



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Pharmacognostic, Physicochemical and Phytochemical Study of *Phyllanthus Amarus* present in Some Appetizer Polyherbal formulations

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ABSTRACT

Phyllanthus amarus S & T (Family: Euphorbeaceae) is an important medicinal plant. It is probably native to America but found throughout to India and almost all tropical countries. Widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and waste lands. Whole plant is used as bitter, antipyretic, antiseptic, astringent, cooling agent, diuretic, in dropsy, gastrointestinal troubles like colic, diarrhoea, dysentery, other genital disease and in jaundice. It also alleviates the anorexia and also used as a diuretic in dropsial affections, gonorrhoea and other problems of genitor urinary tract. Whole plant material was subjected to macro-microscopic, physico-chemical, preliminary phytochemical, TLC to fix the quality standards of this drug. The experiment has resulted a set of diagnostic characters essential for its standardization. The experiments yielded a set of diagnostic characters comparable with standard literatures. This study would be useful for standardization of this raw drug derived from whole plant of *Phyllanthus amarus* S & T.

Keywords: *Phyllanthus amarus*, Whole plant, Physicochemical parameters, Standardization

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Received 01 December 2012, Accepted 18 December 2012

Please cite this article in press as Parmar S *et al.*, Pharmacognostic, Physicochemical and Phytochemical Study of *Phyllanthus Amarus* present in Some Appetizer Polyherbal formulations. American Journal of PharmTech Research 2013.

INTRODUCTION

Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost.¹ *Phyllanthus amarus* S & T is erect annual herb, 10-60 cm tall, main stem simple or branched, terete, smooth or scabridulous in younger parts. It is probably native to America but found throughout to India and almost all tropical countries.² Widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and waste lands.³ Whole plant is used as bitter, antipyretic, antiseptic, astringent, cooling agent, diuretic, in dropsy, gastrointestinal troubles like colic, diarrhoea, dysentery, other genital disease and in jaundice. The drug is highly reputed as a single drug remedy in the treatment of jaundice in traditional medicine. It is carminative, styptic, used in cough and indigestion. It also alleviates the anorexia and also used as a diuretic in dropsial affections, gonorrhoea and other problems of genitor urinary tract.^{4,5}

The decoction of leaves and roots is very bitter and has antiasthmatic, antidiabetic, antidiarrhoeal, anti-inflammatory, antimalarial, antipyretic activity. Fresh juice of leaves is applied locally on cuts and wounds.⁶ Chemical examination of this plant revealed that it consists phyllanthin, hypophyllanthin, isobubuline, quercetin, nirtetralin, lintetralin, phylltetralin, niranthin etc.⁷

Present study include morphological, microscopical examination of whole plant material, various physico-chemical parameters like ash value, extractive value, moisture content and determination of pH of crude drug as well as water extract of *Phyllanthus amarus* which is the ingredient of some polyherbal appetizer formulations and TLC study of crude drug as well water extract in order to identify major chemical constituent present in the drug. This study will provide important tool for standardise the drug.

MATERIAL AND METHODS

Material:-

Whole plant sample of *Phyllanthus amarus* S&T were procured from Ayurlab Herbals Pvt. Ltd, Halol, Gujarat and it was identified authenticated by Dr. Vandana Vyas, Head of Department, Department of Botany, Sheth PT Arts & Science College, Godhra, Gujarat.

Water extract of *Phyllanthus amarus* was also procured from Ayurlab Herbals Pvt. Ltd, Halol, Gujarat. All the reagents and chemicals used for the phytochemical study were of standard grade.

Methods:-

Pharmacognostical Studies

- **Macroscopic Observation:**

The whole plant of *Phyllanthus amarus* was subjected to macroscopic studies which comprised of organoleptic characters of the drugs viz., color, odour, appearance, taste, smell, texture, fracture, etc and extracts were also subjected to performed organoleptic parameters like color, odour and taste.

