PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF TERMINALIA SPECIES WITH SPECIAL REFERANCE TO SUBSTITUTION AND ADULTERATIONS

DISSERTATION

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CERTIFICATE ON PLAGIARISM CHECK

MES ASMABI COLLEGE, KODUNGALLUR

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CERTIFICATE

This is to certify that the project report entitled **"PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF TERMINALIA SPECIES WITH SPECIAL REFERENCE TO SUBSTITUTION AND ADULTERATIONS"** Submitted by **Ms. ATHULYA O M** in partial fulfilment for the Degree of **Master of Science** in **BOTANY** of M.E.S. Asmabi College in the University of Calicut is a bonafide work carried out by her during the fourth semester of the course under the supervision and guidance of **Dr. SHEELA KARALAM B, Chief Scientist, Head Technical Service at Vaidyaratnam Ayurveda Research Institute Thaikkattussery**, during the academic period of 2024.

Dr. GIRIJA T.P
Supervising Teacher

Dr. GIRIJA T.P
Head of the Department

Examiners:

1……

2……

DECLARATION

I hereby declare that the dissertation entitled **"PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF TERMINALIA SPECIES WITH REFERENCE TO SUBSTITUTION AND ADULTERATION"** is a bonafide work for the partial fulfillment of the requirements for the award of the Degree of Master of Science in Botany under the co-guidance of **Dr. GIRIJA T.P, Head of the Research Department of BOTANY, M.E.S. Asmabi College, P. Vemballur, Thrissur.** I also declare that this work has not been submitted for the award of any other Degree/ Diploma/ Fellowship/ Associateship of any other similar title of any University or Institution and it represents the original work done by me under the supervision of **Dr. SHEELA KARALAM B, Chief Scientist, Head Technical Service at Vaidyaratnam Ayurveda Research Institute Thaikkattussery.**

Place: Kodungallur **ATHULYA O M**

Date:

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ABSTRACT

Terminalia arjuna ‘neermaruthu’ is a traditional drug in Ayurveda, widely used for cardiac problems and obesity and so on. It belongs to the family Combrataceae.

The current study is to ensure the substitution and adulteration in *Terminalia arjuna*. For this powdered bark of Terminalia arjuna ‘neermaruthu’, Terminalia elliptica ‘karimaruthu’ and *Lagerstroemia speciosa* ‘manimaruthu’ were screening for phytochemical analysis, TLC, HPTLC and Powder microscopy. The results show that *Terminalia arjuna* and *Terminalia elliptica* has similar compounds in qualitative analysis and they both show similar Rf values in TLC and also shared same peaks in the HPTLC analysis. *Lagestroemia speciosa* give different compounds in the phytochemical analysis also make difference in TLC and HPTLC analysis. In powder microscopy analysis both *Terminalia* species show similar characters and *Lagestroemia speciosa* show some different characters. This study reveals the chance of substitution of *T. arjuna* by *T. elliptica* and adultered by *L. speciosa*.

KEYWORDS: *Terminalia arjuna, Terminalia elliptica, Lagestroemia speciosa,* TLC, HPTLC, Powder microscopy

1. INTRODUCTION

Man has always depended on plants diversity for his many needs including food, clothing, shelter and medicine. There has been growing trend towards natural medicine and the use of dietary supplements for modern health care People throughout the world becoming increasingly dissatisfied with the possible side effects, lack of noticeable long-term results and high cost associated with allopathic drugs. Herbal products have provided a more natural and often more effective alternative.

The use of medicinal plants as a source of medicinal relief from illness can be traced back over five millennia to written documents of the early civilizations of China, India, and Near east, but it is doubtless an art as old as mankind. The wide spread use of herbal remedies and healthcare preparations, as those described in ancient texts such as Vedas and Bible and obtained from traditional knowledge has been traced to the occurrence of natural product with medicinal properties. Traditional herbal medicines from an important part of the healthcare system. Ayurveda, supposed to be the oldest medicinal system in the world, provides potential leads to find active and therapeutically useful compounds from plants. Plants produce a great diversity of substances that could be of therapeutic significance in many areas of medicine. These therapeutic benefits of medicinal plants are often attributed to their antioxidant properties (Mahesh and Sathish, 2008).

According to World Health Organization (WHO) more than 80% of the world's populations relies on traditional medicine contain a wide range of substances that can be used to treat chronic as well as other infectious diseases. The 30 World Health Assembly adopted a resolution urging interested government to utilize their traditional system of medicine with regulations suited to their national health care systems. Utilization of plants for medicinal purpose has been documented long back in ancient literature. However, organized studies in this direction were initiated in 1956 and off late such studies are gaining recognition and popularity due to loss of traditional knowledge: and declining plant population (Verma *et al*., 2012)

Today, most pharmaceutical are not of plant origin but are new synthetic creations. Recently there is a renewed interest in the discovery of phytochemicals. This renewed interest has already developed many chemicals, which still have to be discovered. New modern laboratory techniques have made it easier to discover and identify new

phytochemicals. Moreover, the identification of active compounds in medicinal plants helps to develop new synthetic drugs based on them. This leads to reduce over exploitation of plants helping to minimize the genetic erosion. The isolated active compounds of the plants are secondary metabolites chemical compound that occur naturally in plant with no nutritional value to human life. These active compounds are generally called phytochemicals, play protective role in plants. Each chemical labeled phytochemical works in different ways, not all are same for the human and not all are come from the same plants.

The plant is a biosynthetic laboratory, not only for the primary metabolic compounds like carbohydrate but also for the multitude of compounds like glycosides, alkaloids, terpenoids etc: these exert therapeutic and physiological effects. The compounds that are responsible for medicinal property of the drugs are usually secondary metabolites, A systematic study of a crude drug is embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant material is subjected to phytochemical screening for the detection of various plant constituents (Mahesh and Satish, 2008)

Phytochemicals are simply the chemical derived from the plants. These phytochemicals are also known as plant metabolites. The medicinal value of the most plants lies in some of these chemical substances that they produce which has a physiological action in human body. There are two types of metabolites in plants, primary metabolites which are essential for the vital processes and secondary metabolites which are not essential for the survival but helps plants for other activities such as disease resistance, insect repellence etc. it is well known that plants produce these chemicals to protect themselves but recent researches demonstrate that they can also protect human against diseases.

The phytochemical analysis of plant reveals the presence of various primary and secondary metabolites. Qualitative analysis helps to reveal the type of such compounds. The plants are subjected to extraction procedures to harness the various active metabolic compounds. It is done by different polar and nonpolar solvents (hexane, petroleum ether, benzene, chloroform, ethyl acetate, acetone, butanol, methanol etc) by which all the polar and nonpolar constituents in the plant material can be isolated. The concentrated extract can be tested for the presence of various compounds through standard procedures based on various chemical reactions. Many technique that follows qualitative phytochemical screening include quantitative analysis through chromatography, spectrophotometry,

spectroscopy etc. the biological activities of the extract can be studied through different assay like, Antioxidant assay, antimicrobial assay, Antiproliferative assay etc.

For the project work, plants selected are *Terminalia arjuna* (Roxb.) 'Neermaruthu', Terminalia elliptica Wild. 'karimaruthu', Largerstroemia speciosa (L.) Pers. 'manimaruthu'. Their barks have so many medicinal propertiesand they show resemblance to each other. So they are adultered and substituted by each other. They are collected from various places. Terminalia arjuna is collected from Kodungallur, *Terminalia elliptica* is collected from KFRI Peechi and *Largestroemia speciosa* collected from Kodungallur.

1.1 TERMINALIA ARJUNA

T. arjuna is a tree of the genus *Terminalia*. It is commonly known as arjuna tree. *T. arjuna* grows to about 20-25metres tall. It has a buttressed trunk and forms a wide canopy at the crown, from which branches drop downwards. It has oblong, conical leaves which are green on the top and brown below; Smooth grey bark; It has pale yellow flowers which appear between march and June; Its glabrous, 2.5 to 5cm fibrous woody fruit, divided into five wings, appears between September and November.

The *arjuna* seen across the Indian subcontinent and usually found growing on river banks or near dry river beds in Uttar Pradesh, West Bengal, Bihar, Maharashtra, Madhya Pradesh, Odisha and South and Central India.

Fig. 1: *Terminalia arjuna* tree and its inflorescence

1.1 TERMINALIA ELLIPTICA

Deciduous tree growing to 30m tall, with a trunk diameter of 1m. Deeply tap rooted. Bark is rough and black in colour. The leaves are opposite to subopposite, exstipulate. The fruit is ovoid with five wings not extending beyond the fruit apex. The bark is fire resistant. Water stored in the stem is often tapped and used as a source of potable water in the summer by forest folk.

Fig. 2: *Terminalia elliptica* and its inflorescence

1.2 LAGESTROEMIA SPECIOSA

It is a deciduous tree with bright pink to purple flowers. It is native to tropical southern Asia. Bark creamy brown to light grey, smooth and peeling in papery flakes. Inner bark pale brown and fibrous. Simple, opposite broadly ovate to oblong with prominent abaxial veins.

Fig. 3: *Lagestroemia speciosa* and its inflorescence

SIGNIFICANCE OF THE STUDY

Understand the phytochemical and pharmacognostical profiling of 3 stembarks of different plant species with reference to their substitution and adulteration.

