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Research Article

# Standarization of *Artabortrys hexapetalus*

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**ABSTRACT**

Objectives: To study the morphological, microscopical characters and physiochemical properties of the aerial parts of *Artabortrys hexapetalus* (Annonaceae) to ensure the authenticity of plant standardization parameters of this plant has studied. Methods: In order to study the Microscopical characters, transverse sections of drug has been carried out by free hand. Using multiple reagents, various chemical tests were performed to identifymany microscopic structures.Preliminary phytochemical screening and quantitative estimation have been determined along with HPTLC fingerprinting.CAMAG HPTLC system equipped with Linomat applicator, TLC Scanner, Reprostart and WINCATS software were used for fingerprinting.Results:The microscopic structures of plant revealed the presence of collenchyma,parenchyma cells and palisade cells. Presence of trichomes, parasitic stomata, vascular bundles were also observed.In powder microscopy lignified xylem vessels, stomata and trichomes were clearly seen. Various physicochemical parameters like ash value,moisture content and extractive values were determined to standardize the drug.Chemical tests and HPTLC fingerprinting of multiple extracts revealed the presence of various plant secondary metabolites.Conclusion:These findings will be useful in establishing pharmacognostic and phytochemical standards for identification as well as assessment of purity and quality of this plant, which is definitely gaining the relevance in plant drug research and establishment of plant monograph.

**Keywords:** Artabortrys, standardization, phytochemical screening, fingerprinting, metabolites.

## INTRODUCTION

Herbal medicine has been used throughout history and within every culture to prevent and treat diseases[1]. During the past decade, traditional systems of medicine have become a topic of global importance. Current estimate suggests that, in many developing countries, a large proportion of the population relies heavily on traditional practioners and medicinal plants to meet primary health care needs. Few plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal application. Assurance of the safety, quality & efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries[2]. For safety, quality & efficacy of drugs standardization is done. Standardization can be achieved by pharmacognostical studies, includes morphological, microscopical studies, physicochemical constants, preliminary phytochemical screening, foreign matter, ash values and extractive values. *Artabortys hexapetalus* [(L.F) Bhandari] (Annonaceae) is a medium size climbing shrub, largely cultivated in India[3]. Leaves are oblong, lanceolate, and glabrous. Flowers solitary or paired, often fragrant, usually on woody, hooked, recurved branches (Peduncles)[4]. It is widely distributed throughout the southern part of China and also in the southern part of Asia[5]. As a Chinese traditional folk medicine, its roots and fruits are used for treating malaria and scrofula, respectively[6]. The flower is acrid, bitter; useful in vomiting, diseases of the blood and the heart, leucoderma, headache. A decoction of leaves is given for cholera[3]. Phytochemically it contains alkaloids, anthraquinones, butyrolactones, flavanoids, neolignans, phenolic compounds, terpenoids and volatile oils[7]. In spite of the numerous medicinal uses and chemical constituents attributed to this plant, there is no pharmacognostical report on the anatomical and other physico-chemical standards required for the quality control of the crude drug. Hence the present investigation includes morphological and anatomical evaluation, determination of physicochemical constants and HPTLC of various extracts of *Artabortrys hexapetalus.*

## MATERIAL AND METHODS

*Plant material and extraction*

Fresh aerial parts of *Artabortrys hexapetalus* was collected in the month of December and January from Roshanara Bagh, Delhi and authenticated by Dr. Sunita Garg, NISCAIR, Delhi. The voucher specimen (NISCAIR/RHMD/consult/2014/2546-125) of the test drug was deposited in the NISCAIR herbarium for future reference. The fresh aerial parts were dried under the shade and powdered in a mixture grinder. The powdered aerial parts were packed in a paper bags and stored in air tight container until use. The coarse powdered material of *Artabortrys hexapetalus* were extracted successively by petroleum ether, Dichloromethane, Ethyl acetate, ethanol, and Water solvent. Each time before extraction with next solvents, the coarse powder material was dried in hot air

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Table 1: Microchemical test

| S. No | Test | Observation | Inference |
|---|---|---|---|
| 1. | T.S. + phlouroglucinol +conc. HCL | Lignified fibers and sclerides | Fibers and sclerides present |
| 2. | Powder drug + few drops of water | No swelling | Mucilage absent |
| 3. | Powder drug + Phlouroglucinol + Conc. HCL | Lignified fibers Lignified sclerides | Fibers present Sclerides present. |