- **Microscopic Studies:**

Microscopic identity of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen may have to be treated with chemical reagents. An examination by microscopy alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence. The transverse sections of whole plant of *Phyllanthus amarus* taken, decolorised with chloral hydrate and stained with phloroglucinol and HCl to stain lignified tissues. The sections were then mounted over the slide with the help of glycerine.⁸

- **Powder study of Plant material:**

The whole plant of *Phyllanthus amarus* was dried at 60°C for 4-6 hrs, to make it moisture free and grounded using electric grinder and passed through 60#. For Microscopical examination the powder was first boiled with Chloral hydrate for 10 min and then treated with Phloroglucinol & HCl (1:1). Then the powder was mounted over the glass slide with the help of Lactophenol and observed under microscope.⁸

Physico-chemical studies

Various Physico-chemical parameters as discussed below were performed for both crude powder as well as extract of each plant.

- **Determination of Ash value:**⁸⁻¹⁰

- (a) **Total ash:**

Place 2gm of the ground air dried material, in a previously ignited and tarred crucible (Silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500°C until it is white, indicating the absence of carbon. Cool in a desiccators and weigh. If carbon free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2ml of water or saturated solution of ammonium nitrate. Dry on a water bath, then on a hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30min, then weigh without delay. Calculate the content of total ash in mg/g of air dried material.

b) Acid insoluble ash:

To the crucible containing the total ash, add 25ml of hydrochloric acid, cover with a watch glass and boil gently for 5 minutes. Rinse the watch glass with 5ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to original crucible, dry on hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30min, then weight without delay. Calculate the content of acid insoluble ash in mg/g of air dried material.

c) Water soluble ash:

In the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered-glass crucible or on an ash less filter paper and washed with hot water. The filter paper containing the insoluble matter was transferred to the original crucible and ignited in a crucible for 15 minutes at a temperature not exceeding 450C. The residue was allowed to cool in desiccator for 30 minutes, and it was weighed without delay. The weight of this residue was subtracted from the weight of total ash. The percentage of water soluble ash was calculated with reference to air-dried plant material.

▪ Determination of Extractive Values:⁹**a) Water soluble:**

Place about 5g of coarsely powdered air dried material, accurately weighed, in a glass stoppered conical flask. Macerated with 100ml of chloroform-water (0.25% chloroform in water) for 6 hours, shaking frequently, and then allow to stand for 18 hours. Filter rapidly, taking care not to lose any solvent, transfer 25ml of the filtrate to a tarred flatbottomed dish and evaporate to dryness on a water bath. Dry at 105°C for 6 hours, cool in a desiccators for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg/g of air dried material.

b) Alcohol soluble:

Alcohol soluble extractive was carried out by the same procedure as above using alcohol instead water.

▪ Determination of Moisture content:⁹

Accurately weighed 10 gm of drug was placed in a tarred evaporating dish. After placing the drug in to the tarred evaporating dish it was dried at 105°C for 5 hours. Then sample was weighed. The drying and weighing was continued at one-hour interval until constant

weight was reached. Percentage moisture content was calculated on the basis of sample taken.

▪ **Determination of pH:**⁸

The pH value of a solution was determined potentiometrically by means of a glass electrode, a reference electrode and a digital pH meter. The pH meter was operated according to the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. 1 gm powder and 1 gm extract of each plant was taken and dissolved in 100 ml dimineralized water. The electrodes were immersed in the solution and measured the pH.

Preliminary Phytochemical Study:-⁸⁻¹¹

Preliminary phytochemical study was done to check the chemical class present in the crude powder as well as water extract of *Phyllanthus amarus* namely Alkaloid, Glycoside, Flavonoid, saponin, tannin, sterol and carbohydrate by various chemical tests.

TLC Study:-¹²

Thin layer chromatography was done on crude powder as well as water extract of *Phyllanthus amarus* using Toluene: Ethyl acetate: Formic acid (5.0:4.0:1.0) as solvent system.