OBJECTIVES

- To check the possibilities of drug substitution for *T. arjuna*
- To prepare the monograph of the stembark of 3 species
- To compare the phytochemical contents of 3 stem bark
- To check TLC and HPTLC of stem bark

REVIEW OF LITERATURE

A pharmacognostical and phytochemical evaluation of *Terminalia arjuna* bark shows Macroscopic, microscopic, and phytochemical analysis. This Identified phytochemicals, including tannins, flavonoids, and triterpenoids. It Validated traditional uses and potential therapeutic applications. (Krishnamurthy *et al*., 2017)

Dhingra *et al*., (2018) conducted a study on pharmacology and therapeutic applications of *Terminalia Arjuna's* and it gives its pharmacological activities, including cardio protection and antioxidant effects. And he highlighted potential therapeutic uses and need for further research.

Rao *et al*., (2012) conducted a Pharmacognostical and phytochemical evaluation of Terminalia tomentosa bark. It includes Macroscopic, microscopic, and phytochemical analysis. It Validated traditional uses and potential therapeutic applications.

A Pharmacognostical and phytochemical evaluation of *Terminalia chebula* fruit with referenced to it Macroscopic, microscopic, and phytochemical analysis. It Identified phytochemicals, including tannins and triterpenoids. It gives the idea of Validated traditional uses and potential therapeutic applications. (Singh *et al*., (2019)

Srivastava *et al*., (2015) contacted a study on the topic Development of a method for *Terminalia arjuna* bark's pharmacognostical evaluation. Established a standardized method for quality control. It Contributed to standardization and quality control of herbal medicine.

Singh *et al*., (2022) contacted a study on Research question: Evaluation of Terminalia Arjuna's cardioprotective potential. In vivo cardio protection assays is used as methodology. The study Showed significant cardioprotective activity of *Terminalia arjuna*.

A study on Phytochemical analysis and antioxidant activity of Terminalia tomentosa leaves conducted by the Phytochemical extraction and antioxidant assays. It Identified flavonoids and tannins with antioxidant activity. It provide validated traditional uses and potential therapeutic applications. Kumar *et al*., (2022)

A study on Investigation of Lagerstroemia speciosa's anti-diabetic potential Showed significant anti-diabetic activity through In vivo anti-diabetic assays. Sharma *et al*., (2022)

Mishra *et al*., (2022) contacted a study on Evaluation of *Terminalia Arjuna's* neuroprotective potential. It showed significant neuroprotective activity and it validated traditional uses and potential therapeutic applications through In vivo neuroprotection assays.

Singh *et al*., (2022) contacted a study on Phytochemical analysis and anti-inflammatory activity of *Terminalia tomentosa* bark. It includes Phytochemical extraction and anti-inflammatory assays.

Validated traditional uses and potential therapeutic applications are get from the study of Investigation of Lagerstroemia speciosa's anti-inflammatory potential which Showed significant anti-inflammatory activity. Kumar *et al*., (2022).

Gupta *et al*., (2022) evaluated the anticancer potential of *Lagerstroemia speciosa* through In vitro anti-cancer assays showed significant anti-cancer activity.

Mishra *et al*., (2022) contacted a study on Pharmacognostical and phytochemical evaluation of *Terminalia chebula* bark. It included Macroscopic, microscopic, and phytochemical analysis and it validated traditional uses and potential therapeutic applications.

Review of *Terminalia* species' phytochemistry and pharmacology by Kumar *et al.* (2022) provide the phytochemical and pharmacological activities. It Highlighted potential therapeutic uses and need for further research.

Sharma *et al.* (2022) conducted a study on evaluation of *Lagerstroemia speciosa's* cardioprotective potential. This study helps to know the significant cardioprotective activity.

Pharmacognostic and forensic study of *Terminalia arjuna* bark by Dhingra *et al*., (2013) Aimed to establish standards for forensic identification of unknown plant material. The study included Microscopic macroscopic, preliminary phytochemicals screening and physico chemical evaluation.

The study provides a detailed pharmacognostic and forensic evaluation of *Terminalia arjuna* bark, which can be useful in future experimental studies and forensic identification of unknown plant material.

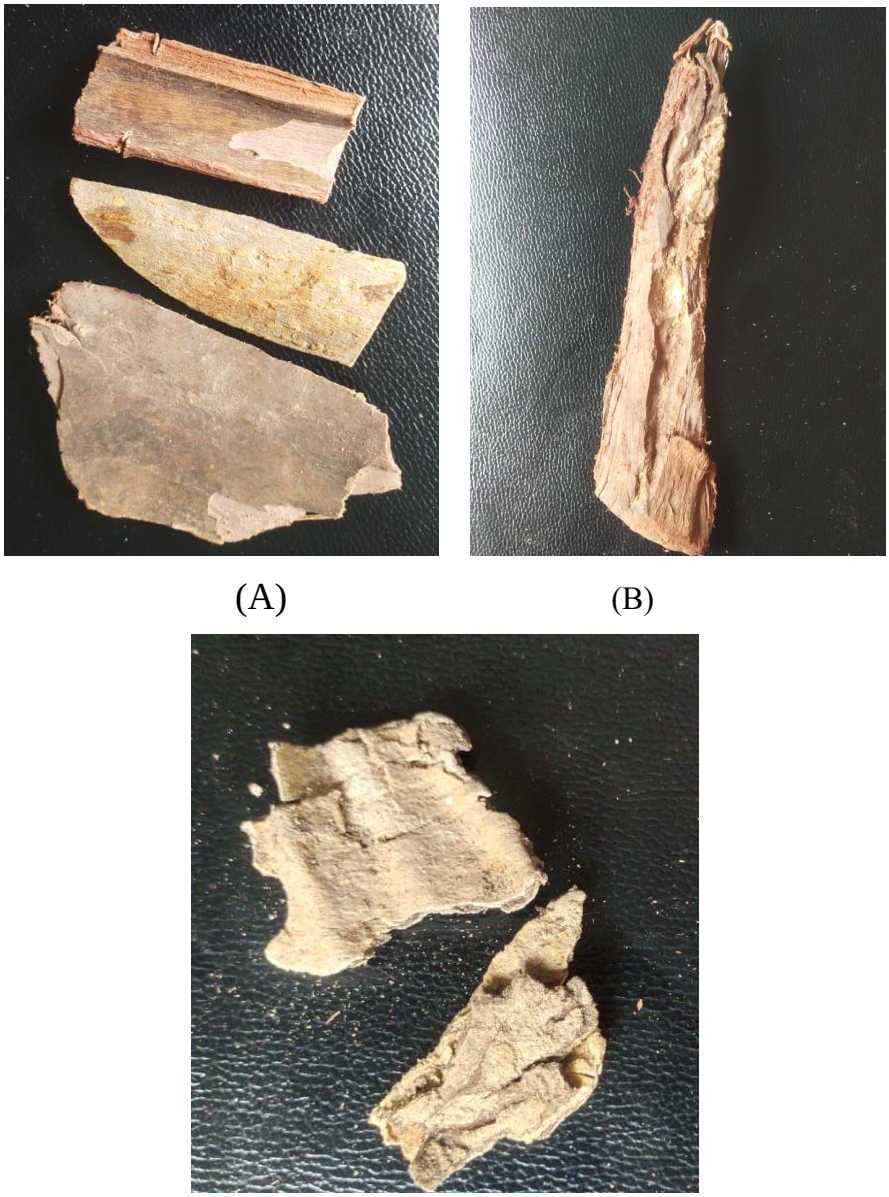
A comprehensive study on pharmacognostic, physico and phytochemical evaluation of *Terminalia arjuna* stem bark by Gunjan M Chaudhari, Regunath T Mahajan which provides a comprehensive evaluation of *T. arjuna* stem bark, including pharmacognostic, physicochemical, and phytochemical studies. The results can be used for future experimental studies and quality control of herbal formulations. It includes phytochemical screening, HPTLC fingerprint and HPLC analysis.

Singh *et al*., (2022) conducted a study on the topic investigation of *Terminalia arjuna's* anti-diabetic potential. It involves In vivo anti-diabetic assays. It helps to Validated traditional uses and potential therapeutic applications.