Table 2: Physicochemical studies

| S. No | Parameter | Results |
|---|---|---|
| 1. | Organoleptic character | |
| | Appearance | Powder |
| | Colour | Green |
| | Smell | Pleasant |
| | Taste | Slight bitter |
| 2. | Loss in weight on drying at 105ºC | 7.2% |
| 3. | Alcohol soluble matter(%) | |
| | In cold alcohol: | 5.2 g% |
| | In hot alcohol: | 7.4 g% |
| 4. | Water soluble matter (%) | 11.18 g% |
| 5. | PH value | |
| | pH of 1% aqueous solution | 6.2 |
| | pH of 10% aqueous solution | 6.5 |
| 6. | Ash value | |
| | Total ash | 6.60% |
| | Acid insoluble ash | 4.78% |
| | Water soluble ash | 2.71% |
| | Sulphated ash | 0.26% |
| 7. | Successive extractives(%) | |
| | Petroleum ether | 1.499% |
| | Dichloromethane | 0.892% |
| | Ethylacetate | 1.224% |
| | Ethanol | 7.486% |
| | Aqueous | 11.18% |
| 8. | Test for extraneous material | |
| | Foreign matter | 0.1% |
| | Sand &silica | Not visible |
| | Insect infestation | Nil |
| | Rodent contaminations | Nil |
| 9. | Inorganic elements | |
| | Fe | 325.028% |
| | Zn | 2.547% |
| | K | 0.90% |
| | Ca | 1.43% |

oven below 50 ºC. The extracts were concentrated under reduced pressure. The extracts were stored at cool place in the dark until use.

*Morphological evaluation*

Aerial parts of *Artabortrys hexapetalus* were subjected to morphological evaluation for parameters like colour, shape, size, taste and texture. Macroscopical examination was carried out the freshly collected aerial parts and to the powder[8].

*Microscopic evaluation*

For anatomical description material was sectioned free-hand, in transverse direction. All parts were cross section with free hand and sections were prepared staining was done to study anatomical structures and Various types of histochemical tests were performed for the identification of starch grain, globules and xylem with the following solutions: hydrochloric and phloroglucinol, for the detection of lignin; iodine solution for starch[9].

*Leaf constant*

For establishing standardization parameter various leaf constants, palisalide ratio, vein islet number, vein termination number and stomatal index evaluated by Khandelwal[9].

*Physicochemical investigation*

For establishing standardization parameter various physicochemical parameters such as ash value, foreign matter, inorganic matter, extractive values, pesticide residue, determination of aflatoxin, determination of heavy metal, microbial contamination are done[10]. These parameters are were determined as per method determined in Indian pharmacopoeia[11].

*Fluorescence analysis*

Many phytochemicals showed fluoresce when suitably illuminated with UV light[12]. Light rich in short wavelengths is very active in producing fluorescence and for this reason strong ultraviolet light produces fluorescence in many substances, which do not visibly fluoresce in day light[13]. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation[14]. The changes in appearance and colour were observed and recorded. Color reaction of petroleum ether, ethyl acetate, chloroform, methanol and water extract and aerial parts was also observed in normal light and UV light[15].

*Qualitative phytochemical investigation*

The preliminary qualitative phytochemical identification had been carried out by using various phytochemical tests like test for steroids, phenol, flavanoids, alkaloids, glycoside, tannins, saponnins, gums and carbohydrates[16].

*HPTLC fingerprinting*

HPTLC study of different extracts was carried out by the method of Harborne and Wagner et al[17,18].

*Application of bands*

Sample (10µl each) were applied in the form of bands on pre-coated silica gel 60GF254 aluminium sheets (20x10 cm) with the help of Linomat V applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software 24.

*Development of chromatogram*

Table 3: Safety parameters

| Test for Pesticide | | |
|---|---|---|
| Name of pesticide | Obsevation | Limit |
| DDT | Not detected | 0.0050ppm |
| HCH (Alpha &Beta) | Not detected | 0.0050ppm |
| Endosulfan | Not detected | 0.0050ppm |
| Alpha endosulfan | | |
| Beta endosulfan | | |
| Endosulfansulphate | | |
| Malathion | Not detected | 0.0050 ppm |
| Parathion | Not detected | 0.0050 ppm |
| Test for Heavy Metals | | |
| Name of heavy metal | Observation | Limit |
| Arsenic | Not detected | 0.2 ppm |
| Lead | Not detected | 0.2ppm |
| Mercury | Not detected | 0.2ppm |
| Cadmium | Not detected | 0.2ppm |
| Test for Aflatoxin | | |
| Name of Aflatoxin | Observation | Limit |
| $B_1$ | Not detected | 1.0ppb |
| $B_2$ | Not detected | 1.0ppb |
| $G_1$ | Not detected | 1.0ppb |
| $G_2$ | Not detected | 1.0ppb |
| Microbial Contamination | | |
| Micro Organism | Observation | Limit |
| Total aerobic microbial count | 591 | $10^5$ |
| Enterobacteriaceae | Less than 10 | $10^3$ |
| Total fungal count | Less than 10 | $10^1$ |

After the application of spots, prepared plates were developed in previously saturated twin trough chamber (20 X10 cm) in linear ascending direction with respective solvents for specified time.

