Pharmacognostic Evaluation of substitute fruit drugs *Kashmarya(Gmelina arborea* Roxb.*)* & *Draksha*(*Vitis vinifera* Linn.)

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ABSTRACT

About: *Pratinidhi Dravyas* are substitutes mentioned in classics based on similar pharmacological activities. Fruits of *Kashmari(Gmelina arborea* Roxb.) and *Draksha(Vitis vinifera* Linn.) have been advised to take as substitutes since long. Drug standardization parameters are taken here to compare basic macro-microscopic, phytochemical similarity of these drugs. Materials and Methods: Fresh fruits were collected documented for macro-microscopic, physicochemical and HPTLC, phytochemical standards. Results: Macro-[microscopic] standards of both drugs documented. Chemical similarity observed among both indicates the idea behind substitution.

(Keywords: *Pratinidhi dravya, Kashmarya, draksha, pharmacognostic*)

INTRODUCTION

Plants are prime sources of therapeutics and diet since time immemorial. Many medicinal plants have been added from vedic era till lexicon period and few have been found unavailable because of many reasons [1]. Recently increased demand on natural resources has faced unavialablity of few valuable medicinal plants [2]. Pratinidhi dravyas are the substances which have similar pharmacological properties as the original drug but may not have a similar appearance [3]. Ayurveda science elaborates in later texts to use a set of medicinal plants in the non-availability of original drug [4]. Fruits of Draksha are advised to take in the scarcity of Kashmari(*Gmelina arborea* Roxb.) [5].

Fruits of Kashmari (Gmelina arborea Roxb.) are among widely described fruits in classical texts of Ayurveda. Though roots are mentioned under Dashamoola, edible fruits advised as rejuvenating, nutritious, beneficial in ailments of blood [6]. Sweet fruits are recommended in many therapeutic conditions like bleeding piles, fever, thirst, gout, atrophy of foetus, internal haemorrhage, anaemia, greying of hairs [7]. Butyric acid, tartaric acid (trace), resinous matter, saccharine matter, cardiac glycosides & Steroids are chief phytochemical constituents of fruit [8]. A medium sized deciduous tree commonly distributed in deciduous forests throughout India. Fruits are drupe, fleshy, ovoid, turning yellow orange when ripe with 2 seeds [9].

Draksha (Vitis vinifera Linn.) cultivated for its fruits ranging from sour to sweet taste which has been a luxurious traded commodity since long [10]. These are widely grown in western parts of India, Punjab, Kashmir, Central Europe, Turkey, Morocco, and Portugal [11]. The grape vine has long woody stems rooted to ground. Stems are covered with flaky bark. It grows up to 35 meters. It has alternate, broad, palmate leaves. The unripe fruits are green and ripe fruits are dark purple in colour [12]. Sugar, gum, tannin, tartaric acid, citric acid, malic acid, potassium chloride, magnesia and alum are chief phytochemical constituents of fruits [13]. These are laxative, cooling, antiallergic, digestant, haemostatic and anti-inflammatory[14]. Due to its medicinal properties, it increases the moistness of our body tissues and softens the dried ones. Hence these are names as Mridweeka in Sanskrit [15].

Both the fruits find mentioned in *madhura skanda, phala varga, virechanopaga dashemani* and they also form a part of the trio - '*Madhura Triphala'*.[16] The *kashaya* preparation of *kashmarya phala* as well as *draksha* was indicated for inducing *virechana* especially indicated in *panduroga* (anemia)[17]. These fruits are also under abhava pratinidhi dravyas; as in the non avaialablity of one drug other can be used. Both these fruits possess Madhura rasa, Sheeta veerya and rejuvenating, beneficial in blood disorders [18].

Table1: Pharmacotherapeutics of Draksha and Gambhari [18]

	Kashmarya Phala	***Draksha***
Rasa	*Madhura*	*Madhura*
Guna	*Guru, Snigdha*	*Snigdha, Guru*
Veerya	*Sheeta*	*Sheeta*
Vipaka	*Madhura*	*Madhura*
Doshaghnata	*Vata-Pittahara*	*Vata- Pittahara*
Karma	*Brimhana, Hridya, Trishnahara, Dahashamaka, Vrishya, Rasyana, Medhya, Keshya, Mutrala, Rakta-pitta shamaka, Vishaghna, Sandhaniya*	*Brimhana, Hridya Balya, Jwarahara, Trishnahara, Daha-shamaka, Vrishya, Mutrala, Ruchya, Swarya, Chakshushya*

MATERIALS AND METHODS

Drug Source

Fresh fruits of *Gmelina arborea* Roxb. And seeded variety of fresh fruits of *Vitis vinifera* Linn. collected from their natural habitat. The samples were thoroughly washed, and dried in shade under controlled conditions to avoid damage and fungal degradation. Few fresh samples kept in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml) solution for microscopic study [19].

Macroscopy

The external features of the fresh fruits were observed by the naked eye and were then documented using Canon IXUS digital camera. The macroscopic features were compared with local flora for authentication [20].

Microscopy

The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axiocam camera under bright field light. Magnifications of the figures are indicated by the scale-bars [21].

Powder microscopy

A pinch of the shade dried and powdered sample of the test drug was mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using using Zeiss AXIO trinocular microscope attached with Zeiss Axiocam camera under bright field light. Magnifications of the figures are indicated by the pre-calibrated scale-bars using Zeiss AxioVision software[22]

Physicochemical study

Powdered test drug samples were subjected for physico-chemical tests like loss on drying at 105ºC, total ash, acid insoluble ash, water-soluble ash, alcohol-soluble extractive and water- soluble extractive based on the standard guidelines [23].

Phytochemical study

Phytochemical tests of samples were conducted for the presence of secondary metabolites like alkaloids, carbohydrates, steroids, saponins, tannins, flavonoids, phenols, coumarins, triterpenoids, carboxylic acids, resin and quinones [24].