3. MATERIAL AND METHODS

3.1 COLLECTION OF PLANT MATERIAL

Fresh bark of *Terminalia arjuna*, *Terminalia elliptica*, and *Lagestroemia speciosa* were collected from various places in Thrissur district. The stem bark was thoroughly washed in order to remove dust. Then it keep for air dried. After that, they were pulverized to powder. The powder barks then kept in an airtight container until required for further laboratory analysis. (Fig1)



(A) (B)

(c)

Fig. 4: Barks (a) *Terminalia arjuna* (b) *Terminalia eliptica* (c) *Lagerstroemia speciosa*

3.2 EXTRACTION

15 gram each of the samples was successively extracted with 125 ml of ethanol using a sequential extraction method for 3 days. The extracts were kept under the refrigerator until the various phytochemical analyses. (Fig2)

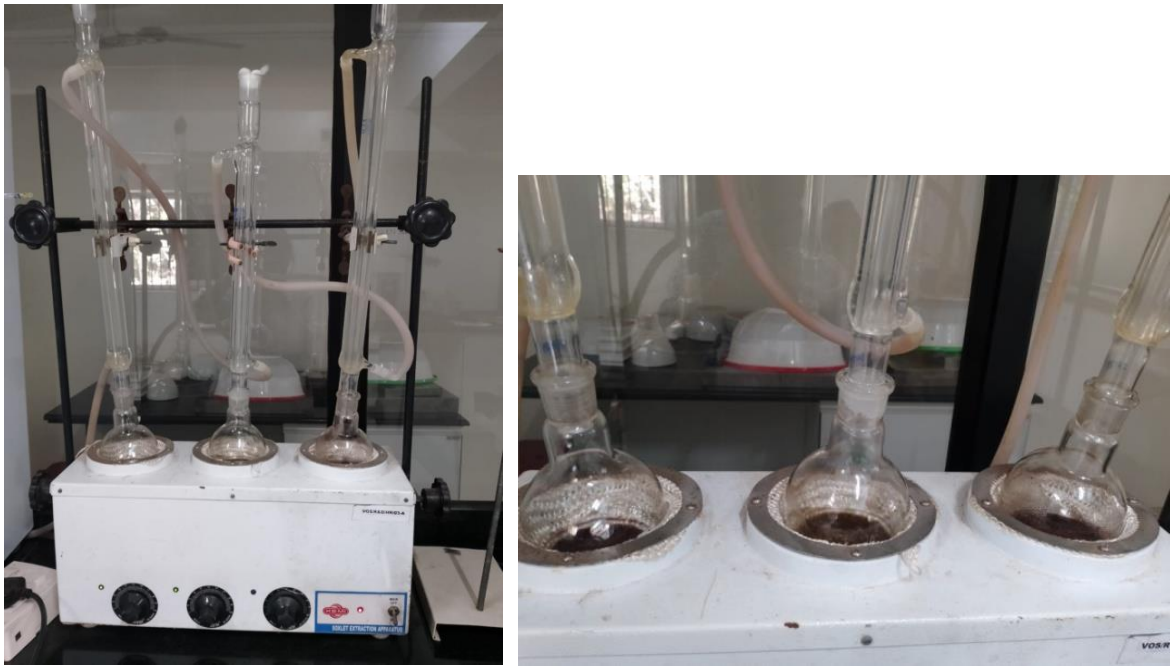


Fig. 5: Sequential extraction of samples

3.3 PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was done for the qualitative detection of the major classes of phytochemicals using standard procedure (harborne,1973). The sample extracts were screened for the presence of bioactive compounds such as alkaloids, steroids, terpenoids, saponin, flavonoids, tannin and glycosides.

3. 3.1. ANALYSIS OF PRIMARY METABOLITES

The extracts were screened for the presence of primary metabolites.

1. TEST FOR CARBOHYDRATE

Molisch's test

To one ml of the extract few drops of molisch's reagent was added and mix well. Followed by the addition of 1 ml of concentrated H_2SO_4 to form a layer below the aqueous solution. A brown ring at the junction between the H_2SO_4 and aqueous extract indicate the presence of carbohydrate.

2. TEST FOR REDUCING SUGER

Benedict test

To 1 ml of extract a few drops of benedict's solution is added and heated in a test tube for two min and allowed to cool. A brick red, dark green or dark orange colour precipitate if formed indicate the presents of reduced suger.

3.3.2 ANALYSIS OF SECONDARY METABOLITES

1. TEST FOR ALKALOIDS

Dragendroff's test

Methanolic extract was warmed with 2% H_2SO_4 for 2 minutes. It is filtered and a few drops of dragendroff's reagent were added and the red precipitate indicates the presence of alkaloids.

2. TEST FOR FLAVONOIDS

NaOH Test

To 1ml sample add 3ml of dilute NaOH, the sample turns yellow color. Add dilute HCl, if yellow color disappeares, it indicates presence of flavonoids.

3. TEST FOR TERPENOIDES

Salkowski test

The extract was mixed with 2 ml of chloroform and concentrated H2S04 (0 ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed indicate the presence of terpenoids.

4. TEST FOR SAPONIN

Forth test

A fraction of extract was vigorously shaken with water observed for persistent foam.

5. TEST FOR PHENOLS

Fecl3 Test

Add 3drops of FeC13 to 5 drops of sample solution taken in test tube. Dark green color indicates the presence of phenol.

6. TEST FOR CARDIAC GLYCOSIDES

Keller-killani test

5 ml of each methanolic extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution (FeCl3) followed by the addition of 1 ml concentrated sulfuric acid. Brown ring was formed at the interface which indicate the presence of deoxy sugar of cardenolides. A violet ring may appear beneath the brown ring. While in the acetic acid layer, a greenish ring may also form just gradually throughout the layer, indicate the presence of cardiac glycosides.

7. TEST FOR TANNINS

Ferric chloride test

About 0.5g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration indicates positive test for tannins.

8. TEST FOR PHLOBATANNIN

HCl test

Boil 1ml of extract taken in a test tube with 1%HCl.Ared precipitate indicates the positive test for phlobatannin.

9. TEST FOR DETECTION OF STEROIDS

Salkowski Test: About 2ml of extract was mixed with 3 ml of chloroform and then shaken with 2 drops of concentrated sulphuric acid. Development of reddish-brown color indicated the presence of steroids. Green coloured fluorescent layers at the bottom indicated the presence of cholesterol.

3.4. THIN LAYER CHROMATOGRAPHY (TLC)

TLC identity tests provided in the monographs include identification of the drug based on its major chemical constituents used as markers. records of fingerprint profiles using densitometric scanner and visualization of spots under UV or using a suitable spray reagent. Commercially available (E Merck) plates with uniform thickness (0.2 mm) coating of silica gel G or silica gel GF254 etc. (as mentioned under individual monographs) of uniform particle sizes (0.25 μ) on plastic. aluminium or glass supports have been used. The plastic or aluminium sheets (20 x 20 cm) can be cut using a scissor to get the required size (5 x 15 cm etc.) indicated in the procedure. However, use of handmade plates is not recommended to reduce the problems related to uniformity and reproducibility of the results. Use analytical reagent (AR) grade solvents for preparing the solvent system and whatman No. 1 paper for filtration of test solution. Application of known volumes of test and references solutions can be done using calibrated capillaries which are available commercially (CAMAG). Alternatively, samples can also be applied as narrow bands using a band applicator (CAMAG). The latter is preferred as it gives better separation of components with close Rf. values. Leave the chamber for 15 minutes for saturation after pouring the solvent system and then place the plate in the chamber.

Densitometric scans presented in the monographs have been performed on Shimadzu CS-9000 scanner. Fingerprint profile got from the outlined procedure can serve as a permanent record for comparing different batches of the sample. An estimate of the amount of marker present in the sample can also be obtained by comparing its peak area in test solut: with that of reference solution. However to know the exact amount of marker, the method given under assay should be followed.

Visualization of spots can be done either by observing the plate under UV light or by use of spray reagents (as mentioned under individual monographs). Rf. values and colours of some prominent spots along with the marker in the test solution track are given to assist identification of the drug. Rf. values are calculated as follows.

$$Rf. = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent front}}$$

3.5. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

This method is a modified form of thin layer chromatography. It is a type of planar chromatography where the separation is done by high performance layers with detection and the sample components are acquired using an advanced work-station.

HPTLC analysis was performed by CAMAG HPTLC system (Switzerland). Samples were applied using CAMAG ATS-IV on aluminum backed precoated Silica gel 60 F 254 TLC plate (Merck India). Mobile phase was standardized as toluene, ethyl acetate (9:1) for n-hexane and 8:2 for chloroform extracts and toluene: ethyl acetate Methanol: Formic acid (7:3:1:0.1) for ethanol extract. The chromatogram was developed in a saturated Twin Trough chromatographic chamber (Camag, Switzerland). The developed plate was visualized under UV at 254 nm and 366 nm and in visible light after derivatizing with Anisaldehyde sulphuric acid reagent followed by heating at 105°C for 5 minutes.

3.6 POWDER MICROSCOPIC CHARACTERIZATION OF SEEDS

Dried barks were powdered in a mill or pounded to a powder in a mortar, all powder passing through a 180 micron IS sieve is treated in this scheme of examination. Take a sufficient amount of bark powder of *Terminalia arjuna, Terminalia elliptica* and *Lagestroemia speciosa*. On each microscopic slide and add 1-2 drops of diluted Safranin. Spread the sample evenly over the slide and mount in glycerine. Observed the slides through the microscope. Repeated the procedure in 2-3 slides to get maximum characters. Transferred the images using the attached camera and software.

4. OBSERVATION

4.1. PHYTOCHEMICAL SCREENING

Ethanol extract was screened for the presence of primary and secondary metabolites using standard procedure. The observations are tabulated in Table 1.

The result showed that *Terminalia arjuna* contain carbohydrate, reducing sugar, flavonoids, glycoside, phlobotanin, terpinoides, coumarin, saponin, steroid, tannin, phenol, while *Terminalia elliptica* contain carbohydrate, reducing sugar, flavonoids, glycoside, terpinoides, coumarin, saponin, steroid, tannin, phenol, alkaloid and *Lagestroemia* contains reducing sugar flavonoids, terpinoides, glycoside, Steroid, tannin, Phenol

SL. No.	COMPOUND	TEST	TERMINALIA ARJUNA	TERMINALIA ELLIPTICA	LAGERSTROEMIA SPECIOSA
1.	Carbo hydrate	Molisch's test	+	+	++
2.	Reducing Sugar	Benedict test	++	++	-
3.	Alkaloid	Dragendroff's test	-	++	-
4.	Flavonoids	Ferric test	+	++	+++
5.	Glycoside	keller killani test	+	+	+++
6.	Phlobotanin	wagner's reagent test	-	-	+
7.	Terpenoid		++	+	+++
8.	Coumarin		+	+	-
9.	Saponin	Form test	+++	++	-
10.	Steroid	Salkowski's test	+	+	+++
11.	Tanin	NaoH test	+	+	+++
12.	Phenol	Lead test	++	++	+++

4.2 THIN LAYER CHROMATOGRAPHY

TLC is a chromatography technique that seperates components in the extract through this technique the Rf value calculated is similar in *Terminalia* species and slightly different in *Lagestroemia speciosa* .

