figure 14: Macroscopic characters of *Azadirachta indica* leaf. Type of leaf – Compound, Shape – Lanceolate, Arrangement – Subopposite or alternate, Apex – Acute, Margin – Serrate, Venation – Reticulate, Base – Oblique, Texture – Glabrous, Color – Slightly yellow green, Odor – Indistinct

information fed into them. Hence, there are zero chances of error using this technology.[1]

MATERIALS AND METHODS

Selection of barks and leaves

About 53 barks and 46 leaves were selected from seven volumes of Ayurvedic Pharmacopoeia of India to study powder microscopy. Fresh bark and leaf samples were self-collected from the National Institute of Science Communication and Information Resources Pusa campus and Botanical Garden, Noida. These samples were identified by Dr. Sunita Garg, Scientist G.

Preparation of database

The selected barks and leaves were subjected to detailed powdered microscopic identification. The specific identifying features of each bark

Table 4: Represents specific distinguishing characters of barks of *Albizia lebbeck* and *Albizia odoratissima*

Features	*Albizia lebbeck*	*Albizia odoratissima*
Common name	Shiris[29-31]	Kala Shiris[32]
Calcium oxalate crystals	Prismatic [Figure 26]	Rosette [Figure 27]
Stone cells		
Occurrence	NA	Isolated
		Group
Walls	NA	Thin walled
Lumen	NA	Wide
Shape	NA	Oval [Figure 28]
Cork	Oval	Thick walled
	Rounded [Figure 29]	Polygonal
		Pentagonal [Figure 30]

NA: Not available

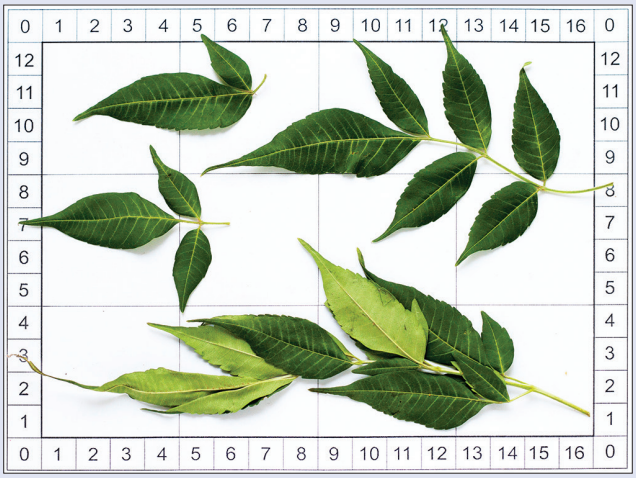

Figure 15: Macroscopic characters of *Melia azedarach* leaf. Type of leaf – Bipinnate, Shape –ovate to oblong lanceolate, Arrangement – More or less opposite, Apex – Acuminate, Margin –Entire to variously serrate, Venation – Reticulate, Base – Slightly unequilateral, acute, or rounded, Texture – Glabrescent, Color – Dark green, Odor – Pungent odor when crushed

Figure 16: Macroscopic characters of *Indigofera tinctoria* leaf. Type of leaf – Compound, Shape – Oblong to oblanceolate, Arrangement – Alternate, Apex – Rounded tip, Margin – Entire, Base – Rounded, Texture – Velvety, Color – Pale green to greenish black, Odor – No characteristic odor

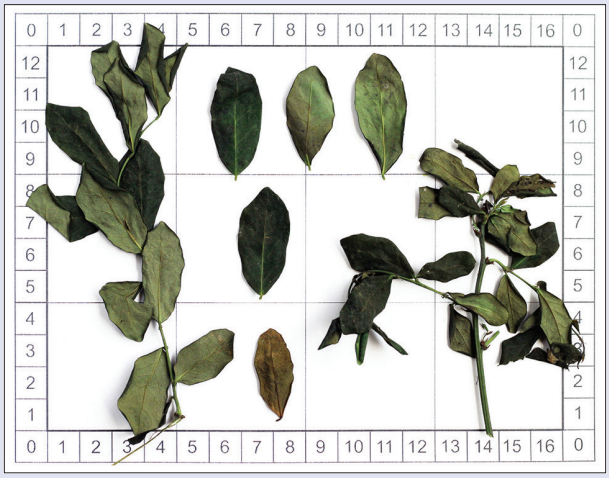

Figure 17: Macroscopic characters of *Indigofera arrecta* leaf. Type of leaf – Bipinnate or tripinnate, Shape – Oblong, Arrangement – Opposite, Apex and base – Acute, Margin – Entire, Color – Dark greenish black