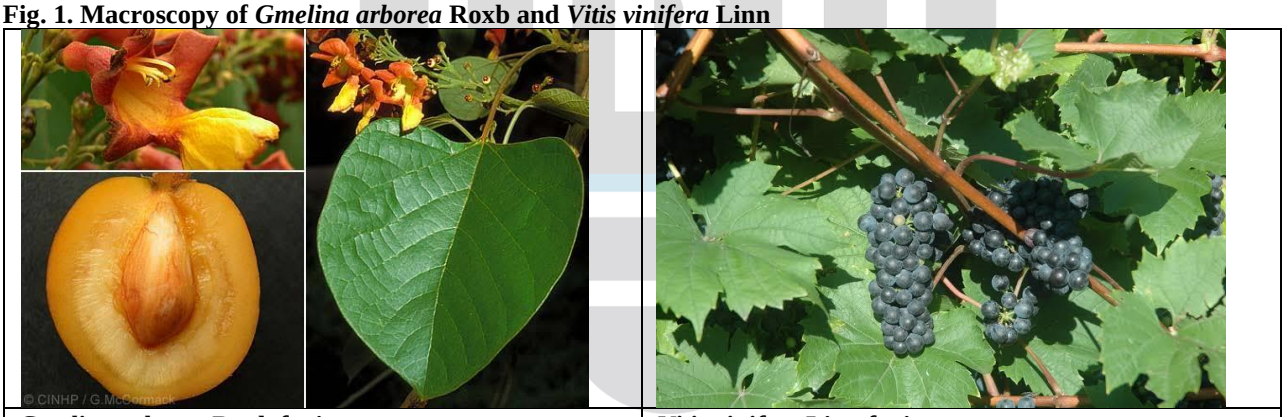
HPTLC[25]

10.0 ml extract of each sample powder was fractionated in a separating funnel with 20.0ml of butanol. Butanol fraction thus obtained was dried and dissolved in 10.0ml methanol. 4 and 8µl of the above samples were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in **Toluene: Ethyl Acetate: Formic acid (5.0: 4.0: 0.2).** The developed plates were visualized in short UV, long UV and then derivatised with Vanillin sulphuric acid (ASA) reagent and scanned under UV 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were recorded.

RESULT

Macroscopy

Fig. 1. Macroscopy of *Gmelina arborea* Roxb and *Vitis vinifera* Linn



***Gmelina arborea* Roxb fruit**	***Vitis vinifera* Linn fruit**

Microscopy

The microscopic section of fruit of *Gmelina arborea* Roxb. shows pericarp differentiated into epicarp and mesocarp. The epicarp has few layers of thin-walled longitudinal cells. Pigment cells are seen frequently in between cells at the outer layers with occasional presence of sclereids and stone cells. The mesocarp has large, thin-walled, multi-layered iso-diametric parenchymatous cells with supply of vascular strands. Endocarp is not appreciated well.

Vitis vinifera Linn. shows pericarp differentiated into a single -layered epicarp and a multi-layered mesocarp. Epicarp consists of thin-walled irregular cells with frequent occurrence of pigment cells stone cells and prismatic crystals of calcium oxalate. Mesocarp is pulpy with large cells and abundant vascular strands. (Figure 2 &3)

Powder microscopy

Powder microscopy of fruit of *Gmelina arborea* Roxb. shows multi-layered epicarp with sclereids underneath the thick layers of straight-walled epidermis. Mesocarp shows scattered cells of vessels, sclerenchyma and trichomes. Stone cells and cells containing starch are seen with pigmented layers.

Vitis vinifera Linn. Fruit shows epicarp with pigment cells and remains of pollen grains. Mesocarp shows presence of stone cells. Stone cells were derived from endocarp of *Vitis vinifera* Linn. when it was macerated with glycerine. (Figure 4&5)