RESULTS AND DISCUSSION

Photograph of Herbarium sheet of *Phyllanthus amarus* was shown in Figure 1, it was identified as *Phyllanthus amarus* S & T.

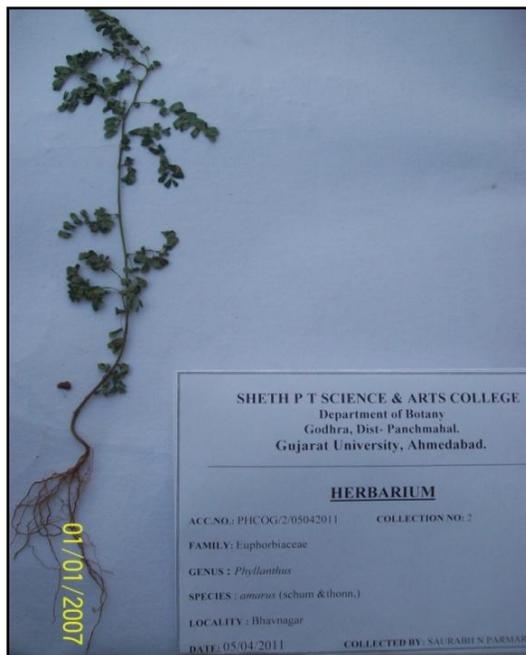


Figure 1: Herbarium sheet of *Phyllanthus amarus* S & T

Pharmacognostical studies

Macroscopic observation:

Macroscopic observation taken as organoleptic characters of crude powder as well as water extract which were given in Table 1, which showed almost similarity.

Table 1: Organoleptic characters

<i>Phyllanthus amarus</i>	Organoleptic characters		
	Colour	Odour	Taste
Crude powder	Yellowish brown	Characteristic	Bitter
Water extract	Yellowish brown	Characteristic	Bitter

Microscopic Observation:

Stem: Detailed section showed (Figure 2) a layer of epidermis, embedded with stomata, at places bearing papilla and covered with thick cuticle, a narrow band of chlorenchymatous hypodermis lies underneath this, followed by 2-3 rows of cholrenchymatous cortex, pericycle is characterised by discontinuous ring of groups of thin walled fibres, phloem is narrow, parenchymatous, cambium is distinct, xylem consists of radial rows of vessels, tracheids, thin walled fibres, parenchyma and uni to biseriate medullary rays. Pith is wide and parenchymatous. Cells getting disintegrated on drying developing cavities in the centre, cluster and rosette crystals of calcium oxalate traversed through out the parenchymatous cells of cortex and pith.

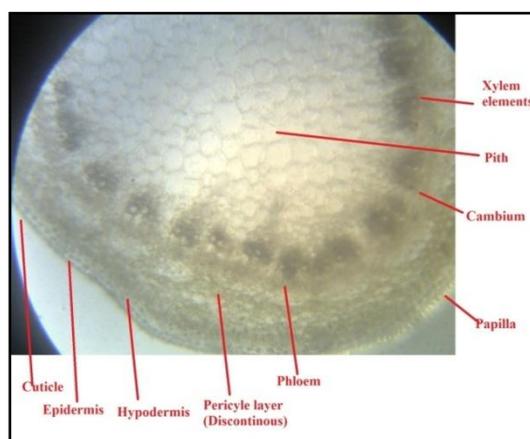


Figure 2: Detailed microscopy of *Phyllanthus amarus* stem

Leaf: Detailed section showed (Figure 3) that leaf passing through midrib is slightly elevated on the lower side and flat on the upper side, shows a layer of upper epidermis its cells being bigger in size than the lower one and covered with thin cuticle, at pplace it is papillose and embedded with stomata, underneath the upper epidermis lies a layer of palisade in continuation with the midrib. Merestele of the midrib consist of radiate xylem and an arc of phloem, underneath the layer of lamina lies 2 to 4 rows of spongy parenchyma traversed with obliquely cut vascular bundles and prismatic and rosette crystals of calcium oxalate.