T.arjuna	*T. elliptica*	*Lagestromia speciosa*
0.075	0.075	0.075
0.113	-	-
-	-	0.126
-	0.291	-
0.443	0.443	0.443
-	-	0.506
-	0.645	-
-	-	0.670
0.746	-	-
0.835	0.835	-
-	-	0.848
-	-	0.860
0.873	-	0.873
-	0.898	0.898
0.924	-	-
-	-	0.936
-	0.949	-

Table2: Rf values of bark chloroform extract

4.4 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

HPTLC is a powerful analytical technique that enables the separation, identification, and it can provide chromatographic fingerprints that can be visualized and stored as electronic images (Johnson *et al*., 2011). HPTLC profiles of *T. arjuna*, *T.elliptica* and *L.speciosa* developed for extracts like ethanol. The developed chromatograms were visualized and documented in various UV and visible spectrum and Rf value for each separated band were recorded.

The Rf value for choloroform seq.extracts of *T.arjuna*, *T.elliptica*, *L.speciosa* were presented in Table 3 and the HPTLC profiles were shown in Figure 6. At 254nm, major band were observed at 0.11 for *T.arjuna* and two bands a 0.10, 0.88 in *T.elliptica* and 7 bands at 0.07, 0.10, 0.12, 0.34, 0.48, 0.58, 0.59 for *L.speciosa*. few bands are shown at 366nm 0.10, 0.91 for both *T.aarjuna* and *T.elliptica*. In L.speciosa 3 bands got at 0.12, 0.33, 0.90. Common bands are got for terminalia species while *Lagestromia* not posses any common band with others

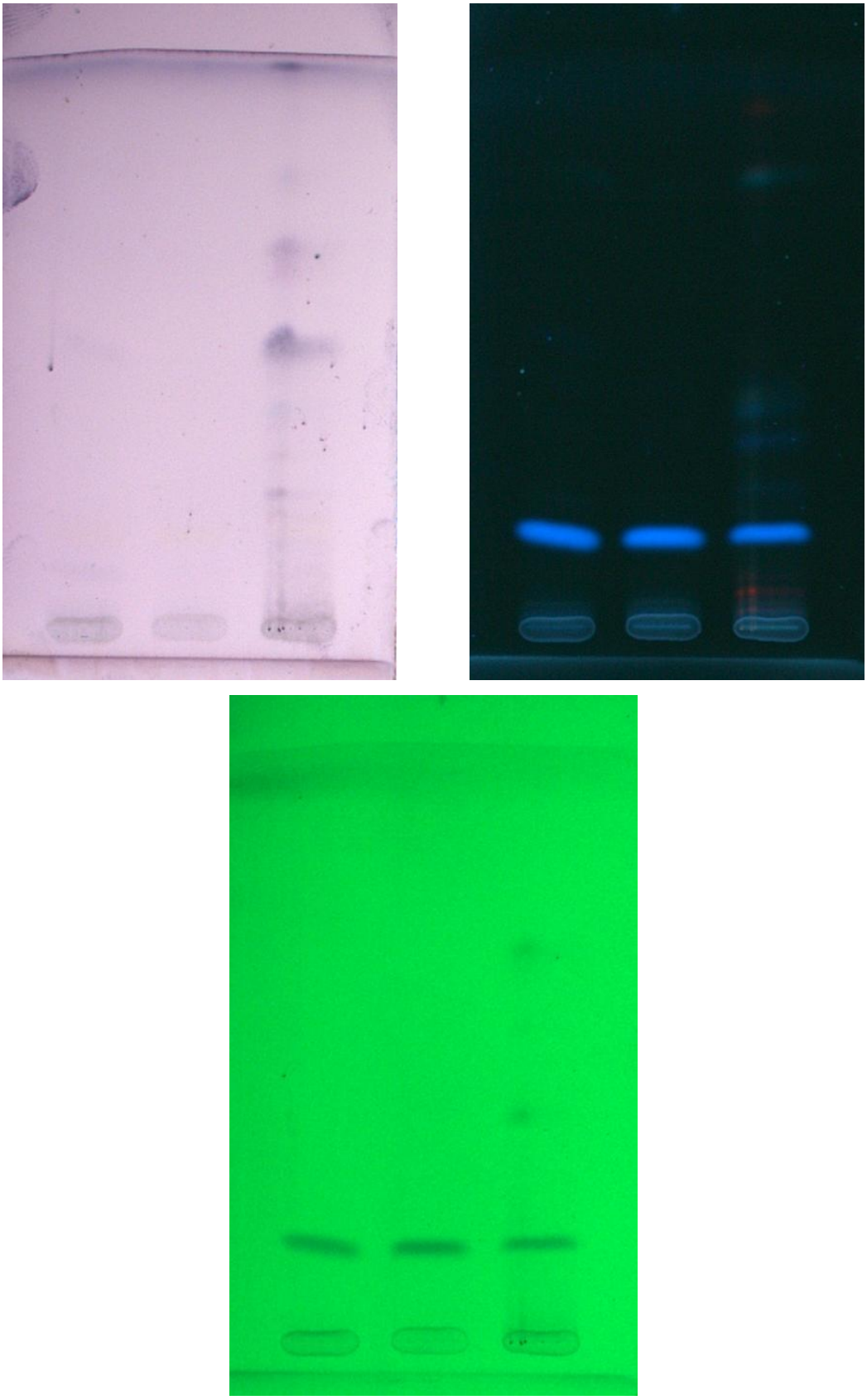
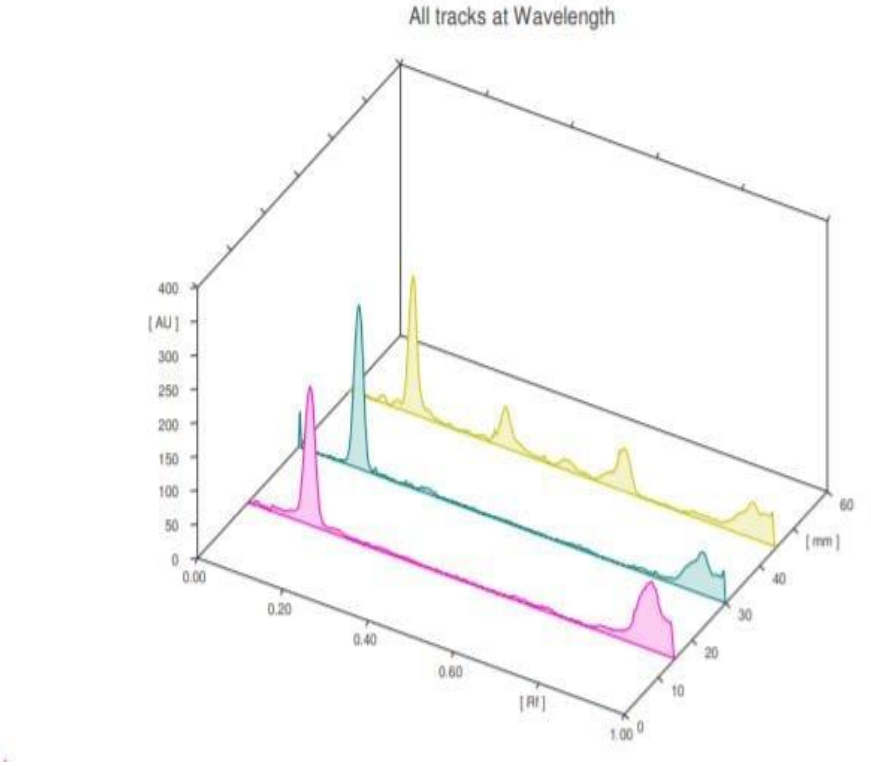


Fig 6. HPTLC analysis of 3 samples

winCATS Planar Chromatography Manager

Measurement Table

Wavelength	254
Lamp	D2 & W
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	405 V



Graph 1.HPTLC of 3 sample in 254 nm.