*Detection of spots*

The developed plates were dried by hot air to evaporate the solvents from the plate. The developed plate was sprayed with anisaldehyde sulphuric acid as spraying reagent and dried at 100 ºC in hot air oven for three minutes. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 nm and 334 nm, respectively. The $R_f$ values and finger print data were recorded by WIN CATS software[19].

## RESULTS

*Morphological study*

A medium size climbing shrub, often scandent and 8-10 ft long producing flowers and fruits. Color of shrub is greenish and fades to yellow with age, and is extremely fragrant. Leaves are oblong, lanceolate, shortly acuminate, glabrous, shining, and acute at the base. Leaves are 6-15cm long, 2-4.5cm wide. Flowers are yellow, solitary or in pairs on hooked and curved branches. It is an important parameter for evaluation of crude drug.

*Microscopy study*

Chemo-microscopy revealed the presence of lignified fibers, sclerides, mucilage, tannins and starch grain (Table 1).

*TS of petiole*

Transverse section of the petiole is circular at the lower side and flat on its upperside. Underneath the epidermis lies a continous patch of sclerenchyma. In the centre of the petiole there is presence of pith outside the pith the cells of parenchymatous ground tissues and vascular strand is there. Between the vascular strand and the endodermis pericycle is present. Outside the endodermis parenchymatous cells are there (figure 1a).

*TS of leaf*

Transverse section of the leaf passing through the midrib is convex on the lower side, a collenchymatous band underneath both the epidermis, a centrally located arc of bicollateral vascular bundle. Detailed TS shows a layer of upper and lower epidermis (figure 2d) embedded with paracytic stomata (figure 2c) and bears multicellular trichomes (figure 2f). Midrib shows 4-5 rows of collenchymatous band underneath both the epidermis and

Table 4: Fluoresence characteristics of powdered drug

| S. No | Chemical treatment | Ordinary light | UV long WL | UV short WL |
|---|---|---|---|---|
| 1. | Powdered Drug | Green | Greenish purple | Green |
| 2. | Powdered Drug treated with distilled water | Green | Green | Grey green |
| 3. | Powdered Drug treated with 50% HCl | Greenish brown | Black | Purplish green |
| 4. | Powdered Drug treated with 50% $H_2SO_4$ | Blackish yellow | Black | Greenish black |
| 5. | Powdered Drug treated with 50% $HNO_3$ | Green | Black | Green |
| 6. | Powdered Drug treated with conc. HCl | Greenish brown | black | Purplish green |
| 7. | Powdered Drug treated with conc. $H_2SO_4$ | Black | Purplish green | Black green |
| 8. | Powdered Drug treated with conc. $HNO_3$ | Brown | Black | Greenish |
| 9. | Powdered Drug treated with petroleum ether | Green | Dark green | Green |
| 10. | Powdered Drug treated with $CHCl_3$ | Green | Brown | yellow green |
| 11 | Powdered Drug treated with $CH_3OH$ | Green | Light brown | yellow green |
| 12. | Powdered Drug treated with ethylacetate | Green | Light green | Dark green |
| 13. | Powdered Drug treated with 10% $fecl_3$ | Brownish | Black | Light green |
| 14. | Powdered Drug treated with 10% NaOH | brown green | Black | Blackish green |
| 15. | Powdered Drug treated with ammonia | Green | Green brown | Green |
| 16. | Powdered Drug treated with picric acid | Yellowish green | Black | light green |
| 17. | Powdered Drug treated with Iodine | Brownish green | Black | green black |
| 18. | Powdered drug treated with Glaciel acetic acid | Yellow | Purplish green | yellowish green |
| 19. | Powdered drug treated with $CH_3OH$ and NaOH | Yellowish green | Black brown | Green |