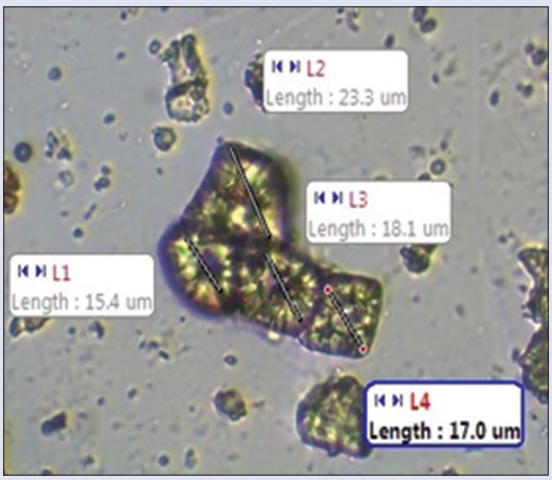

Figure 18: Stone cells present in *Ficus lacor* bark powder. Abundant stone cells with size ranging from 15-25 µm in length

Table 5: Represents specific distinguishing characters of leaves of *Azadirachta indica* and *Melia azedarach*

Features	*Azadirachta indica*	*Melia azedarach*
Common name	Neem[33-36]	Bakayana[37]
Epidermis	Straight Anticlinical	Wavy
Trichomes	Simple Covering Warty [Figure 31]	Glandular [Figure 32] Stellate [Figure 33]
Stomata	Anomocytic Actinocytic [Figure 34]	Anomocytic [Figure 35]
Calcium oxalate crystals	Prismatic [Figure 36]	Needles [Figure 37]

and leaf were studied along with few selected adulterants and substitutes. These features of 100 plants were uploaded in the software tool for identity of powdered herbals through analytical microscopy (TIPHAM). The powder microscopy of bark samples of four *Ficus* species, that is, *Ficus lacor*, *Ficus racemosa*, *Ficus religiosa*, and *Ficus benghalensis* along with *Albizia lebbeck* and *Albizia odoratissima* and leaves of *Azadirachta indica*, *Melia azedarach*, *Indigofera tinctoria*, and *Indigofera arrecta* is discussed in the result section as an example.

Processing of crude drug

Standard operating procedure for preparation of slides of powdered bark and leaf samples was prepared with varying grinding techniques – industrial and laboratory blade grinding. Five samples were procured from industry [Figure 1] and microscopical features were compared using Motic microscope among various samples of these commonly used herbal drugs. It was concluded that grinding technique does not affect significantly probability of various microscopical features. The microscopical features were found to be stable and specific, which can be used to determine the botanical identity of the drug.[21]

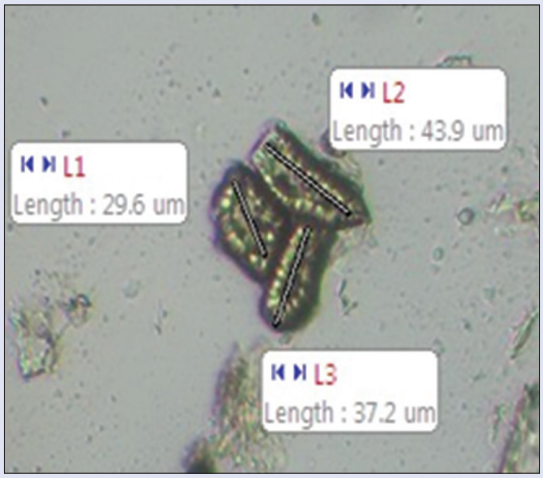

Figure 19: Stone cells present in *Ficus lacor* bark powder. Abundant stone cells with size ranging from 25-45 µm in length

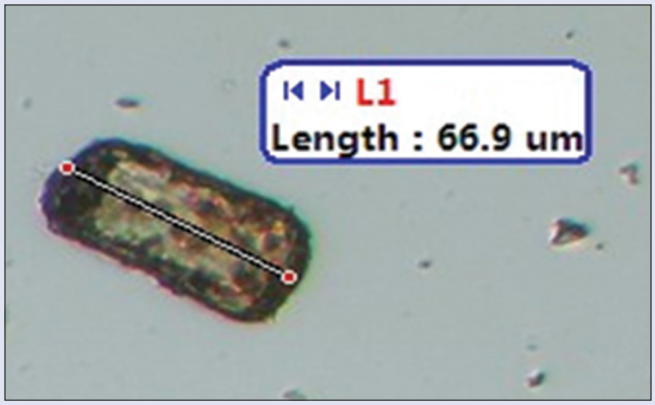

Figure 20: Stone cells present in *Ficus racemosa* bark powder. Stone cells larger in size than *Ficus lacor*, that is, greater 60 µm in length