Measurement Table

Wavelength	366
Lamp	D2 & W
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	405 V

User : avscmpr
Tuesday, June 04, 2024 3:59:11 PM

Approved :. . . . . .
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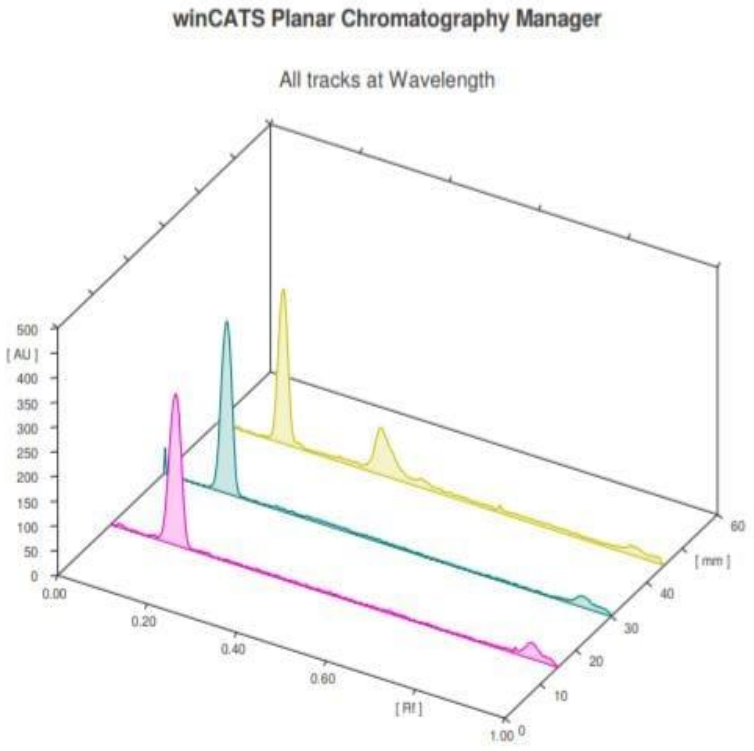


Graph 2. HPTLC of 3 samples in 366 nm.

4.4 POWDER MICROSCOPIC CHARACTERIZATION OF SEEDS

The study compared the anatomical characteristics of three barks.

CHARACTERS	T.ARJUNA	T.ELLIPTICA	L.SPECIOSA
Fibres	With narrow and wide lumen	With narrow lumen	With wide lumen
Fragments of parenchymatous cells with numerous starch grains and coloured content	present	present	present
Stone cells	Absent	Absent	Group of stone cells
Starch grains	Small starch grains	Medium sized	Large and small
Prismatic crystals	Absent	Present	Elongated type of prismatic crystals
Surface view of fragments of cortical cells	Present	Present	Present
Reddish brown coloured cell content	Present	Present	Present
Pitted stone cells	Absent	Absent	Present
Rosette crystals	Numerous number	Less in number	absent
Fragments of parencymatous cells with starch grains and oil globules	Present	Present	Present
Fragments of parenchymatous cells	Absent	Present	Present

with the starch grains and rosette crystals			
Trichomes	Absent	Absent	Aseptate trichomes are present
Group of fibres overlapped with parenchymatous cells containing starch grains	present	present	present
Reddish cell content with oil granules	absent	present	present
Crystal fibres	absent	present	present

Table 3: microscopic characteristic features of 3 barks

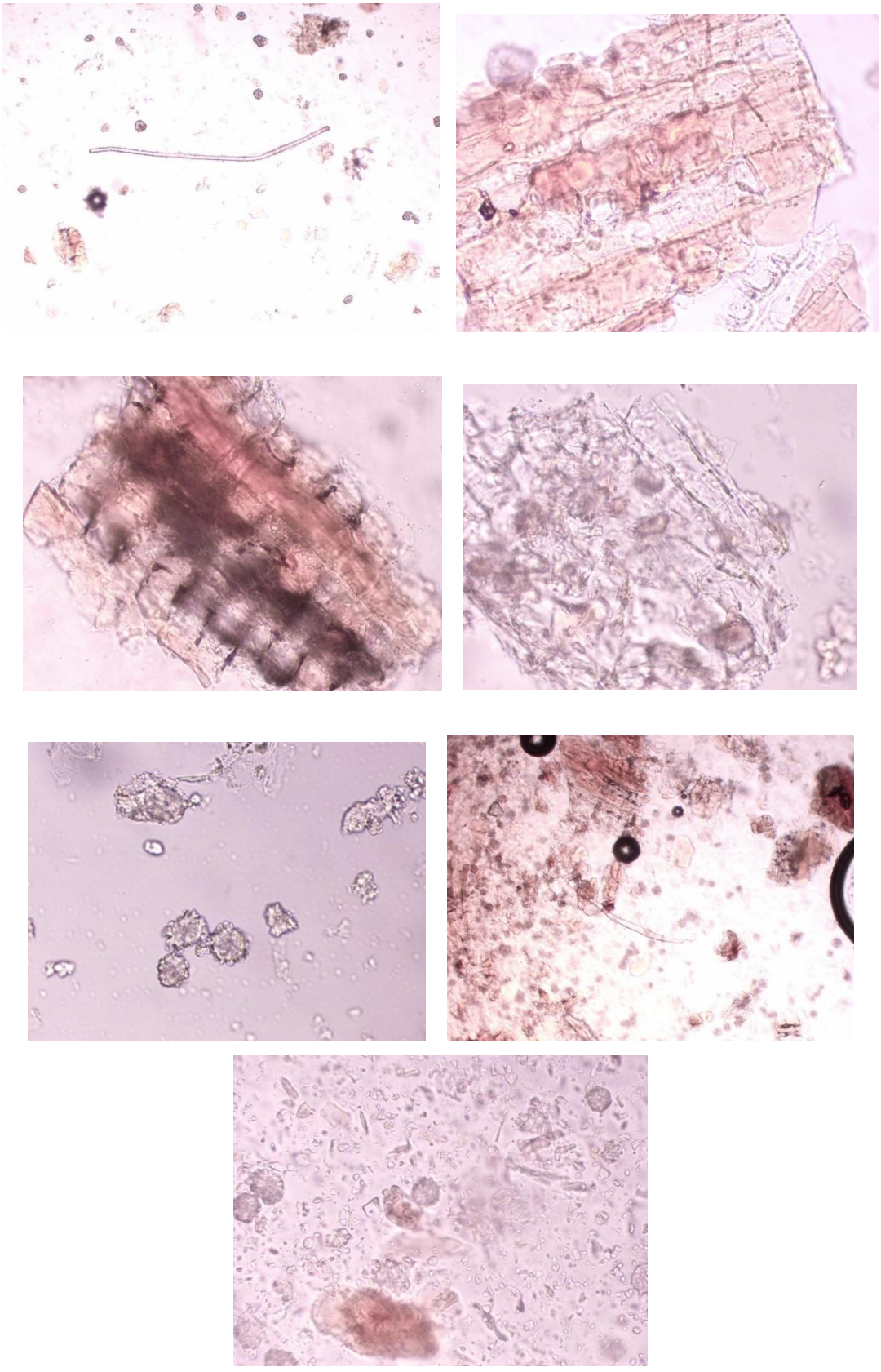
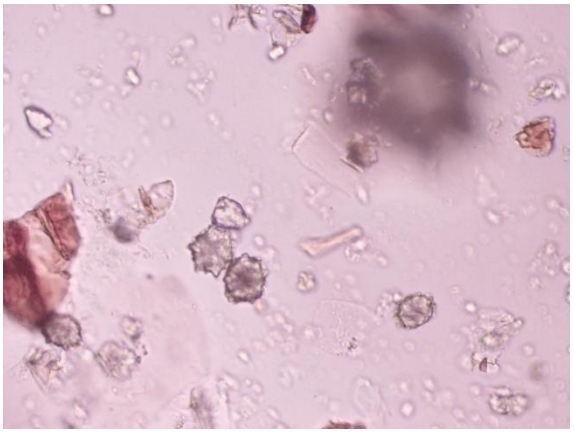
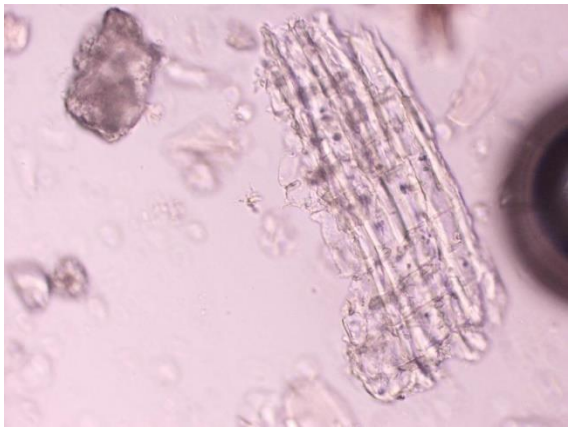
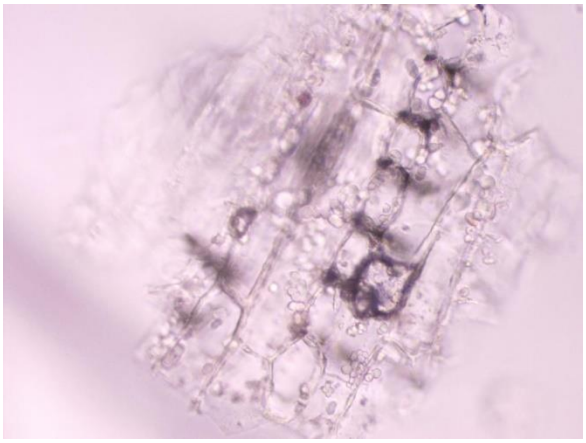
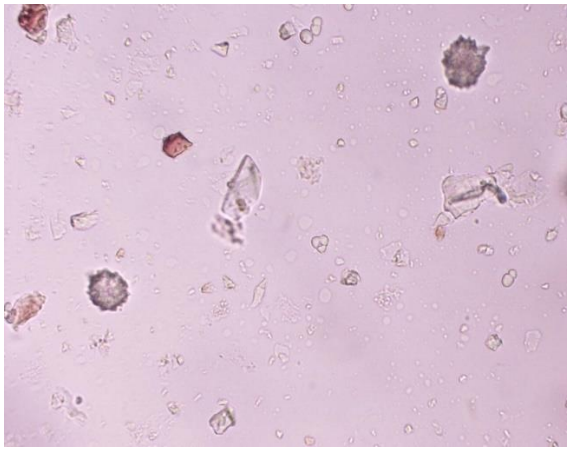
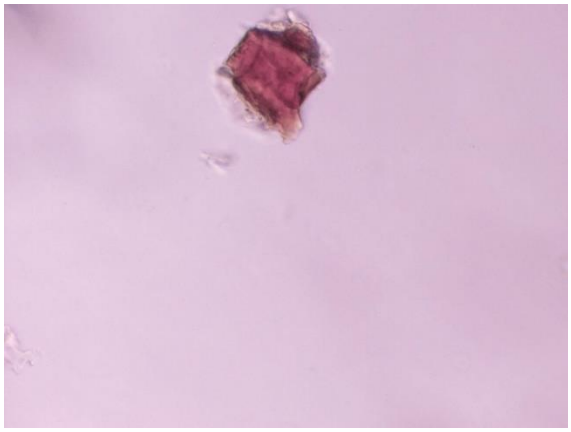
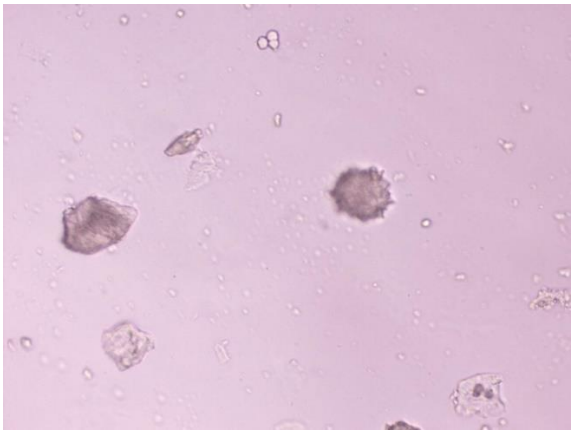
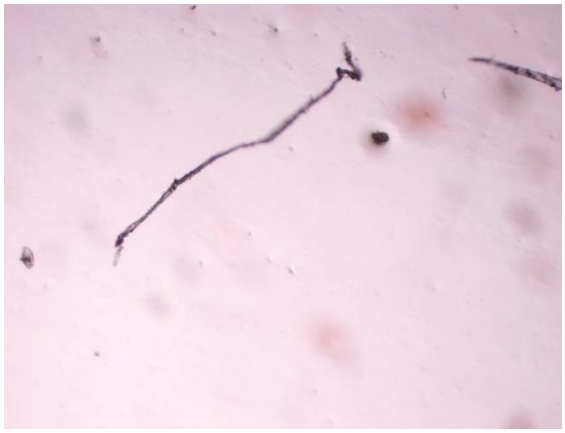
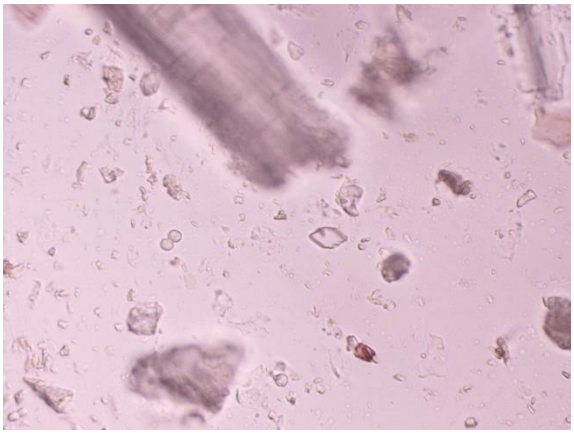