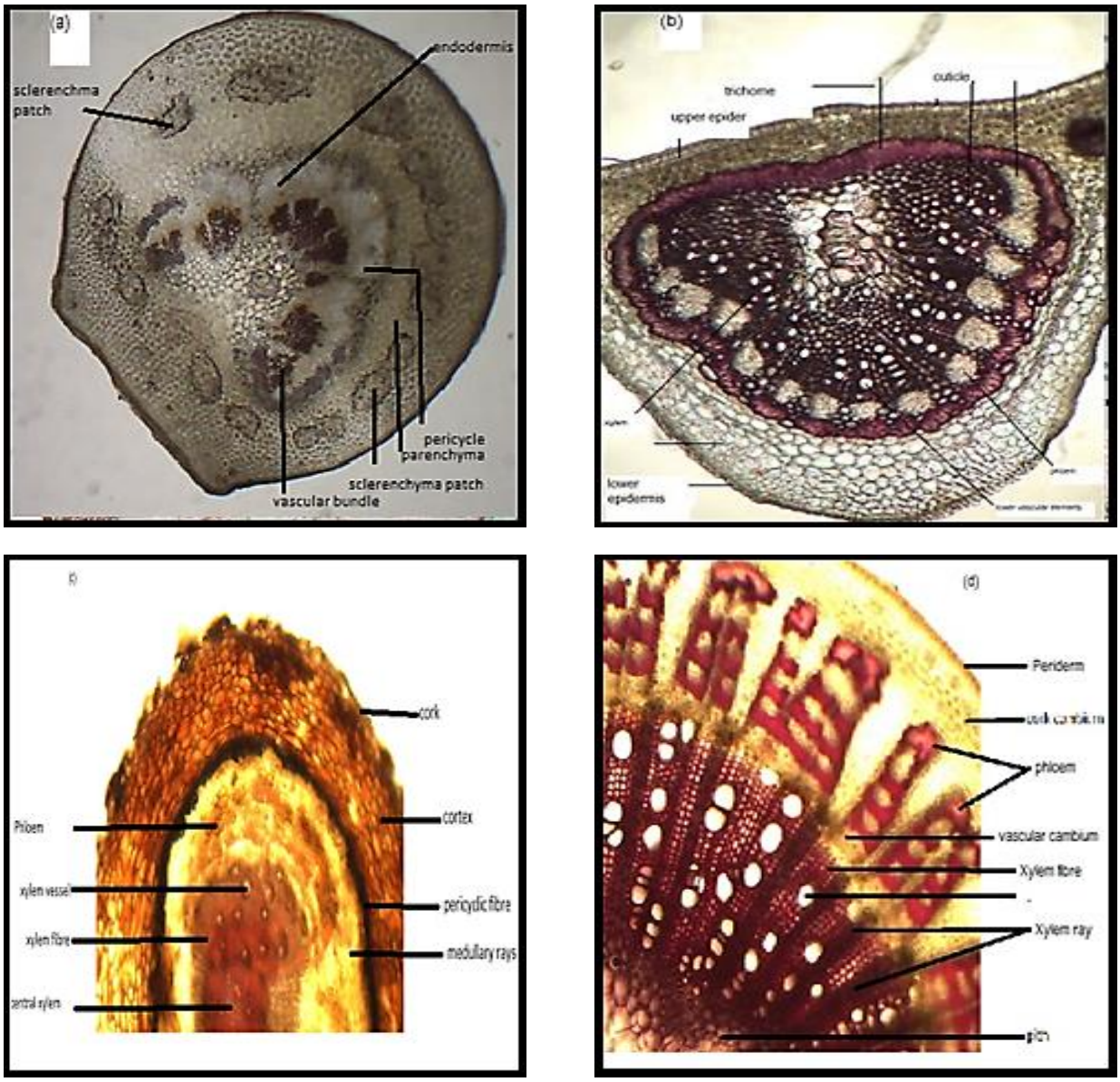


Figure 1: Transverse section of a) Petiole b) leaf c) root d) Stem at 10 X.

Table 5: Fluororesence characteristics of extracts

| S. No | Extract | Ordinary light | UV Short WL | UV long WL |
|---|---|---|---|---|
| 1. | Petroleum ether extract | Dark green | greenish black | Reddish in colour |
| 2. | Dichloromethane extract | Greenish Black | Greenish black | Black |
| 3. | Ethyl acetate extract | Dark brownish yellow | Greenish black | Purple black |
| 4. | Ethanol extract | Brown | Greenish black | Black |
| 5. | Methanol extract | Light brown | Greenish black | Yellowish black |
| 4. | Aqueous extract | Dark brown | Brown | Brownish black |

centrally located arc of bicollateral meristele embedded in the parenchymatous ground tissue. Lamina shows a row of palisade underneath the upper epidermis (figure 2e) and 2-3 rows of spongy parenchymatous tissue transverse with vascular strands (figure 1b).

*TS of root*

TS of root are irregularly circular in outline exhibiting distinct ridges and grooves. Cork of root shows outer one row of brownish cells and cortex is wide, parenchymatous (figure 3a, b, d). This is followed by the cortex made up 6-7 or more rows of oval shaped cells. Group of thick walled pericyclic fibres are present at the lower end of cortex. Endodermis is distinct, a narrow band of phloem surrounding the centrally located solid core of xylem composed of radially arranged isolated vessels, fibres and parenchyma (figure 3c). Wood is composed of many vessed elements, wood fibres, wood parecnchyma and medullary rays (figure 1c).