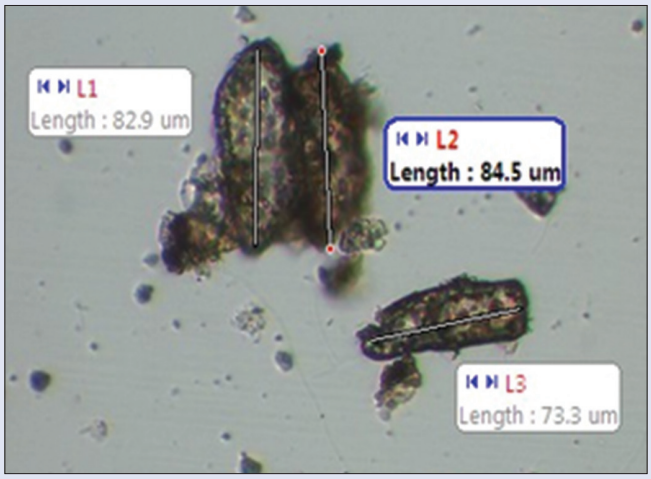

Figure 21: Stone cells present in *Ficus racemosa* bark powder. Stone cells larger in size than *Ficus lacor*, that is, greater 60 µm in length

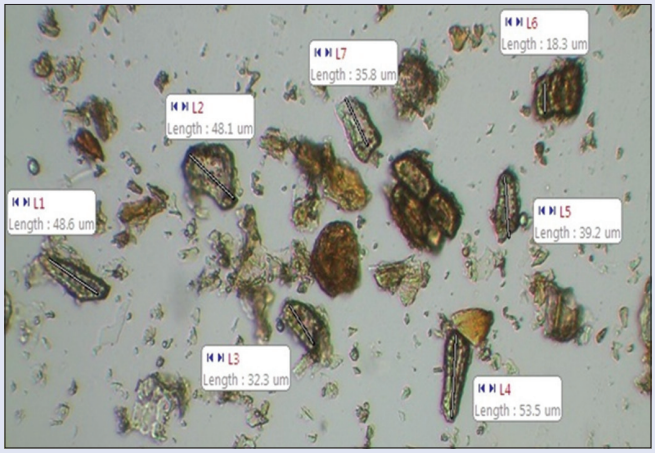

Figure 22: Stone cells present in *Ficus religiosa* bark powder. Few stone cells ranging 18–73 µm in length

Table 6: Specific distinguishing characters of leaves of *Azadirachta indica* and *Melia azedarach*

Features	*Indigofera tinctoria*	*Indigofera arrecta*
Common name	Nili[38]	Bengal indigo[39]
Trichomes	Oval head Multicellular [Figure 38]	Simple Unicellular [Figure 39]
Calcium oxalate crystals	NA	Prismatic [Figure 40]

NA: Not available

Standard operating procedure for preparation of slides

Two types of slides were prepared for the visualization of microscopical features present in a bark. The slide preparation method was optimized to determine the dilution of powdered bark and leaf in water to visualize common as well as distinguishing characters.

Slide I: A 500 mg of moderately fine (44/85) and fine (85) powdered material was soaked overnight in 10 ml of water (1:20) for 24 h. Subsequently, the contents were poured in a Petri plate and slide was prepared by mounting the contents on a clean and dried slide with a brush and observed under Motic microscope moticam 3.0 MP, AE 2000. Most of the features were visible except stone cells and sclereids, which require treatment by oxidizing agent.

Slide II: The slide was prepared by potassium chlorate treatment which is used as an oxidizing agent used for disruption of stone cells and sclereids. However, calcium oxalate crystals and starch grains are destroyed using this method. A 200 mg of powdered material was boiled with 5 ml of 50% nitric acid. To this, added a pinch ~100–150 mg of potassium chlorate. The contents in a Petri plate are poured after the effervescence ceases. The contents were mounted on a clean and dried glass slide with the help of a brush; observed under Motic microscope.