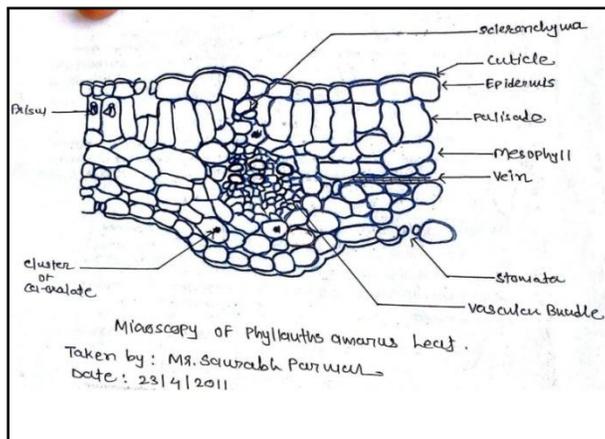


Figure 3:Detailed microscopy of *Phyllanthus amarus* leaf

Root:

Transverse section of root (Figure 4) is circular in outline with central lignified wood occupying more than 2/3 area of the root. Cortex and Phloem are narrow. In the young root, outermost region is occupied by a layer of epidermis but in older roots the layer of cork cells containing tannin are seen. Cortex is parenchymatous, contains simple starch grains and tannin. Inner cortex is characterised by the presence of lignified sclereids isolated or in groups of 2-5. The phloem is parenchymatous. Xylem is composed of radially arranged xylem vessels with bordered pitted thickening. Xylem fibres are thick walled; parenchyma and medullary rays which are usually biseriate contain starch grains.

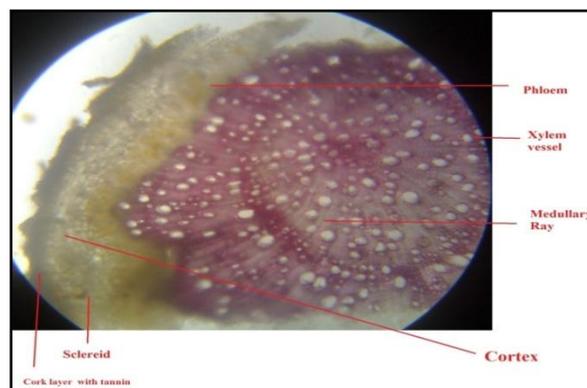


Figure 4:Detailed microscopy of *Phyllanthus amarus* root

Fruit:

Detail section (Figure 5) showed the outermost layer of tangentially running, narrow, rectangular cells of epicarp covered with thin cuticle, underneath this lies a layer of hypodermis consisting of big sized oval shaped, tangentially arranged parenchymatous cells, followed by a layer of small sized rectangular shaped radially arranged chlorenchymatous cells and then 3-4 rows of collapsed parenchymatous cells, the innermost layer of pericarp being made up of radially

arranged compactly placed big sized rectangular thick walled parenchymatous cells; a layer of endocarp of radially arranged parenchymatous cells being located underneath to this.