*TS of stem*

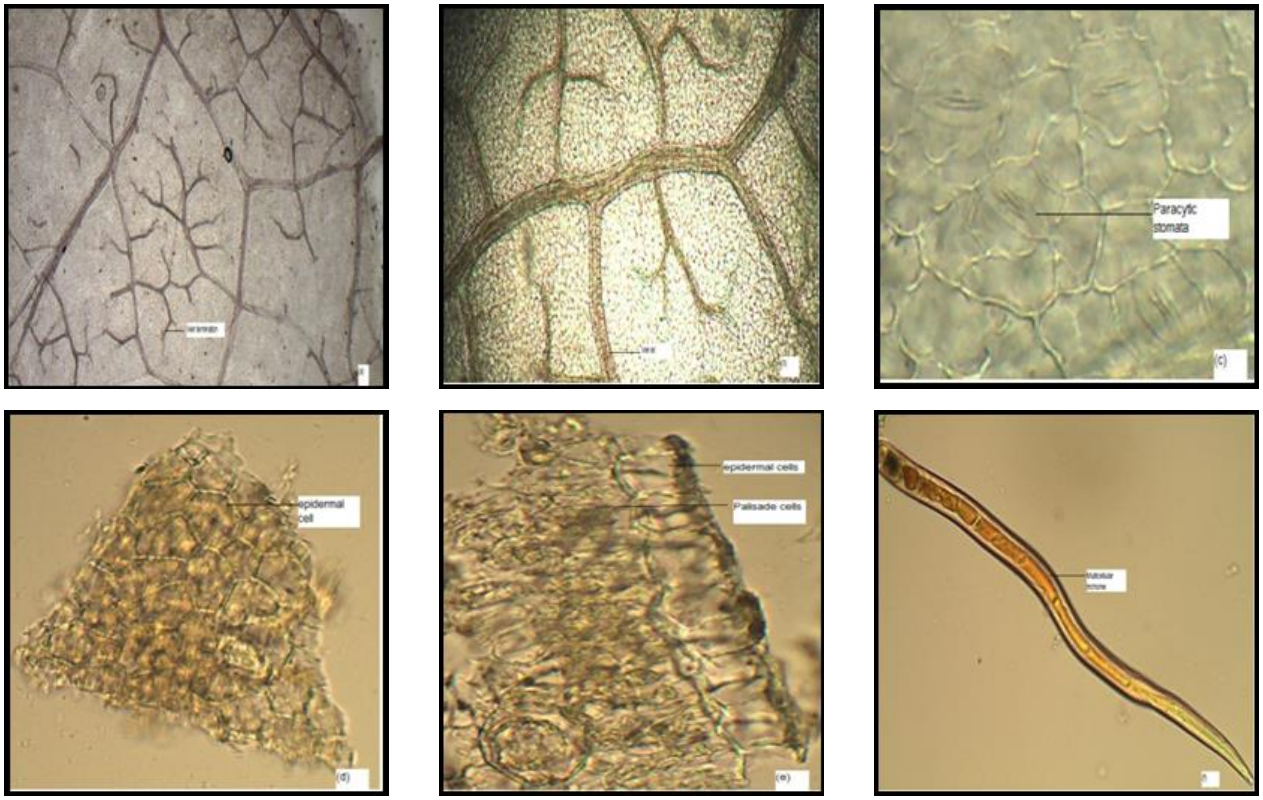


Figure 2: Powdered characteristics of leaf a) Vein termnation b) Vein lets c) stomata d) epidermal cells e) epidermal &Paliade cells f) Multicellular trichomes at 40X.

Table 6: Phytochemical screening of extracts

| Test | Petroleum ether extract | Dcm | Ea | Ethanolic | Aqueous |
|---|---|---|---|---|---|
| Alkaloids | - | - | + | + | + |
| Flavanoids | - | - | - | - | + |
| Tannins | - | - | + | + | + |
| Saponins | - | - | - | - | + |
| Glycosides | - | - | - | - | + |
| Steroids | - | + | - | + | + |
| Steroidal terpenes | + | - | - | - | - |
| Phenolic | - | - | - | + | + |
| Gums and mucilage | - | - | - | - | + |
| Carbohydrates | - | - | - | + | + |

Table 7: HPTLC fingerprinting of extracts

| Extracts | No. of constituents | Rf value |
|---|---|---|
| Dichloromethane | 7 | 0.05,0.46,0.52,0.57,0.64,0.72,0.81 |
| Ethyl acetate | 4 | 0.04,0.12,0.21,0.61 |
| Ethanol | 5 | 0.05,0.09,0.23,0.55,1.01 |

Diagrammatic TS of the stem is circular in outline, shows outer layer of epidermis bearing single layer of circular cells, hypodermis lying underneath this consists of collenchymatous cells. These cells are followed by a continuously running band of parenchymatous phloem, composed of sieve tube, companion cell, parenchyma and uni to biseriate medullary rays running in continuation with xylem rays; a ring of xylem lying underneath this is varying in height, composed of vessels. Pith is wide, parenchymatous and attached at the xylem ring (Figure 1d).