Development of parameters to study powder microscopy of crude drugs

A bark powder is identified microscopically by various features such as stone cells, sclereids, calcium oxalate crystals, medullary rays, fibers, starch grains, and cork. Bark powder cannot be merely authenticated by observing the presence or absence of these features as these are common in all barks. The variation lies in the size and shape of the stone cells and sclereids, their occurrence, and type of wall and lumen. The program TIPHAM is developed keeping in mind the specificity of these parameters for a particular bark. Similar case stands for authentication of the leaf

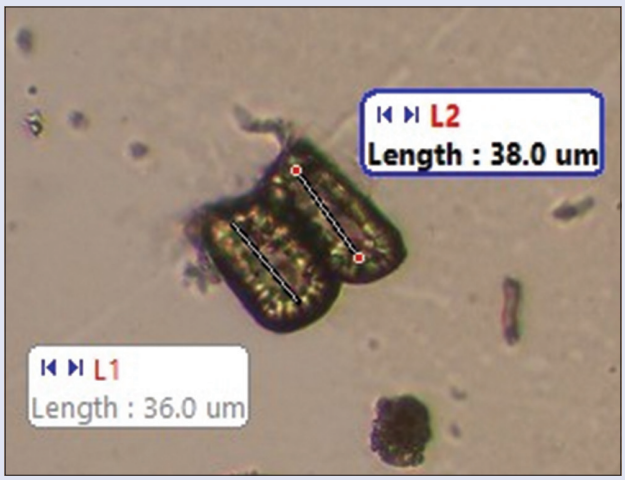

Figure 23: Stone cells present in *Ficus benghalensis* bark powder. Isolated or group of stone cells about < 50 μm in length

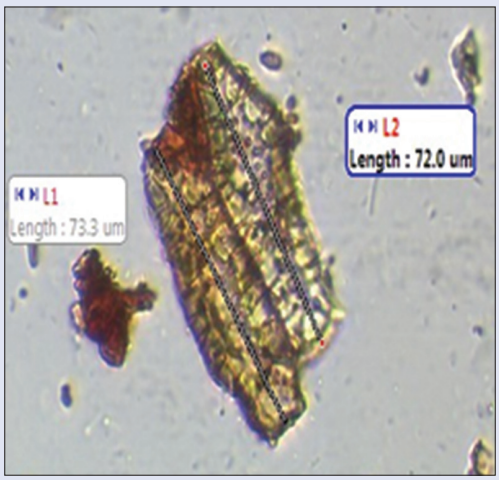

Figure 24: Sclereids present in *Ficus religiosa* bark powder. Abundantly present < 100 μm in length

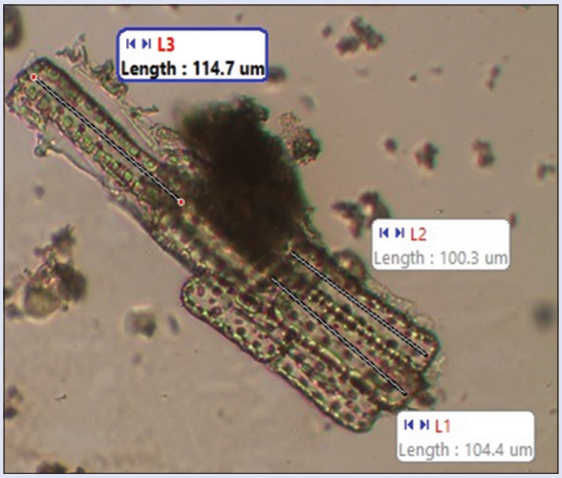

Figure 25: Sclereids present in *Ficus benghalensis* bark powder. Few in number > 100 μm in length

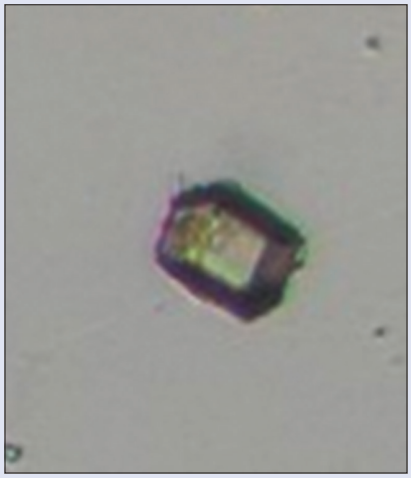

Figure 26: Prismatic crystals present in *Albizia lebbeck* bark powder. Prismatic crystals of calcium oxalate observed in *Albizia lebbeck* bark powder

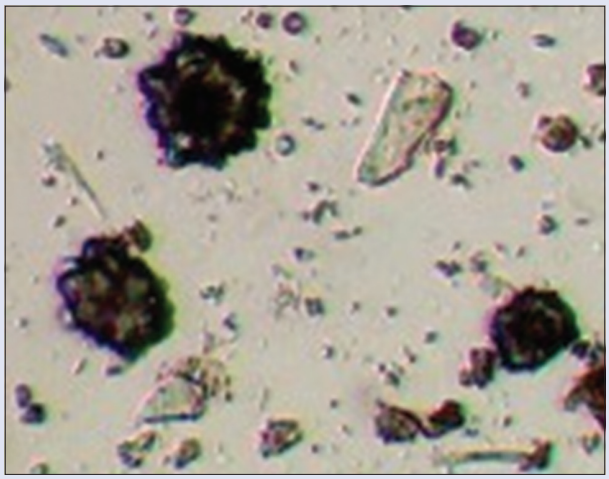

Figure 27: Rosette crystals present in *Albizia odoratissima* bark powder. Rosette crystals of calcium oxalate which is a distinguishing character from *Albizia lebbeck* bark powder