Figure 2: Microscopy of *Gmelina arborea* Roxb. fruit

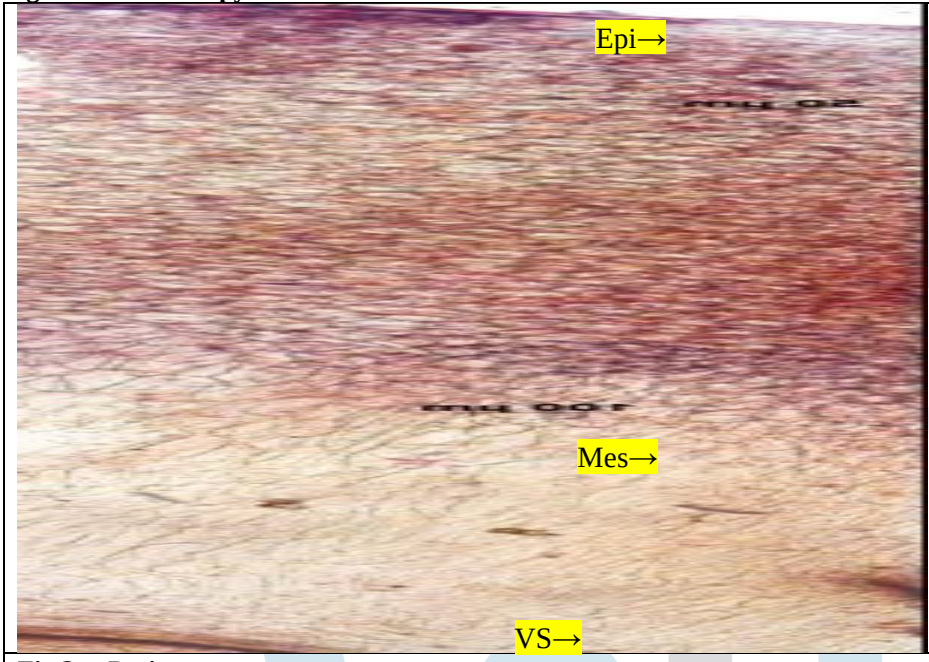


Fig 2.a. Pericarp

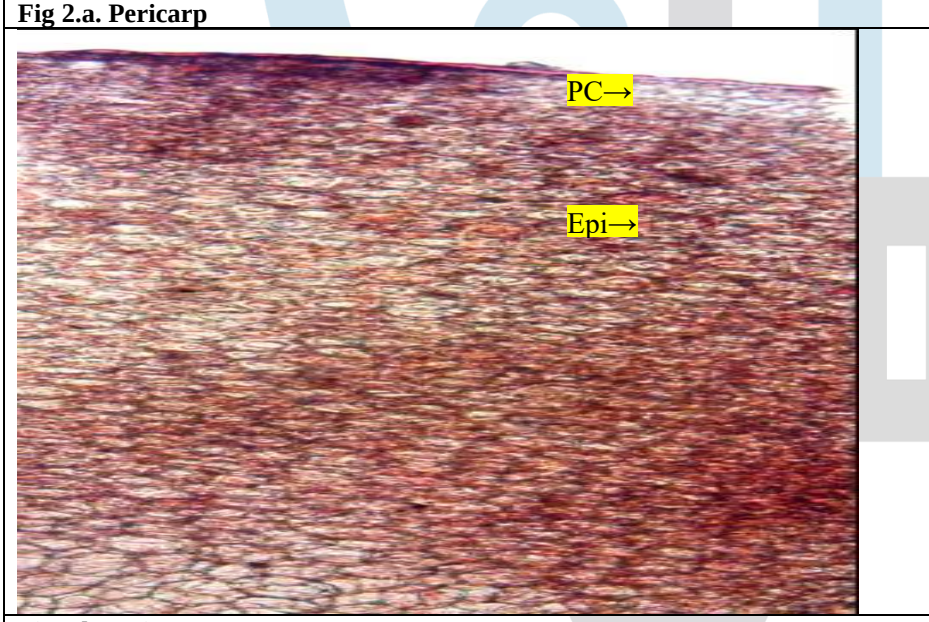


Fig 2.b. Epicarp

Epi – epicarp; Mes – mesocarp; PC – pigment cell; VS – vascular strand

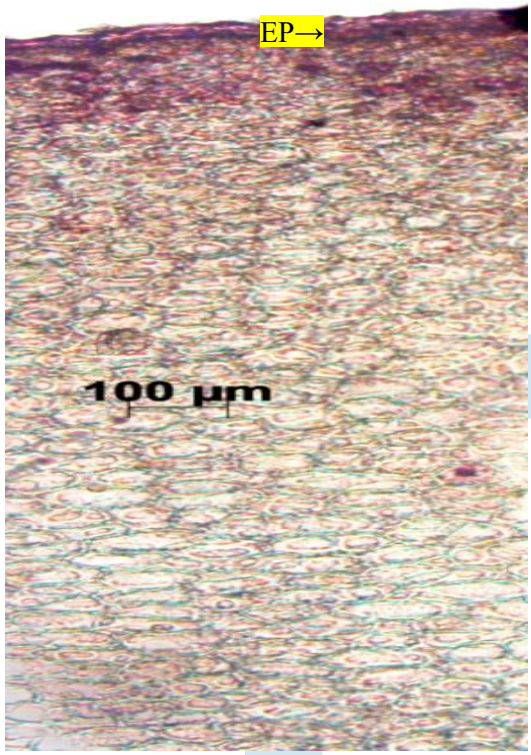


Fig 2.c. Pericarp

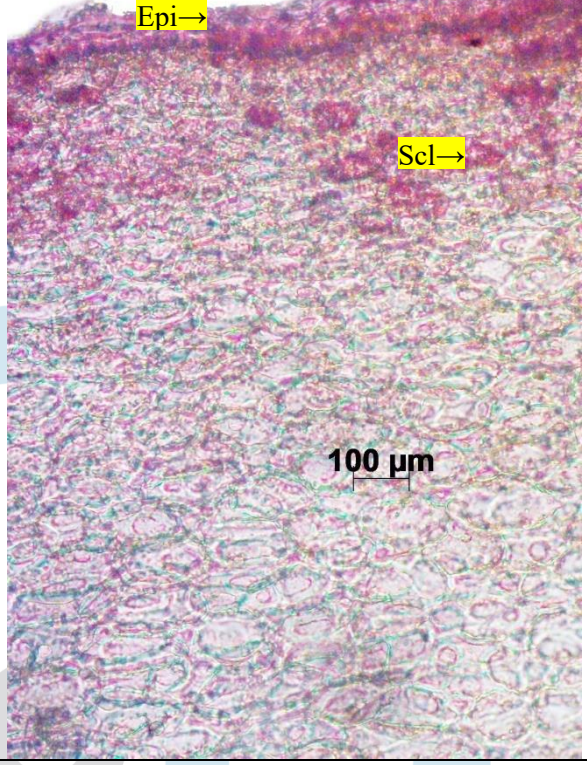