Fig7.Powder microscopy of Terminalia arjuna

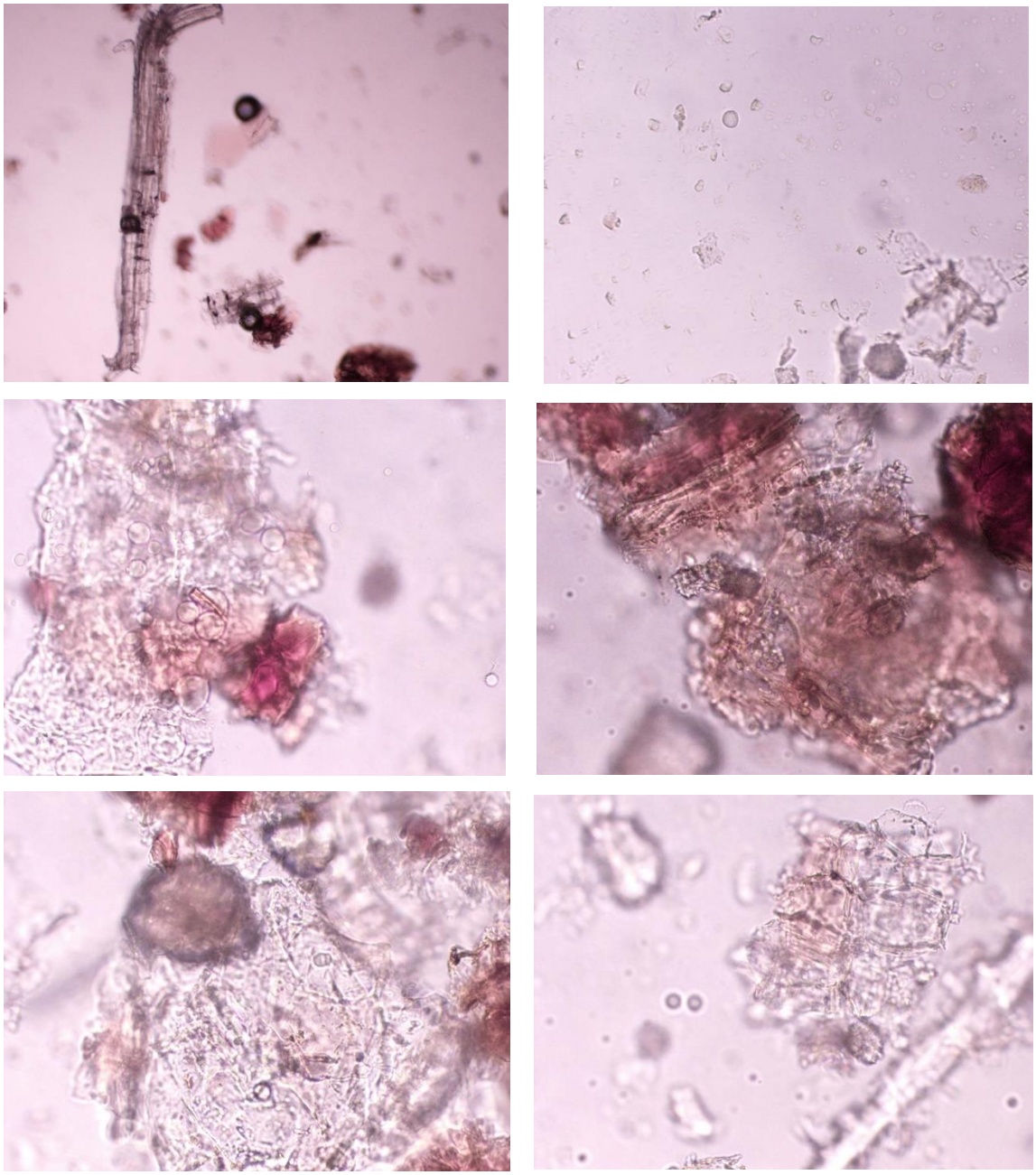
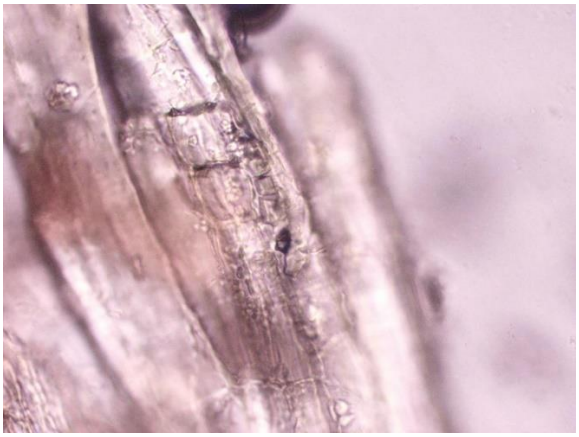
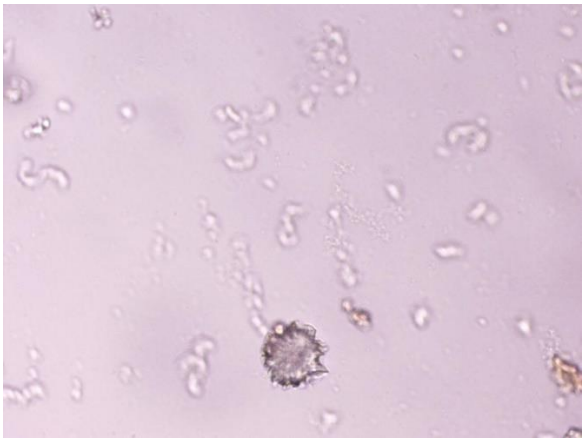
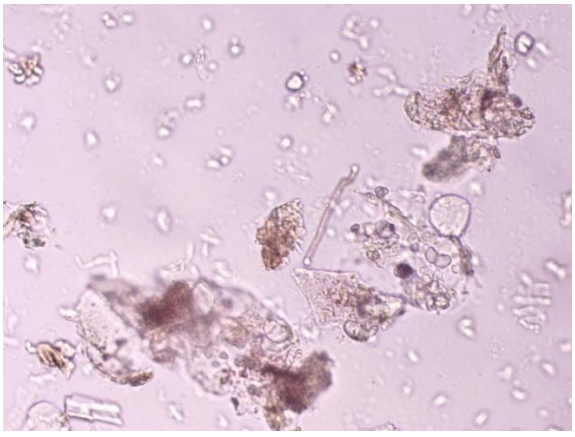
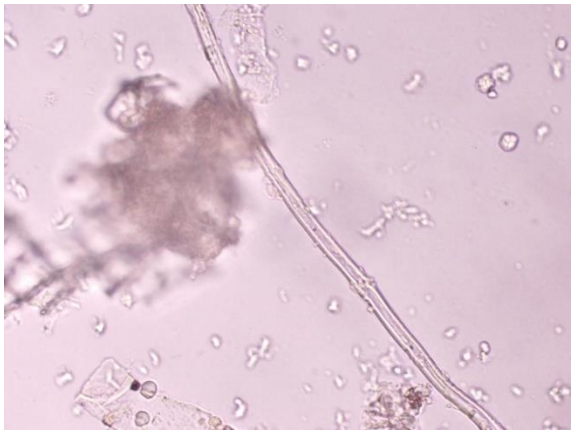
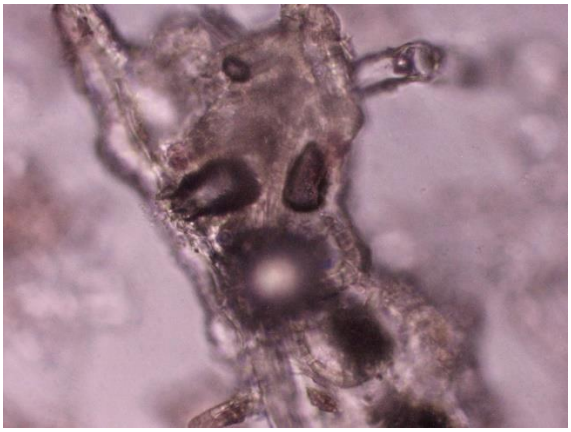
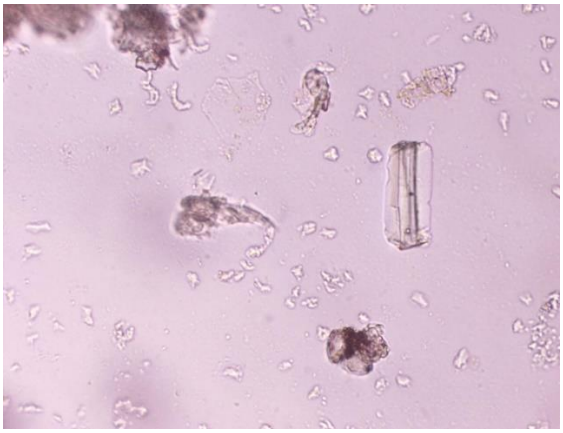
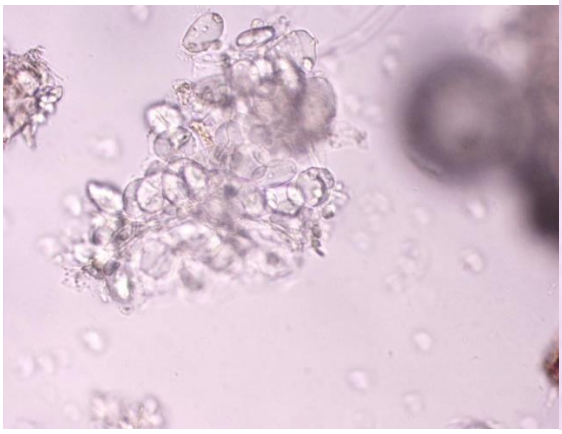