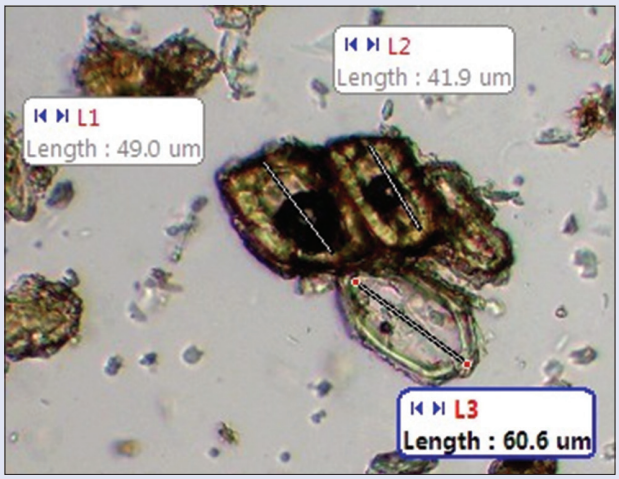

Figure 28: Stone cells present in *Albizia odoratissima* bark powder. Isolated or group of oval stone cells which are found to be absent in *Albizia lebbeck* bark powder

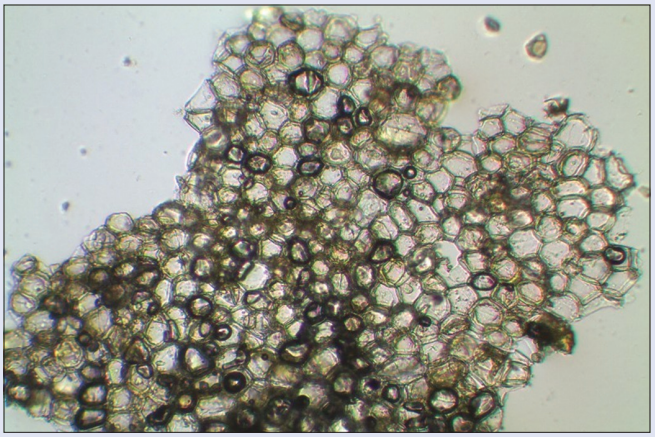

Figure 29: Cork cells present in *Albizia lebbeck* bark powder. Oval-to-rounded cells due to disintegration of parenchymatous cells which is a characteristic feature of *Albizia lebbeck* bark powder cavities in cork

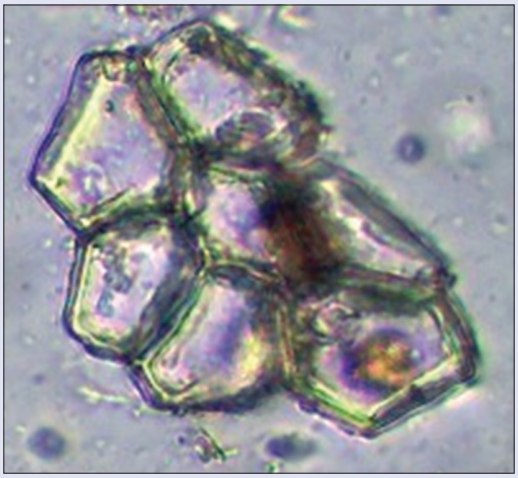

Figure 30: Cork cells present in *Albizia odoratissima* bark powder. Polygonal-to-pentagonal cork cells

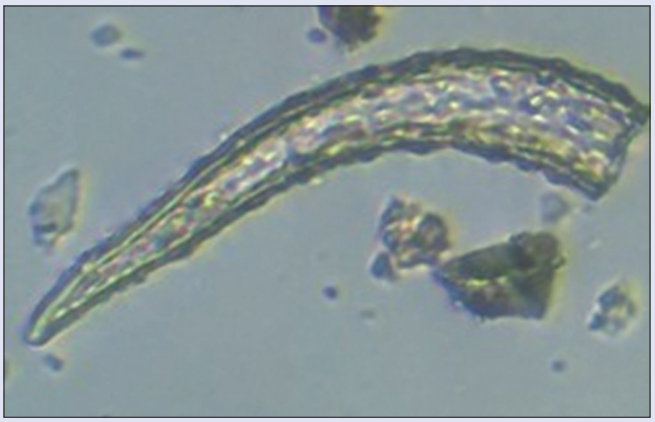

Figure 31: Trichomes present in *Azadirachta indica* leaf powder. Simple, covering, and warty trichomes