Fig 2.d. Epidermis, epicarp and Sclereids

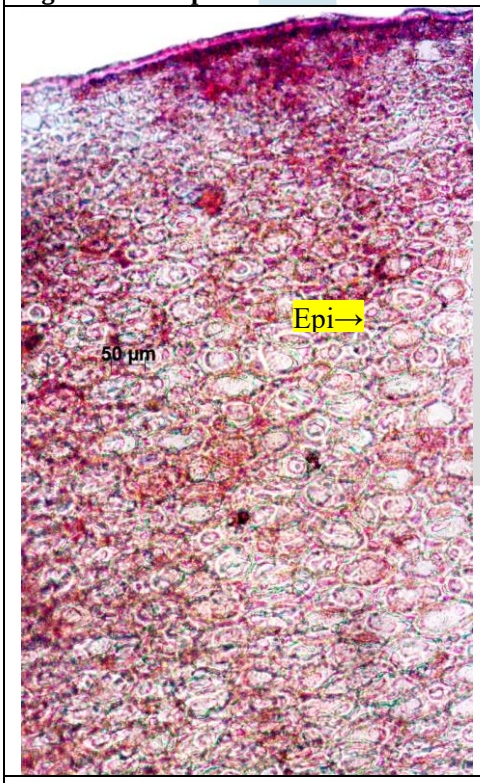


Fig 2.e. Epicarp

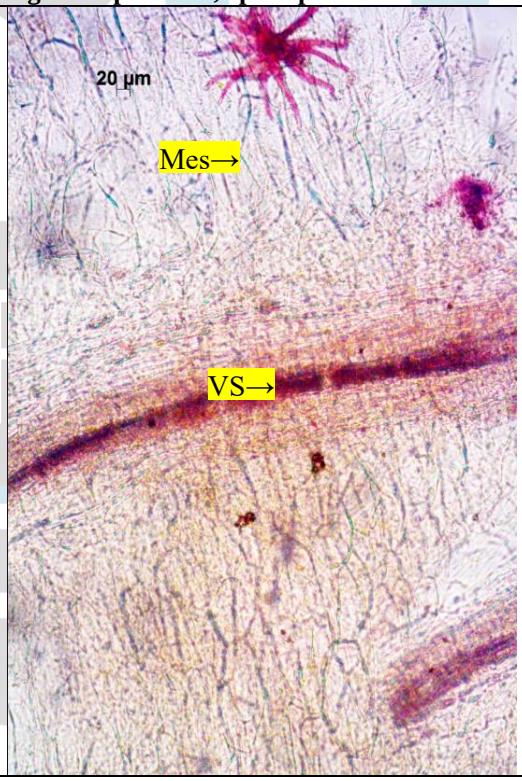


Fig 2.f. Mesocarp and vascular strand

Figure 3: Microscopy of *Vitis vinifera* Linn. fruit

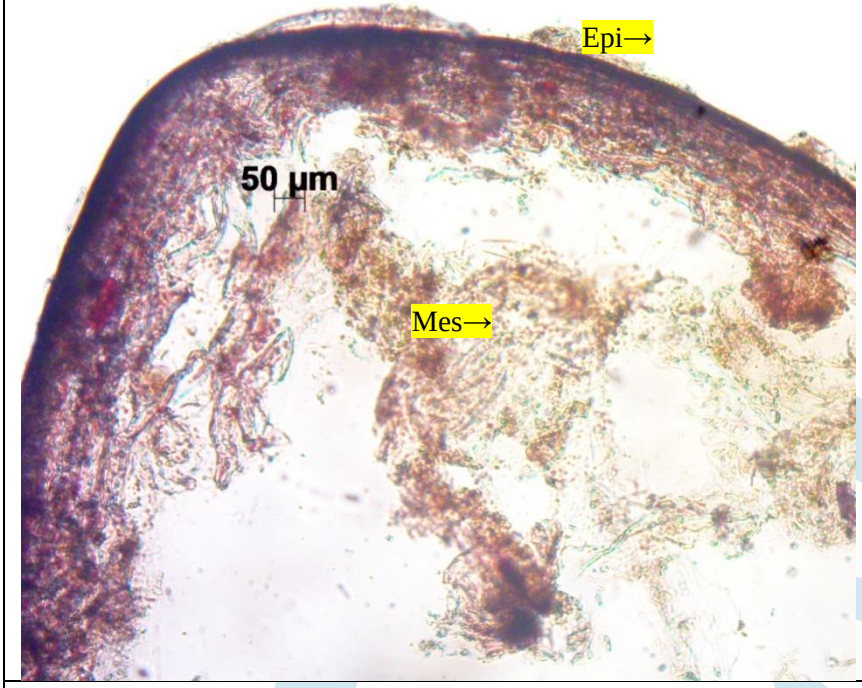


Fig 3.a. Pericarp (Epicarp and mesocarp)