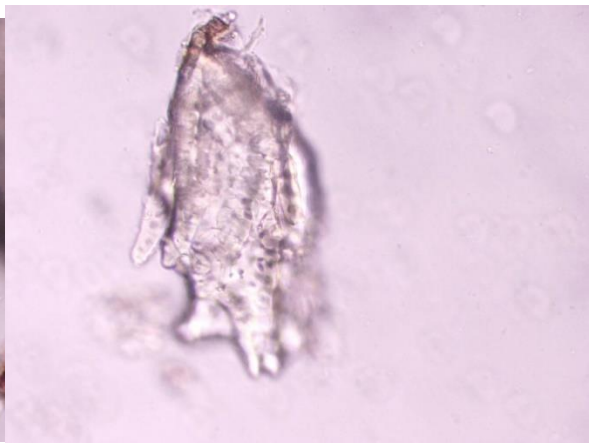
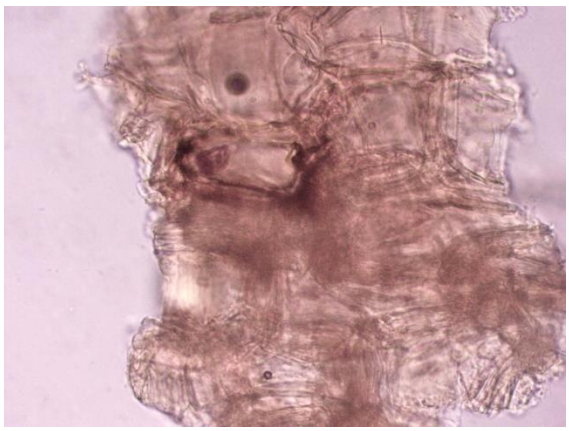
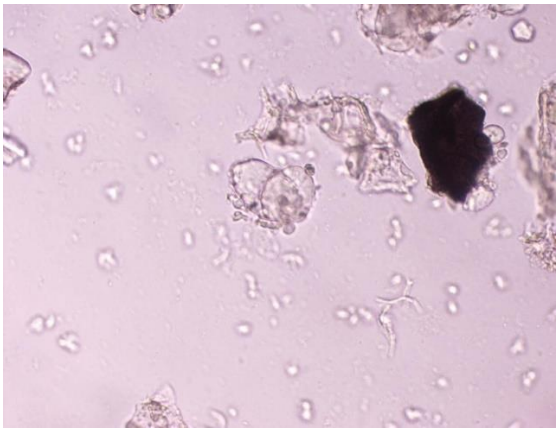
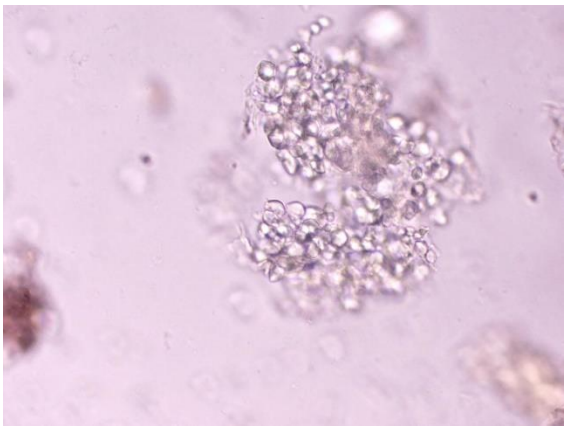
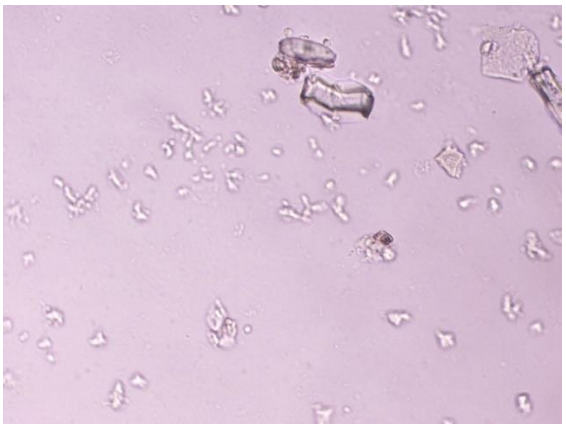
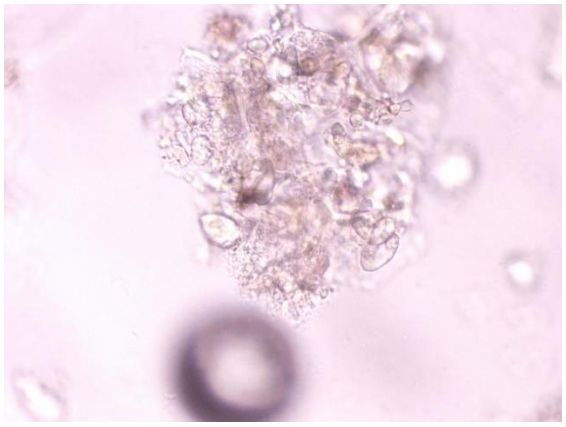
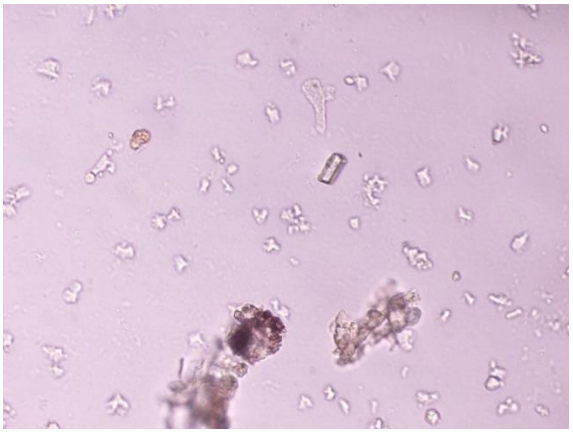
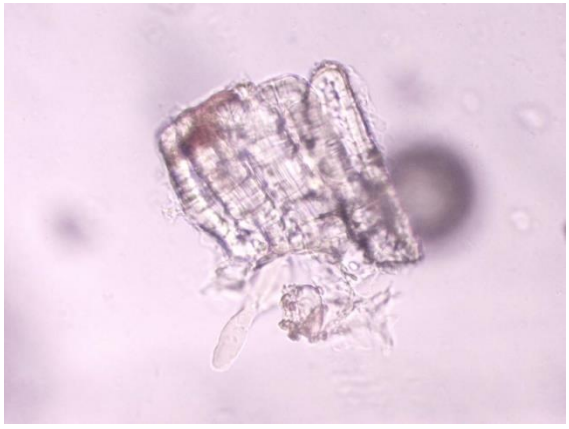
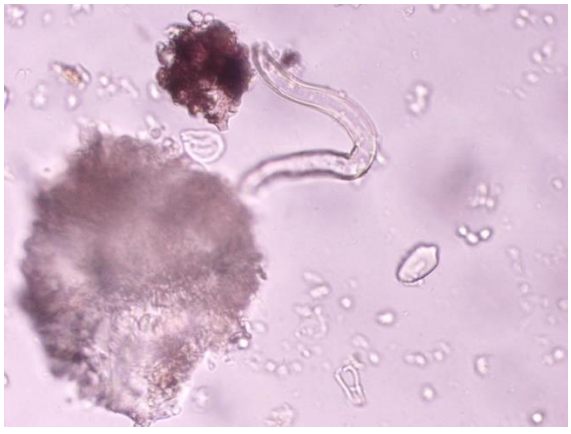