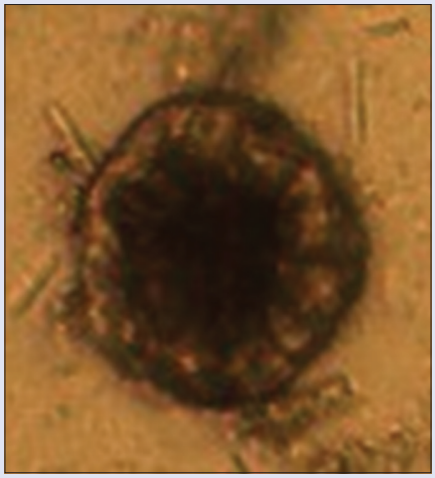

Figure 32: Trichomes present in *Melia azedarach* leaf powder. Glandular trichomes present which is a distinguishing character from *Azadirachta indica* leaf powder

Figure 33: Trichomes present in *Melia azedarach* leaf powder. Stellate trichomes present which is a distinguishing character from *Azadirachta indica* leaf powder

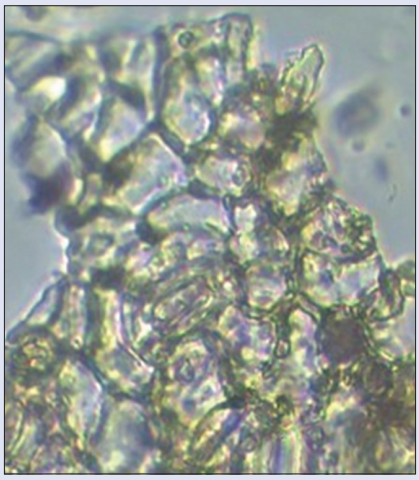

Figure 34: Stomata present in *Azadirachta indica* leaf powder. Anomocytic and actinocytic stomata present with straight and anticlinical epidermal walls

powder. The powder microscopy of bark was studied based on specific identifying features and their subfeatures as shown in Table 1. The

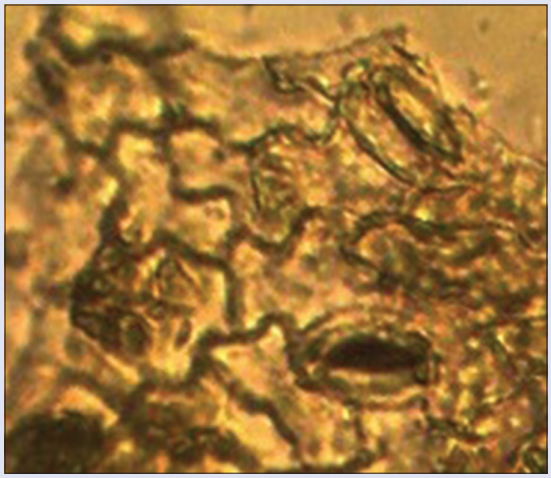

Figure 35: Stomata present in *Melia azedarach* leaf powder. Anomocytic stomata present with wavy epidermal walls

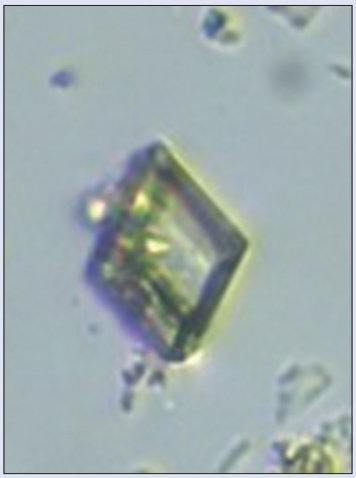

Figure 36: Calcium oxalate crystals present in *Azadirachta indica* leaf powder. Prismatic crystals of calcium oxalate

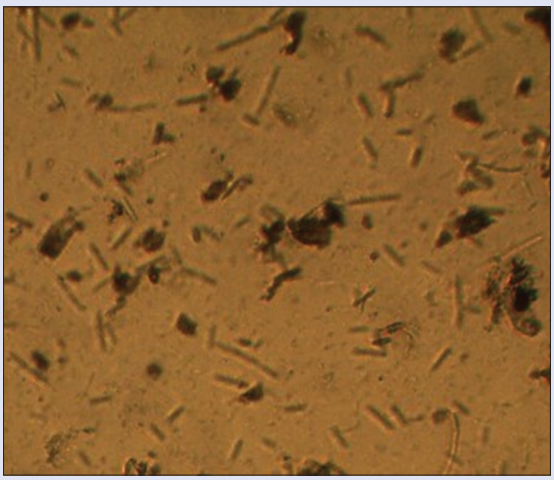

Figure 37: Calcium oxalate crystals present in *Melia azedarach* leaf powder. Needle-shaped crystals of calcium oxalate

powder microscopy of leaves was studied based on following parameters as shown in Table 2.