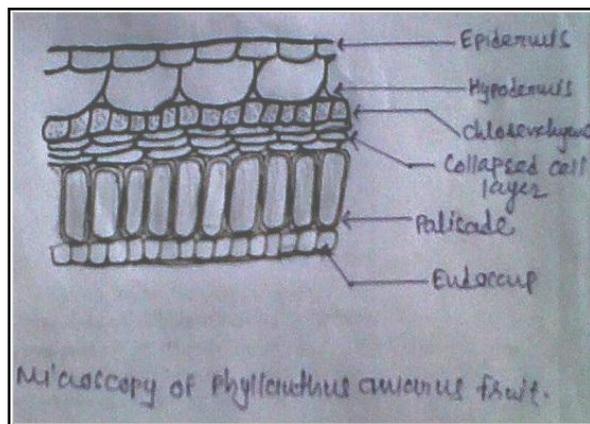
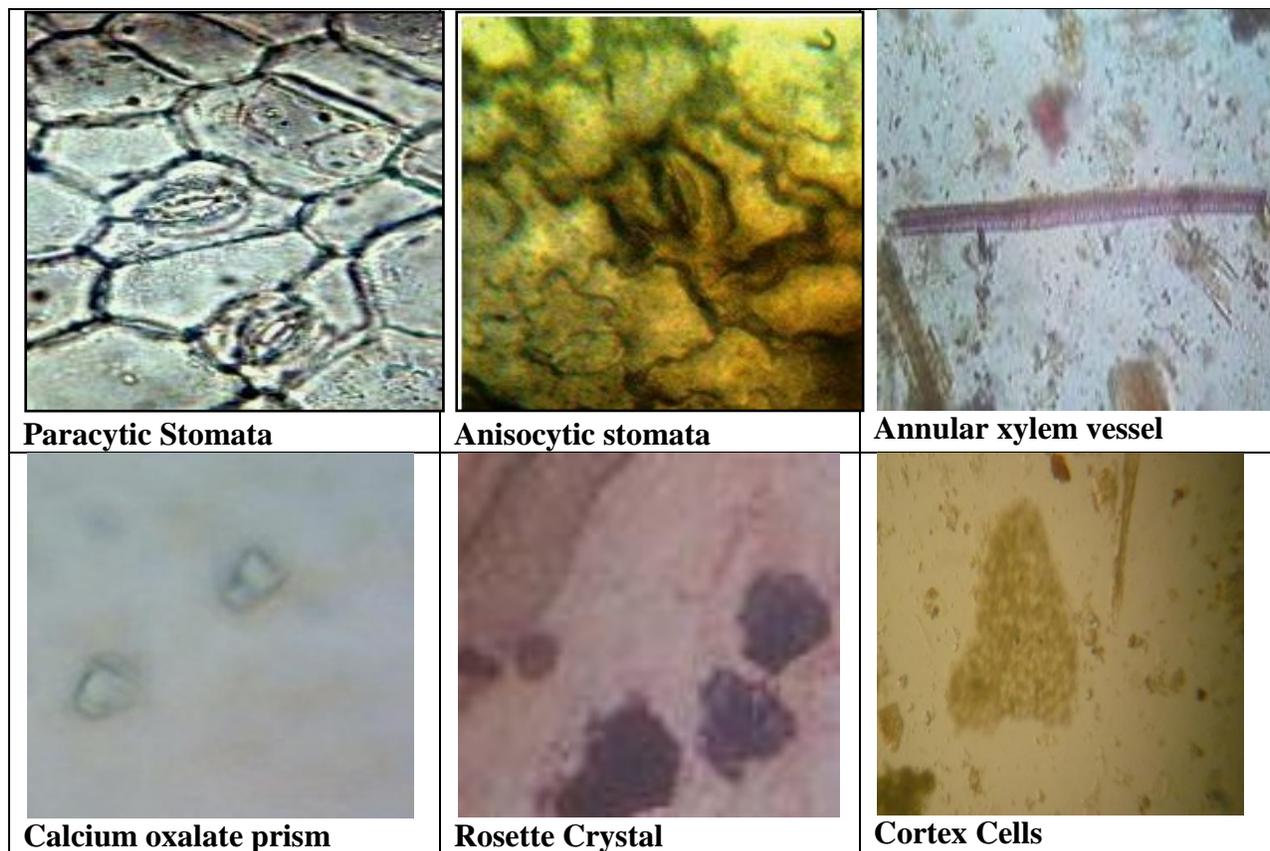


Figure 5: Detailed microscopy of *Phyllanthus amarus* fruit

Powder Study:

Showed (Figure 6) upper and lower epidermis of leaf with anticlinal walls, embedded with paracytic and anomocytic stomata. They being more on lower epidermis, the epidermis cells are bigger in size; transversely cut fragments of lamina showing upper epidermis and row of palisade underneath it. Rosette and prismatic crystal of calcium oxalate in parenchymatous cells, fragments of pitted spiral vessels and fibres in stem.