Fig8.Powder microscopy of Terminalia elliptica

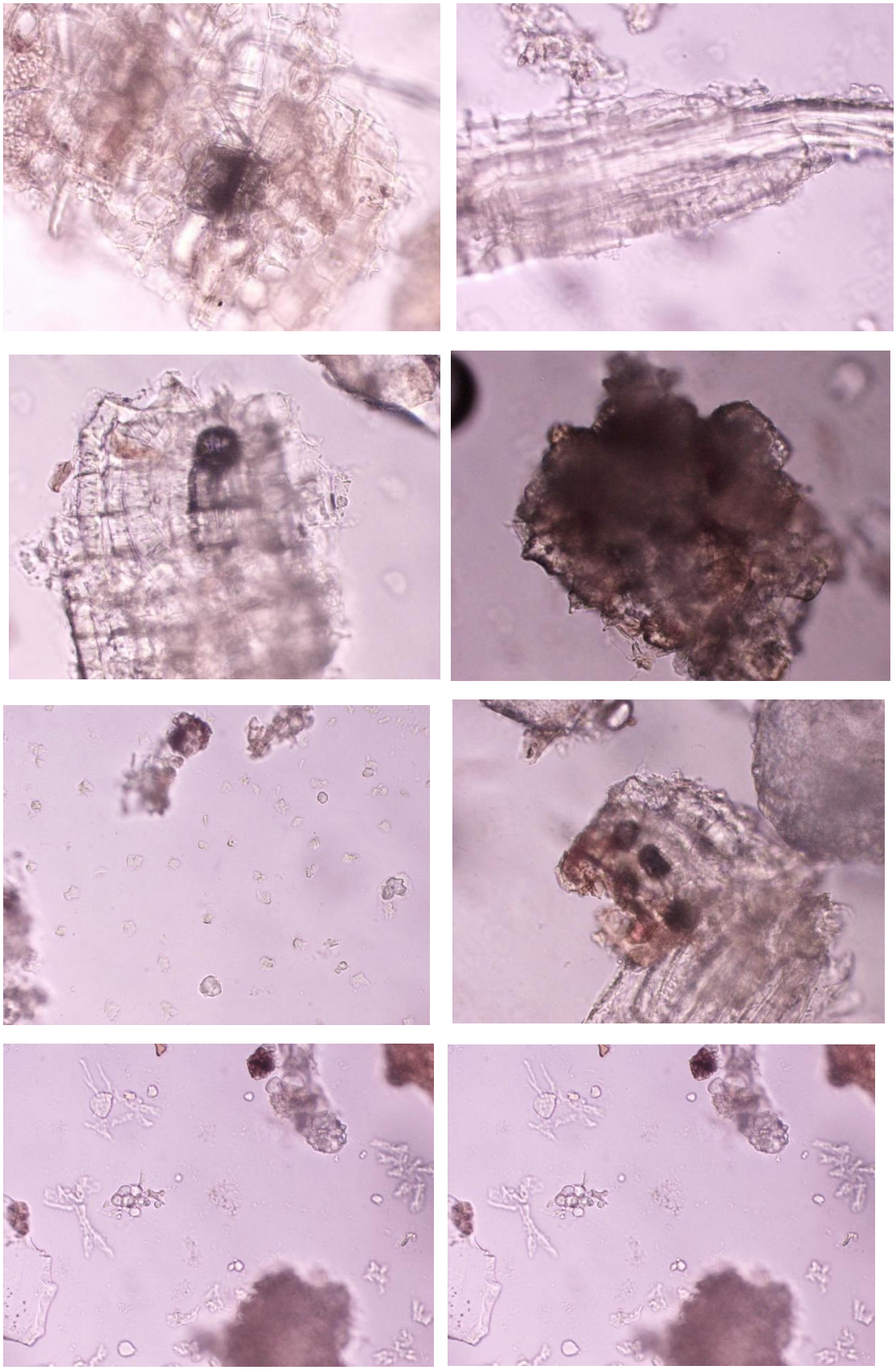


Fig 9.Powder microscopy of Lagestroemia speciosa

5.DISCUSSION

Phytochemical analysis serves as a bridge between traditional herbal knowledge and contemporary science, validating the therapeutic potential of traditional remedies through scientific evidence.

In this study, the phytochemical screening of ethanol extract of barks of *Terminalia arjuna*, *Terminalia elliptica*, and *Lagestroemia speciosa* was conducted and it shows similar compounds in both Terminalia species and slightly different in *Lagestroemia speciosa*

TLC and HPTLC profiling of the chloroform extract of barks provides the similarities and differences of the plant barks. In TLC 7 values are get for both *Terminalia* species and *Lagestroemia speciosa* got 10 values. Similar Rf values are got for the both *T.arjuna* and *T. elliptica* and show difference in *L.speciosa*.

HPTLC analysis gives same bands in both *Terminalia* species at 366 nm. and dissimilar bands in *L.speciosa*. In 256nm the *T. arjuna* shows only one peak whereas *T.elliptica* shows 2 peaks and L. speciosa show 7 peaks.

Powder microscopy reveals the anatomical features of the 3 barks. it also helps to known about the similarity and dissimilarity between the 3 barks. So many characters were got from this study. It also helps to evaluate the similarity in both *Terminalia* species and difference of *L.speciosa* from others.

In the project the aim was to explore the scope of substitution and adulteration in *T. arjuna*. The study provide the chances for being *T. elliptica* can be used as substitute for *T.arjuna* and *L.speciosa* comes as a adulterant and this study also aid in species identification while also understanding the differences and similarities between them.

6. SUMMARY AND CONCLUSION

The current study went through various phytochemical analysis for understanding phytochemical features of barks of *Terminalia arjuna*, *Terminalia elliptica* and *Lagestroemia speciosa*. These analyses included preliminary qualitative analysis, TLC profiling, HPTLC profiling and Powder microscopic analysis.

The study found that 'neermaruthu' bark contain various phytoconstituents except alkaloid and phlobotanin, In 'karimaruthu' contains phytoconstituents except phlobotanin while 'manimaruthu' consist of phytoconstituents except reducing sugar, alkaloid, coumarin and saponin.

TLC profiling shows similar values in both Terminalia species and in *Lagestroemia* shows more number of values and it is some more different from them.

In HPTLC profiling also help to recognized the similarity and difference between the 3 barks. The study gives similar peaks in both Terminalia species and different peaks in *Lagestroemia speciosa*.

Through powder microscopy the comparison of anatomical feature can be done successfully. It also reveal the similarity between both *Terminalia* species and identified the characters in *Lagestromia* speciosa.

T. arjuna is a widely used as herbal in ayurvedic system of medicine. Most commonly the stem bark is used for medicinal preparations. The stem bark used for the treatment of obesity, cardiac problems etc. By analysing the phytochemical profiling between the stem barks of *T.arjuna* and *T.elliptica* shows almost similar activity. Based up on the similarity *T.elliptica* may be possible to substitute for *T.arjuna*. Cardioprotective based analytical methods must be performal in *T. elliptica* to prove its validity before the substitution practice of these two *Terminalia* species.

Currently *L.speciosa* stem barks are adulterated with *T.arjuna*. The cardio protective nature of *L. speciosa* is already proved in some scientific papers. Before the substitution of *T. arjuna* with *L.speciosa* higher instrumental activity must be perform to compare their strength towards cardio protective activity. Phytochemicaly these two barks are shows remarkable variations with each other.

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