Development of tool for identity of powdered herbals through analytical microscopy

TIPHAM was based on Microaided identification-1979 (MICROAID). The program MICROAID is available in the UK which contains databank of 174 powdered vegetable drugs.[22,23] Such software is not available in India. MICROAID consists of a common program which contains all the features of a medicinal plant irrespective of whether it is a bark, leaf, seed, or fruit [Figure 2], whereas in TIPHAM features have been segregated individually for the leaf and bark.

Our group has developed IT tool software on a pilot scale for identification of crude drugs. Database has been generated for about 100 crude drugs. Powdered samples were given to different volunteer students to check validity and application of software. Desirable results were obtained, but it needs to be further supported by feeding more data that can be achieved by working on other plant parts as well.

Flow chart for tool for identity of powdered herbals through analytical microscopy

The web address (www.tipham.com) enables the user to visit the webpage. This webpage will require the user to fill the login id and password to use the software [Figure 3]. Subsequently, user will be taken to the homepage of the tool [Figure 4]. Once the user selects the "Home" option, templates for both bark and leaf will appear [Figures 5-7]. The specific identifying characters are entered in the bark or leaf template and click "Search." Software will search for that particular bark or leaf powder which contains similar features as entered from the database.

Experimental procedure

All the chemicals used in the experiments were of analytical grade. Potassium chlorate was procured from Sigma-Aldrich. Potassium chlorate and 50% nitric acid form a liquid known as Schulze's maceration fluid and is used to disintegrate hard woody substances such as sclereids and stone cells. This fluid is a powerful oxidizing agent and it rapidly oxidizes and removes lignin from vegetable tissues.[24] Two types of slides were prepared – overnight soaking with water for 24 h and other by treatment with potassium chlorate.

RESULTS

Microscopy on powdered samples of 54 barks and 46 leaves has been performed along with few selected adulterants and allied species. The given below is an example of powder microscopy of four bark species of *Ficus* and two species of *Albizia* along with leaves of *A. indica*, *M. azedarach*, *I. tinctoria*, and *I. arrecta*. These samples have been analyzed for specific distinguishing features. Figures 8-13 represent self-collected samples of barks of *F. lacor*, *F. racemosa*, *F. religiosa*, *F. benghalensis*, *A. lebbeck*, and *A. odoratissima*, respectively, whereas Figures 14-17 represent self-collected samples of leaves of *A. indica*, *M. azedarach*, *I. tinctoria*, and *I. arrecta*, respectively. Tables 3 and 4 represents specific distinguishing characters of barks of four species of *Ficus* and *A. lebbeck* and *A. odoratissima*, respectively, whereas Tables 5 and 6 indicate specific distinguishing characters of leaves of *A. indica*, *M. azedarach*, *and I. tinctoria*, *I. arrecta*, respectively. These specific identifying characters are entered in the bark template of TIPHAM and click "search." Software will search for that particular bark powder which possesses these features from the database. Hence, a bark or a leaf powder can be identified within seconds.

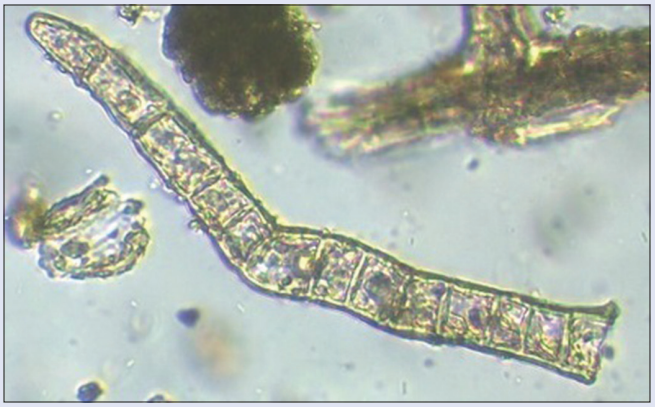

Figure 38: Trichomes present in *Indigofera tinctoria* leaf powder. Abundant multicellular trichomes with oval head