*Qualitative microscopy*

Various types of leaf constants, ash values, inorganic element, extractive values and moisture content were important to determine purity of the drug (Table 2). In leaf constants stomatal index and palisade ratio were found to be 13.63-15 and 1-7 respectively. Whereas vein islet and vein termination number were ascertained as 28-30 and 115-116 respectively (figure 2 a, b). Stomatal numbers

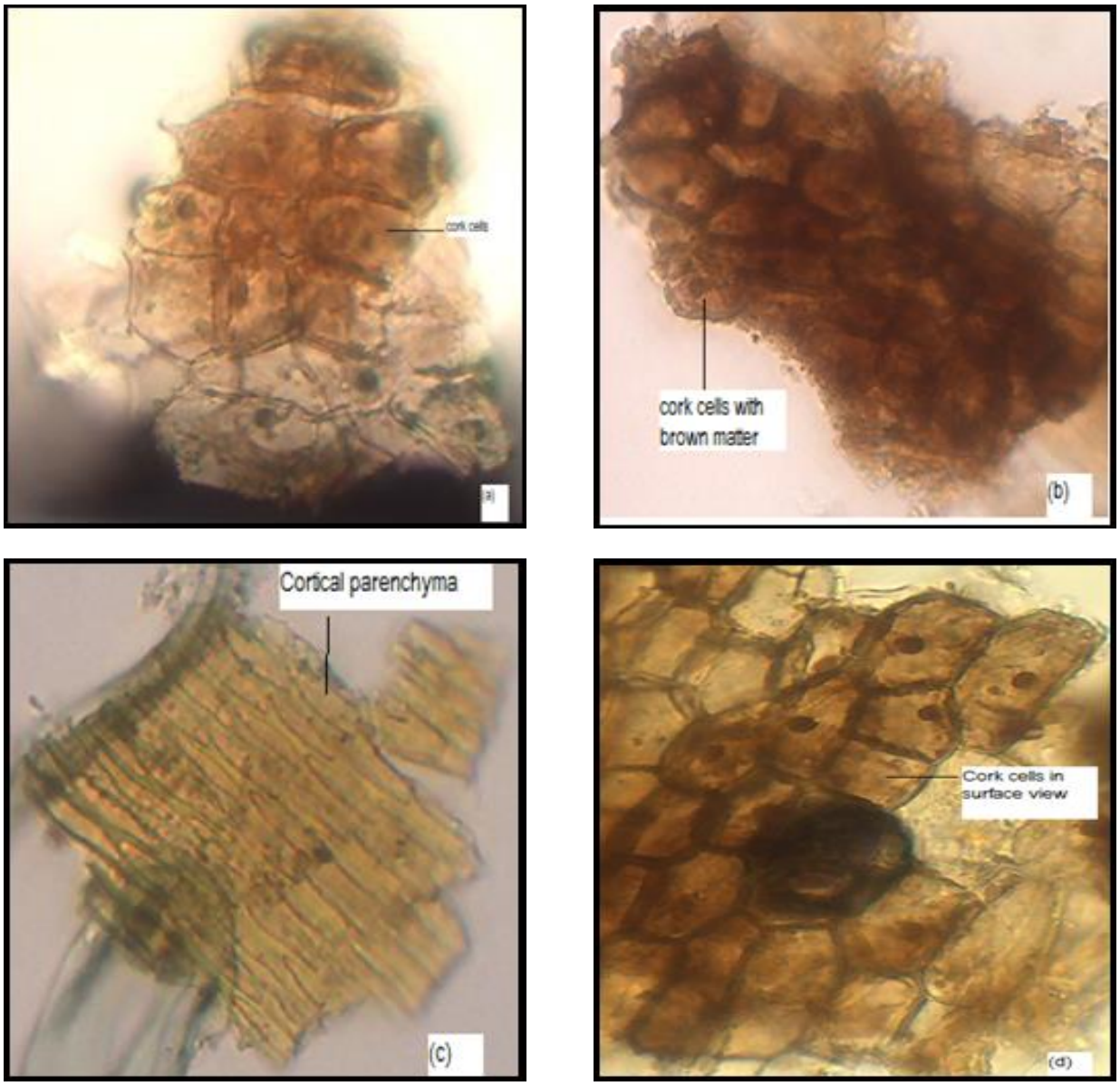


Figure 3: Powdered microscopy of root at 40X a) cork cells b) cells with brown matter c) cortical parenchyma d) cork cells in surface view at 40X.

were found to be on upper surface 28-30 and on lower surface 18-20.

*Physico-chemical analysis*

Various types of leaf constants, ash values, inorganic element, extractive values and moisture content were important to determine purity of the drug. The ash content of drug also showed presence of calcium, magnesium and sulphate while absence of sodium, potassium and phosphate types of inorganic compounds. Inorganic elements (Fe, Zn, k and Ca) have been measured by AAS in plant samples and the results are recorded in the table 3.