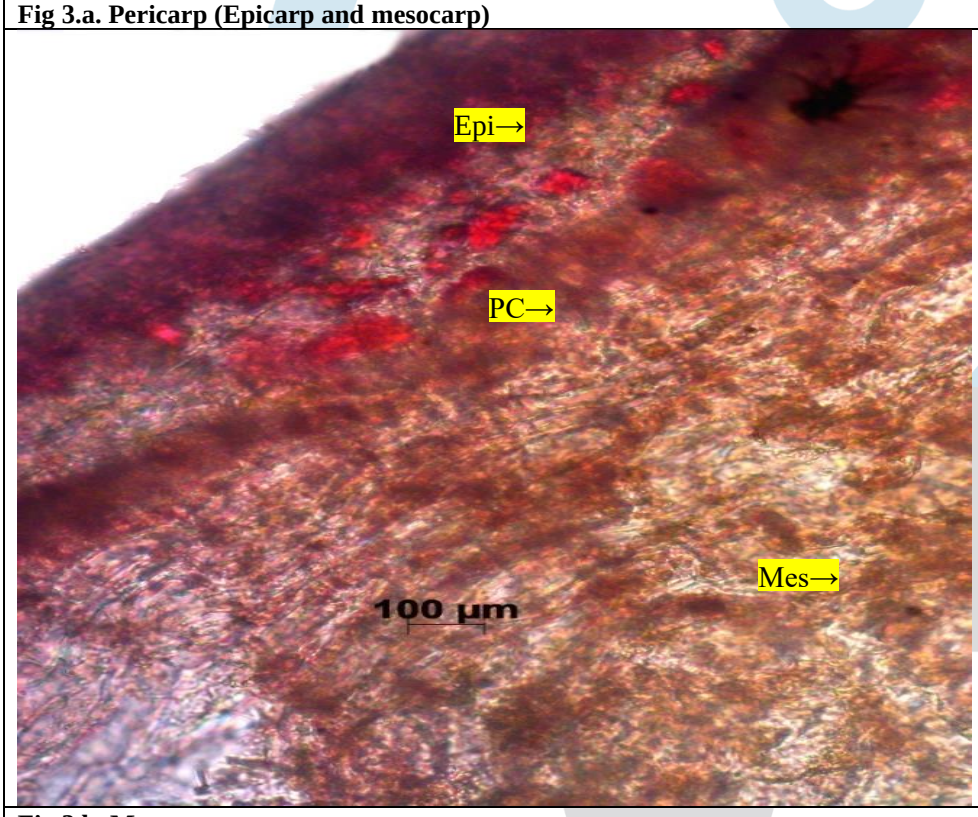


Fig 3.b. Mesocarp

Epi – epicarp; Mes – mesocarp; PC –pigment cells

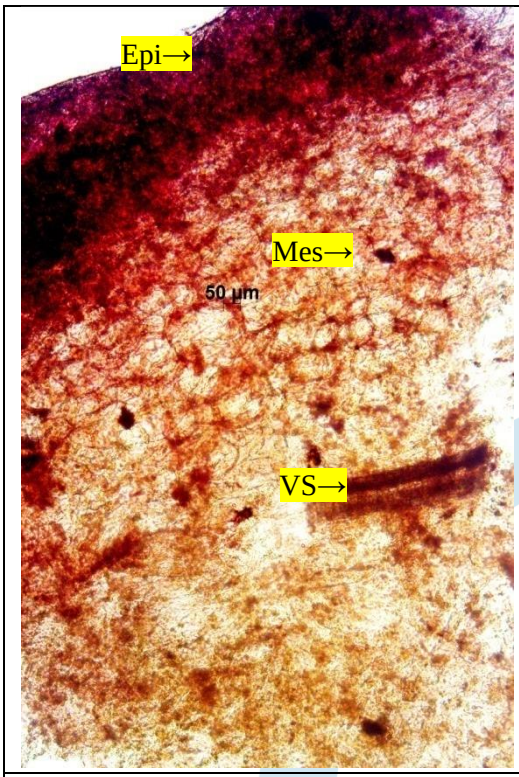


Fig 3.c. Mesocarp containing vascular strand

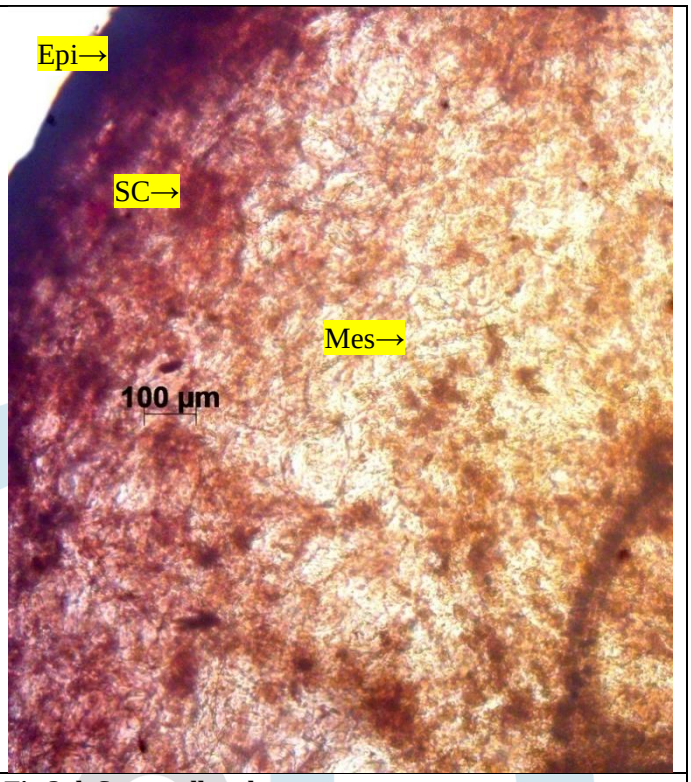


Fig 3.d. Stone cell and mesocarp

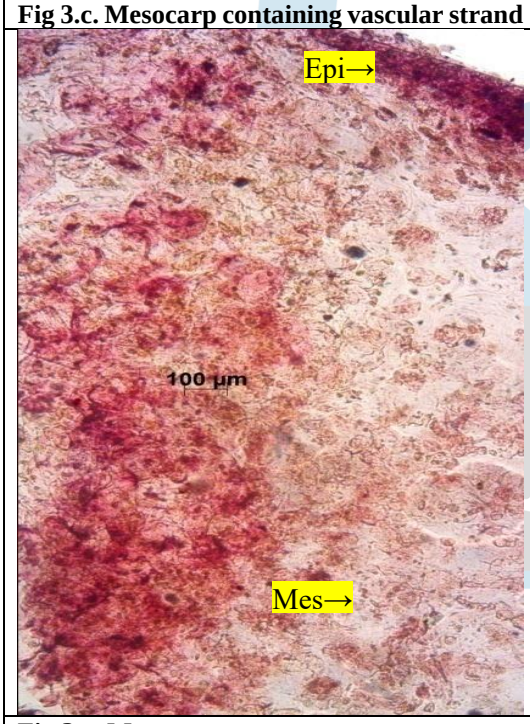


Fig 3.e. Mesocarp

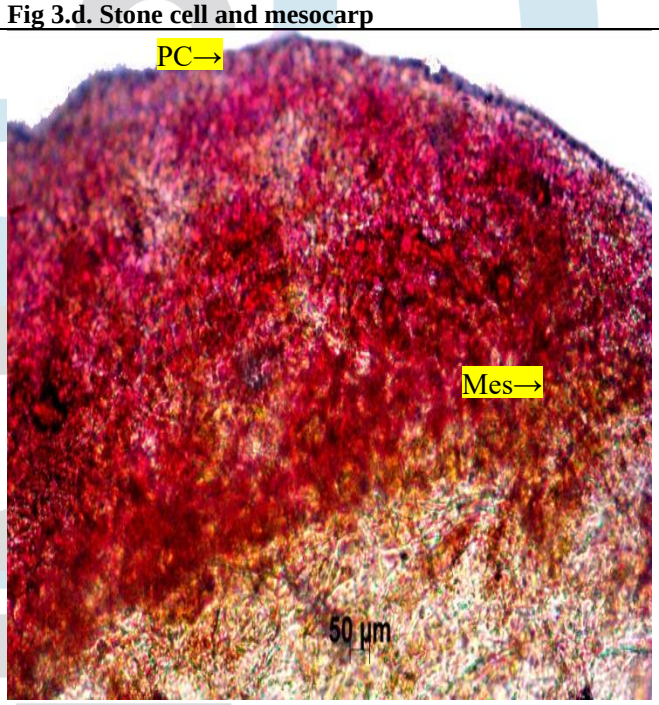


Fig 3.f. Pigment cell, Stone cell and Mesocarp

Epi – epicarp; Mes – mesocarp; PC – pigment cells; SC –stones cells; VS – vascular strand