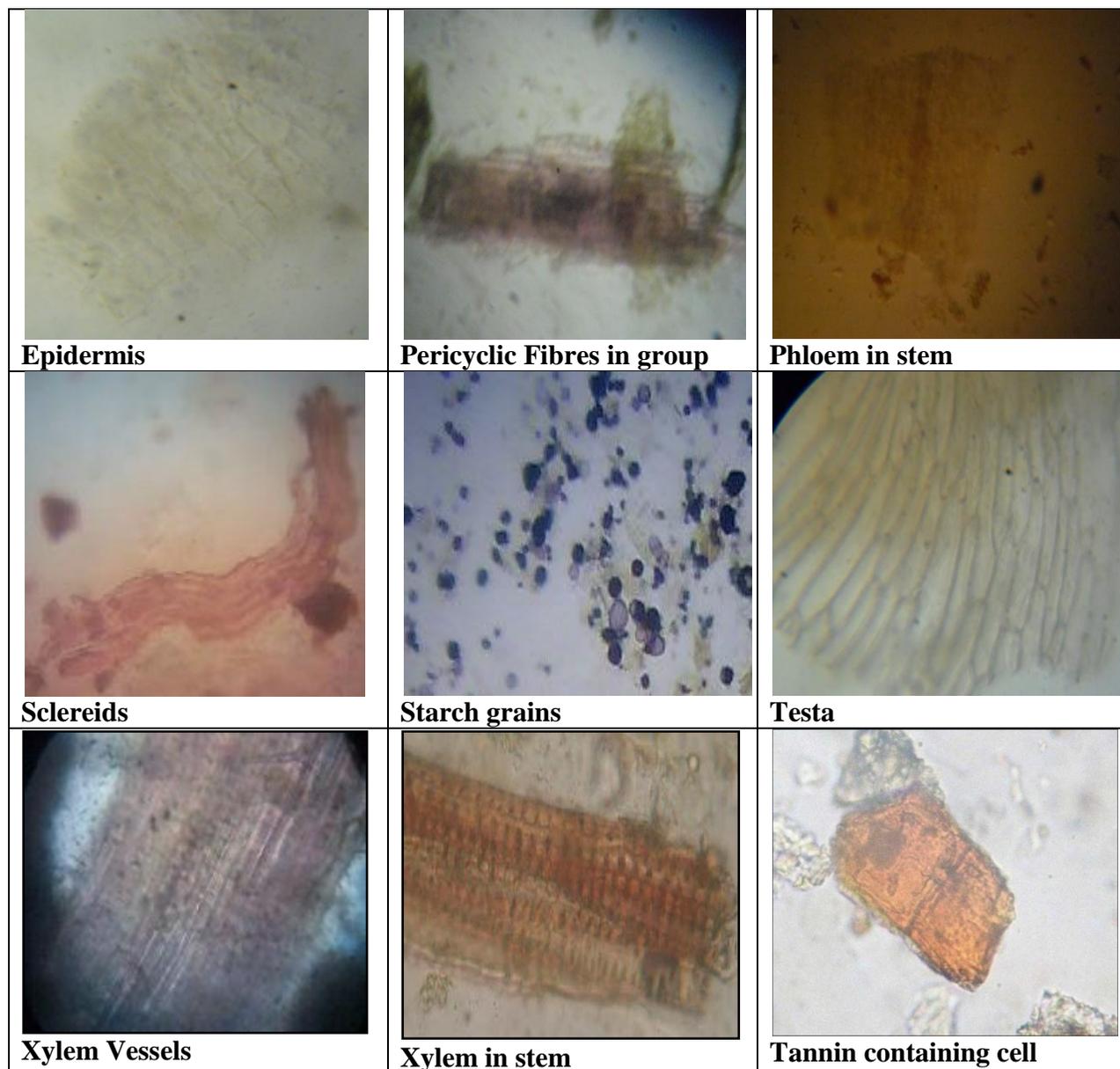


Figure 6: Powder study of *Phyllanthus amarus* crude powder

[Track 1 *Phyllanthus amarus* water extract; Track 2 *Phyllanthus amarus* crude powder; Track 3 Phyllanthin Standard]