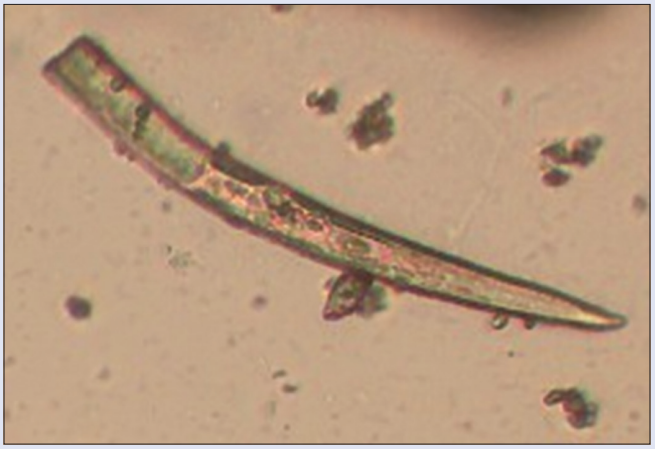

Figure 39: Trichomes present in *Indigofera arrecta* leaf powder. Simple and unicellular trichomes

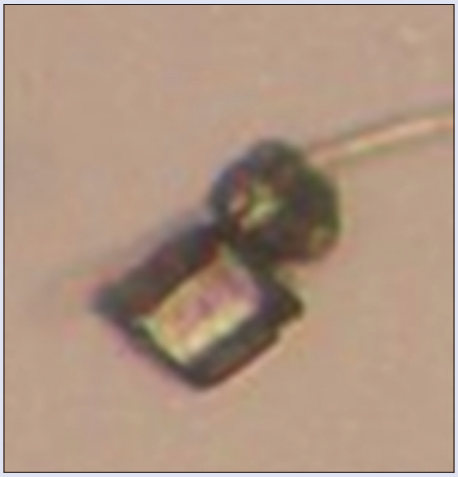

Figure 40: Calcium oxalate crystals in *Indigofera arrecta* leaf powder. Prismatic crystals of calcium oxalate which were not observed in *Indigofera tinctoria* leaf powder

DISCUSSION

The first step for quality control and authentication of any herbal drug is to study its morphology followed by its anatomy or microscopy. Powdering of crude drugs in industries is a dust generating process. Ayurvedic herbal industries are also shifting toward good manufacturing practices. Therefore, industries are willing to purchase grounded herbs, which make it absolutely necessary to check its botanical identity at first stage. This software is beneficial from industrial standpoint because most of the herbal drugs supplied today are in powder form. Hence, there are more chances of adulteration as it is very easy to spoil a drug in powdered state. Industry emphasizes for powder microscopy as it is effortless. There is no need to cut sections and minimum equipment and expertise required. This software will facilitate to do the same. The botanical identity of any plant can be confirmed within seconds. Moreover, this software will be beneficial for pharmacopoeial laboratories.

Different bark powders are more or less similar in appearance. Based on the powder microscopic features, the bark powders of four *Ficus* species can be distinguished. *F. lacor* showed wide range of stone cells in terms of size and shape as compared to other three species of *Ficus*, whereas sclereids are found to be absent in *F. lacor* and *F. racemosa* but abundant in *F. religiosa* and few in *F. benghalensis*. *A. lebbeck* bark powder showed prismatic crystals of calcium oxalate, devoid of stone cells, and contains cork cells possessing oval or rounded cavitations which is the most distinguishing character for this bark powder. On the other hand, *A. odoratissima* contains rosette calcium oxalate crystals, oval stone cells, and polygonal-to-pentagonal cork cells.

A. indica leaf powder showed straight epidermal walls embedded with anomocytic and actinocytic stomata; simple, covering, and warty trichomes along with prismatic crystals of calcium oxalate, whereas *M. azedarach* leaf powder showed wavy epidermal walls embedded with actinocytic stomata; glandular and stellate trichomes with needle-shaped crystals of calcium oxalate. *I. tinctoria* leaf powder revealed the presence of oval head multicellular trichomes, whereas *I. arrecta* possess simple unicellular trichomes.

These specific features when entered in the TIPHAM software, the tool will identify these distinguishing characters and reveal the botanical identity of that particular bark or leaf powder.

CONCLUSION

Powder microscopy of bark and leaves has been performed and information is fed into TIPHAM program. The databank will also comprise of information about closely related species of a plant part and adulterants making the identification more effective. This IT tool software has been developed on a pilot scale for identification of about 100 crude drugs, but it needs to be further supplemented by incorporating more data on other plant parts as well.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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