Figure 4: Powder-microscopy of *Gmelina arborea* Roxb

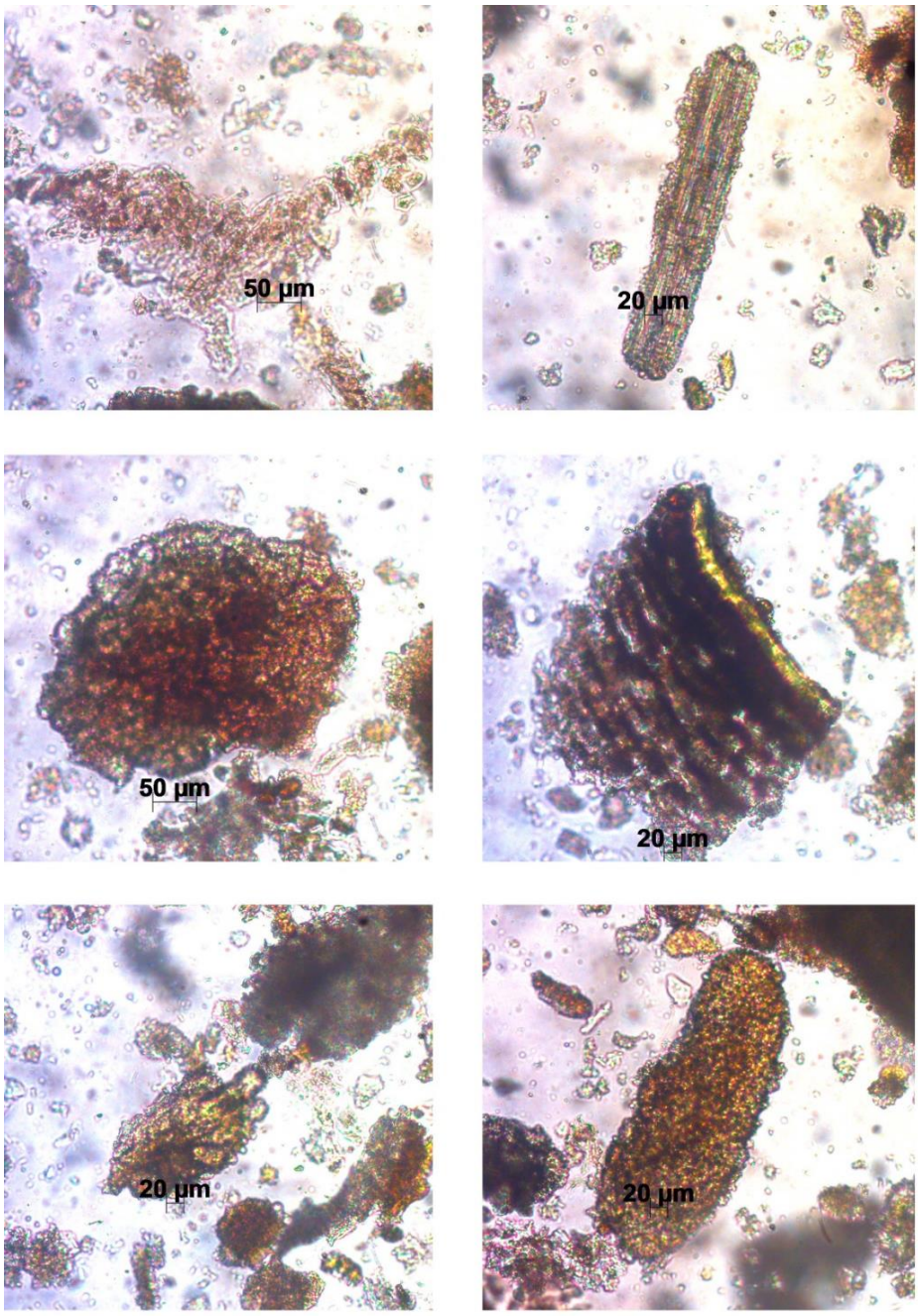
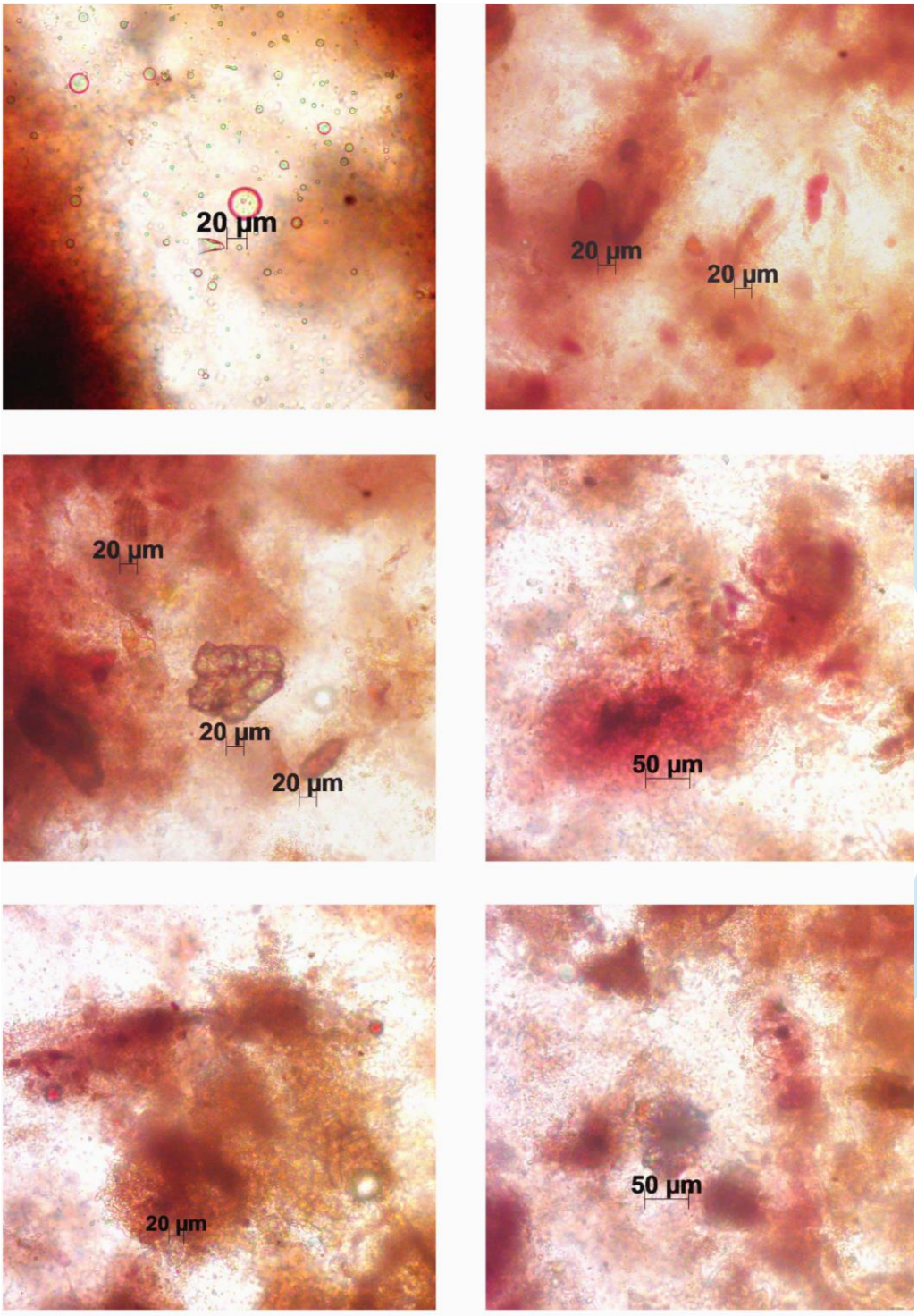