*Safety parameters*

The crude drugs were screened for the presence of microbial contamination. Total aerobic microbial count, Enterobacteriaceae and fungal count, pesticide residue, as per the method laid down in Indian pharmacopoeia (2010). The results are recorded in table 4.

*Fluorescence characteristics*

*Fluorescence characteristics of powdered drug*

All the powders were treated with routinely used reagents and characteristic changes were observed and summarized in table-4.

*Fluorescence characteristics of extracts*

Fluorescence characteristics of the extracts were observed in day light as well as in ultraviolet radiation. The results were recorded in table-5.

*Preliminary phytochemical screening*

Preliminary phytochemical investigation revealed the presence of plants secondary metabolites such as carbohydrates, alkaloids, saponins, tannins, glycosides, flavonoids and steroids. The results were recorded in table 6.

*HPTLC Fingerprinting*

HPTLC fingerprinting of different extracts of *A. hexapetalus* had been carried out by using various types of solvent system for separation of as many as

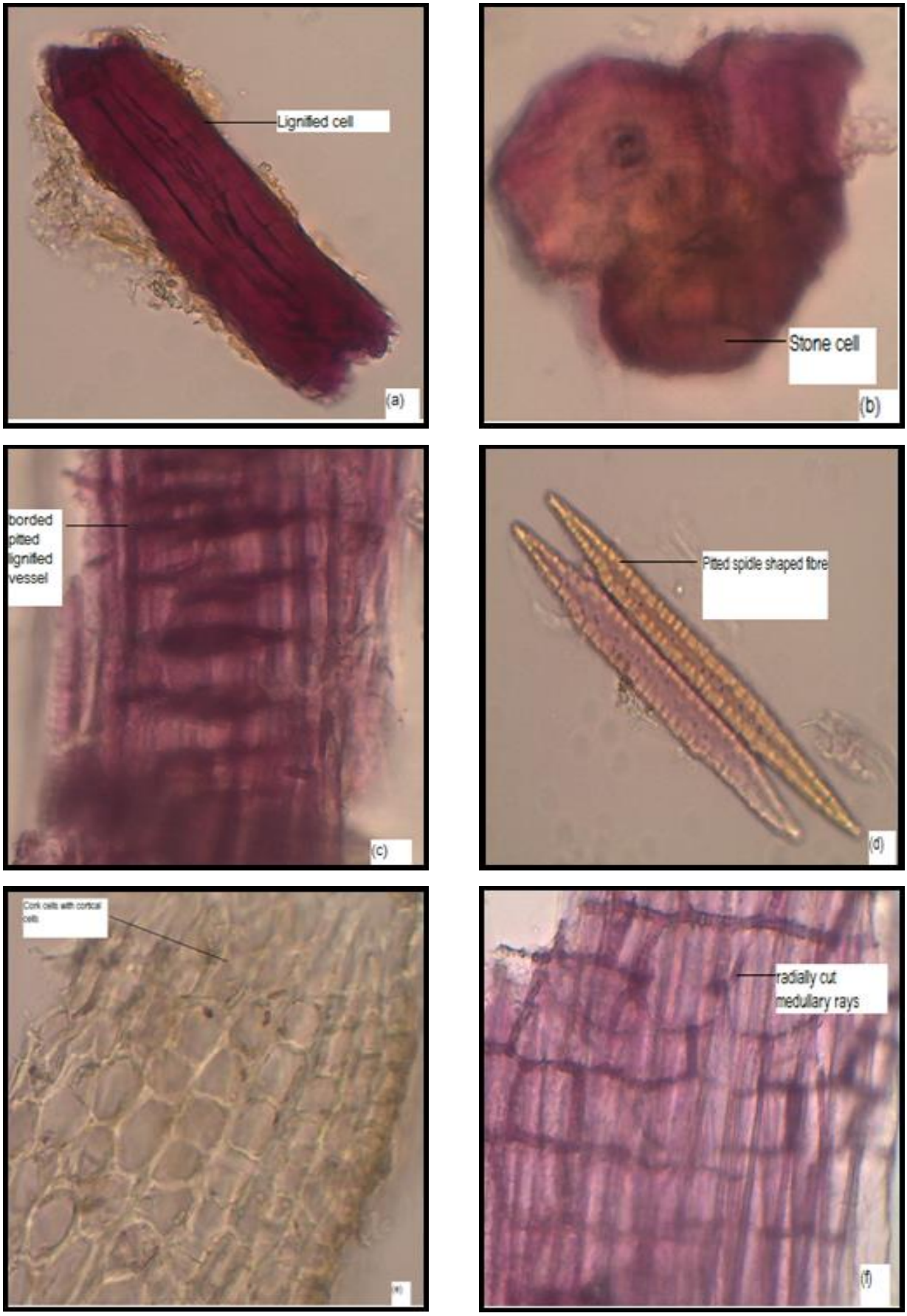


Figure 4: Powdered microscopy of stem: a) Lignified vessel b) stone cells c) borded pitted lignified xylem vessel d) pitted spindle shaped fibres e) cork cells with cortical cells f) radially cut medullary rays at 40X.