Table 2: Physico-chemical parameters for *Phyllanthus amarus*

<i>Phyllanthus amarus</i>	Ash Value			Extractive value		Moisture content	Determination of pH
	Total Ash	Acid insoluble ash	Water soluble ash	Water soluble	Alcohol soluble		
Crude powder	6.58 ± 0.09	1.75	4.42	16.39 ± 0.088	22.51 ± 0.192	4.23 ± 0.001	4.25
Water extract	4.28 ± 0.057	0.55	3.1	87.58 ± 0.355	12.23 ± 0.97	4.64 ± 0.014	4.76

Physico-chemical parameters

Various physicochemical parameters were performed on crude powder as well as water extract of *Phyllanthus amarus* given in Table 2.

Results showed that ash value of crude powder as well as water extract of *Phyllanthus amarus* were in limit as per Ayurvedic pharmacopoeia of India. Total ash value of crude powder and water extract were 6.58 and 4.28 respectively. Results showed that water soluble extractive value of crude powder is 16.39 while alcohol soluble extractive value was 22.51, while water soluble extractive value of water extract was more than 70%, which indicated good quality of water extract. Water soluble and alcohol extractive values for crude powders were fulfilling their specific limits as per Ayurvedic pharmacopoeia of India. Results showed that moisture content for crude powder and water extract were less than 5% w/w; fulfilling the pharmacopoeial limit and pH for all crude powder as well as water extracts were less than 5.

Preliminary Phytochemical Investigation

Crude powder and water extract of *Phyllanthus amarus* were investigated for the preliminary phytochemical analysis. Results were given in Table 3. Result indicated that crude powder as well as water extract of *Phyllanthus amarus* had alkaloid, glycoside, flavonoid, saponin, tannin, sterol and carbohydrate. This showed better quality of extract was used in formulation.

Table 3: Preliminary Phytochemical investigation

Sample		Phytoconstituents						
		Alkaloid	Glycoside	Flavonoid	Saponin	Tannin	Sterol	Carbohydrate
<i>Phyllanthus Amarus</i>	Crude powder	Positive	Positive	Positive	Positive	Positive	Positive	Positive
	Water extract	Positive	Positive	Positive	Positive	Positive	Positive	Positive

TLC study

Thin layer chromatography of *Phyllanthus amarus* whole plant crude powder, water extract and standard phyllanthin was carried out using Toluene: Ethylacetate: formic acid (5:4:1) as solvent system. As a result crude powder obtained R_f at 0.13, 0.28, 0.65, 0.80; water extract obtained R_f at 0.12, 0.28, 0.43, 0.65 and 0.80 while standard phyllanthin gave R_f at 0.65 viewed under 254 nm. Result showed the presence of spot of phyllanthin at R_f 0.65 in crude powder as well as water extract which was comparable with as that of standard phyllanthin.

R_f value of phyllanthin as well as other compound were given in Table 4 while the image of TLC was given in Figure 7.

Table 4: TLC study of *Phyllanthus amarus*

No. Of obtained bands	Rf value at 254 nm		
	Water extract	Crude powder	Std. Phyllanthin
1	0.13	0.12	0.65
2	0.28	0.28	
3	0.65	0.43	
4	0.80	0.65	
5		0.80	

**Figure 7: TLC study of *Phyllanthus amarus* under 254 nm**

CONCLUSION

Preliminary phyto-chemical as well as various aspects of the sample were studied and described along with physico-chemical, microscopic and TLC studies in authentication, adulteration for quality control of raw drugs. The plant of *Phyllanthus amarus* exhibit a set of diagnostic characters, which will help to standardise the drug as well as appetizer formulation.

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