Figure 5: Powder Microscopy of *Vitis vinifera* Linn



Physicochemical study

Table 1. Physicochemical standards

Parameter	**Results n= 3 %w/w Avg ± SD**	
	Gmelina arborea Roxb.	*Vitis vinifera* Linn
Loss on drying	8.26±0.00	26.89±0.01
Total Ash	4.28±0.02	2.78±0.01
Acid Insoluble Ash	0.17±0.01	0.09±0.01
Water soluble Ash	4.11±0.01	2.67±0.02
Alcohol soluble extractive value	22.18±0.01	46.27±0.01
Water soluble extractive value	48.06±0.01	47.49±0.01

Phytochemical study

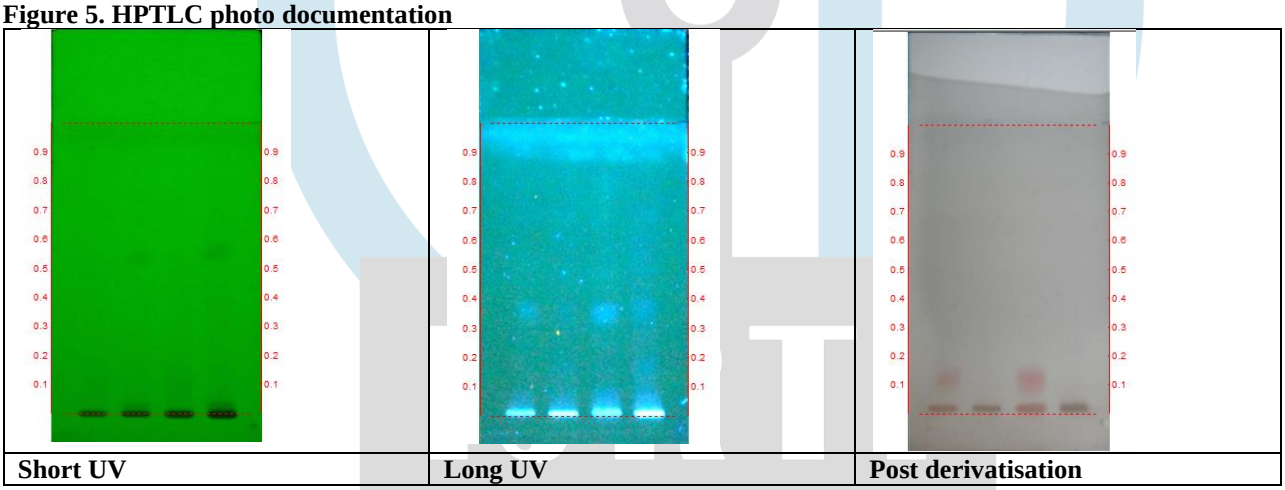
Table 2: Preliminary phytochemical study

Test	**Inference**	
	***Gmelina arborea* Roxb**	***Vitis vinifera* Linn**
Alkaloid	+	+
Steroid	+	
Carbohydrate	+	+
Tannin	+	-
Flavanoids	-	+
Saponins	-	-
Tri terpenoid	-	+
Coumarins	-	-
Phenols	-	-
Carboxylic acid	+	+
Amino acids	-	+
Resin	+	+
Quinone	-	-

(+) – Present; (-) – Negative

HPTLC

Figure 5. HPTLC photo documentation



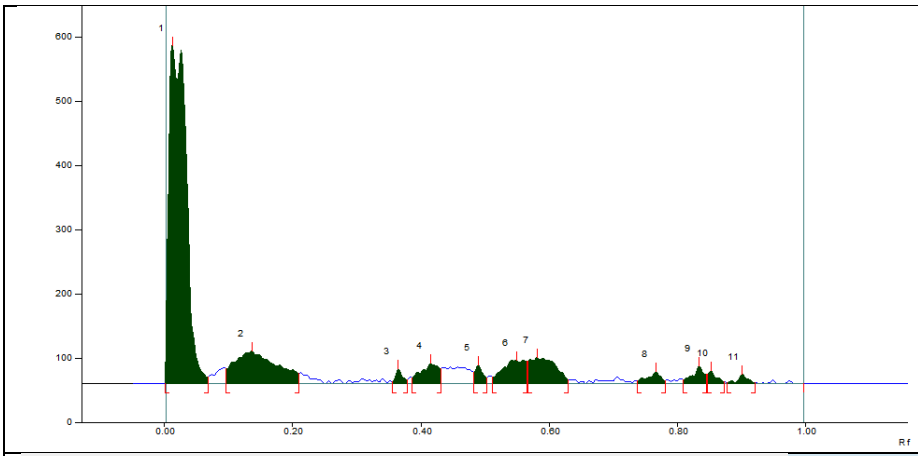
Short UV	**Long UV**	**Post derivatisation**