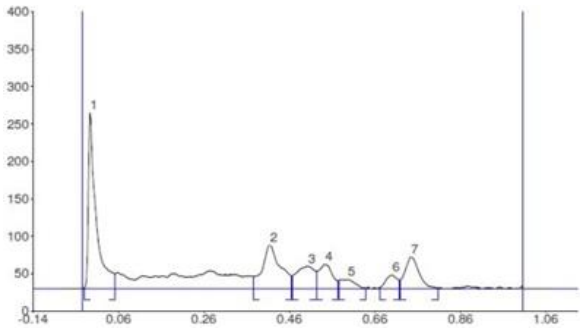

Figure 5: TLC profile of DCM extract

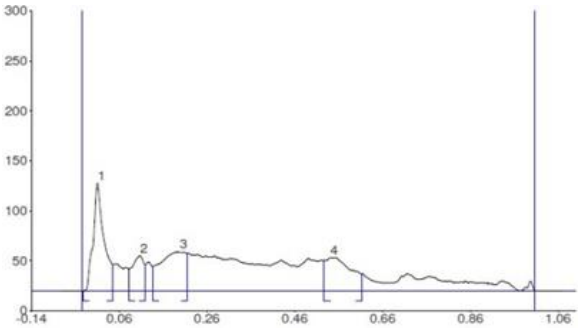

Figure 6: TLC profile of EA extract

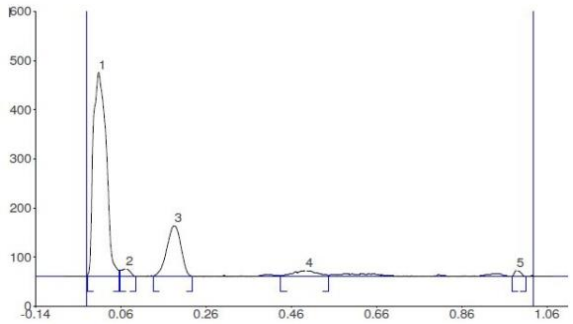

Figure 7: TLC profile of Ethanolic extract

phytochemicals. Results revealed that the presence of several constituents in the extracts. The number of constituent in the extract and their retention factor (Rf) are summarized in Table 7 and chromatographic profile had been shown by Figure 5, Figure 6 and Figure 7.

## DISCUSSIONS

The observations in the present study were undertaken with the aim of developing the pharmacognostic and physicochemical standards of *A. hexapetalus*. Morphologically the test drug is a shrub producing flowers and fruits. Leaves are green in colour, shape is oblong, lanceolate and acute at base. Flowers are extremely fragrant and yellow in colour present on hooked branches. The morphological characteristics were as per previous findings[3,4]. Microscopic characteristics revealed the presence of abundant covering trichomes, parasitic stomata, and oval shaped conjoint collateral vascular bundles. Presence of columnar palisade cell, which is the important distinguish characters, is also observed. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The stomatal index, palisade ratio, vein islet and vein termination numbers was determined in the quantitative microscopy and they can be used to differentiate with closely related other Artabortrys species. Physicochemical constants, safety parameters including HPTLC are helpful for the correct identification and would also serve authentication of this plant in the herbal or pharmaceutical industry. The physicochemical investigation of the drugs is an important task in detecting adulteration or improper handling of drugs. The estimation of moisture content of the drug is essential requirements in evaluation, as it supports bacteria, fungi or yeast growth. Also determinations of ash value and acid-insoluble ash value have equal importance in the evaluation and identification of inorganic impurities in crude drugs[20]. There is presence of important plant secondary metabolites such as tannins, alkaloids, phenolic substances, flavanoids, steroids and carbohydrate in *Artabortrys hexapetalus*, could make the plant useful for treating different ailments of living organism[21]. Thus the preliminary screening tests may be useful in the detection of bioactive principles and results were presented in table. Powdered drug was treated with different reagents and examined under UV light (Short and long wavelength). Different reagent showed different colour with the drug at different wavelengths and the results were presented in table. Different extracts were also examined under different wavelength. A number of solvent were tried individually as well as in combination for separation of different components of extract, but the satisfactory resolution was obtained in the solvent system Toluene: Ethylacetate: Formic acid (5:4:1 v/v) for ethanolic and Toluene: Ethylacetate: Glaciel acetic acid (9:1:0.5 v/v) for Dichloromethane and ethyl acetate extract. HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Due to presence of useful phytoconstituents this plant is useful in traditional system of medicine for the treatment of various diseases; it is the need of herbal era to standardize the plant for the development of quality control parameters and for proper identification as well as differentiate between closely related species.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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