Track 1 -– *Draksha (V vinifera)* 4µl
Track 2 — *Kashmarya (G arborea*)4µl
Track 3 -– *Draksha (V vinifera)*8µl
Track 4 - – *Kashmarya(G arborea)* 8µl
Solvent system – Toluene: Ethyl Acetate: Formic acid (5.0: 4.0: 0.2)

Table 3: R_f values

Short UV		**Long UV**		**Post derivatization**	
Draksha (V vinifera)	*Kashmarya (G arborea)*	*Draksha (V vinifera)*	*Kashmarya (G arborea)*	*Draksha(V vinifera)*	*Kashmarya(G arborea)*
-	-	-	-	**0.13 (Pink)**	-
0.56 (Green)	0.56 (Green)	0.56 (F. blue)	0.56 (F. blue)	-	-

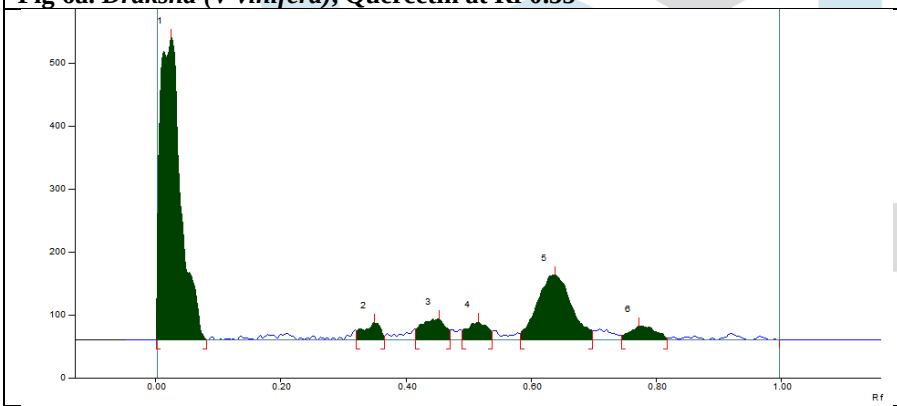
*****F – Fluorescent; L –Light; D – Dark



Track 3, ID: Draksha (Vitis vinifera)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	524.6 AU	64.32 %	0.07 Rf	10.3 AU	10293.2 AU	61.08 %
2	0.10 Rf	23.7 AU	0.14 Rf	50.8 AU	6.22 %	0.21 Rf	16.0 AU	2383.7 AU	14.14 %
3	0.36 Rf	2.7 AU	0.36 Rf	22.5 AU	2.76 %	0.38 Rf	5.2 AU	178.5 AU	1.06 %
4	0.39 Rf	9.3 AU	0.42 Rf	31.8 AU	3.90 %	0.43 Rf	23.5 AU	636.7 AU	3.78 %
5	0.48 Rf	17.0 AU	0.49 Rf	28.2 AU	3.46 %	0.50 Rf	9.2 AU	268.5 AU	1.59 %
6	0.51 Rf	10.1 AU	0.55 Rf	36.5 AU	4.47 %	0.57 Rf	34.1 AU	938.0 AU	5.57 %
7	0.57 Rf	34.8 AU	0.58 Rf	40.4 AU	4.95 %	0.63 Rf	6.1 AU	1198.2 AU	7.11 %
8	0.74 Rf	4.0 AU	0.77 Rf	18.7 AU	2.29 %	0.78 Rf	4.7 AU	277.7 AU	1.65 %
9	0.81 Rf	6.1 AU	0.84 Rf	27.2 AU	3.33 %	0.85 Rf	13.9 AU	338.2 AU	2.01 %
10	0.85 Rf	14.5 AU	0.85 Rf	20.0 AU	2.45 %	0.87 Rf	3.0 AU	182.9 AU	1.09 %
11	0.88 Rf	1.0 AU	0.90 Rf	15.0 AU	1.84 %	0.92 Rf	1.4 AU	156.7 AU	0.93 %

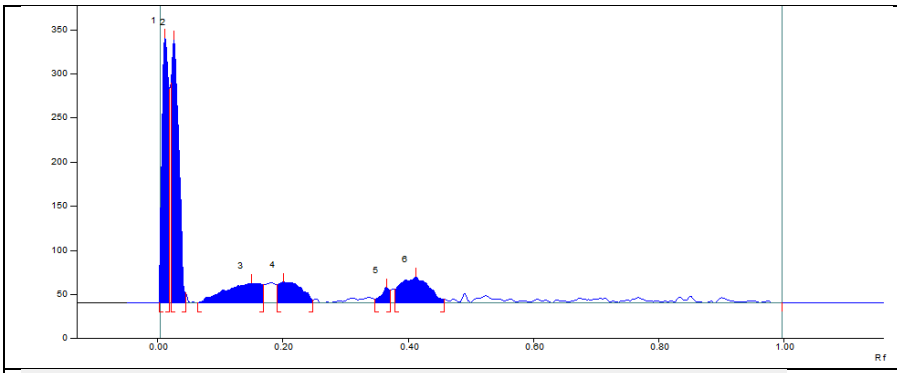
Fig 6a. ***Draksha (V vinifera)*****, Quercetin at Rf 0.55**



Track 4, ID: Gambhari (Gmelina arborea)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	478.4 AU	69.05 %	0.08 Rf	0.2 AU	11327.0 AU	62.59 %
2	0.32 Rf	14.9 AU	0.35 Rf	27.9 AU	4.03 %	0.37 Rf	6.3 AU	535.0 AU	2.96 %
3	0.42 Rf	16.9 AU	0.45 Rf	33.0 AU	4.76 %	0.47 Rf	15.3 AU	907.7 AU	5.02 %
4	0.49 Rf	15.1 AU	0.52 Rf	28.4 AU	4.10 %	0.54 Rf	13.4 AU	655.7 AU	3.62 %
5	0.58 Rf	9.9 AU	0.64 Rf	103.3 AU	14.91 %	0.70 Rf	15.0 AU	4007.9 AU	22.15 %
6	0.75 Rf	6.7 AU	0.77 Rf	21.9 AU	3.16 %	0.82 Rf	2.5 AU	662.7 AU	3.66 %

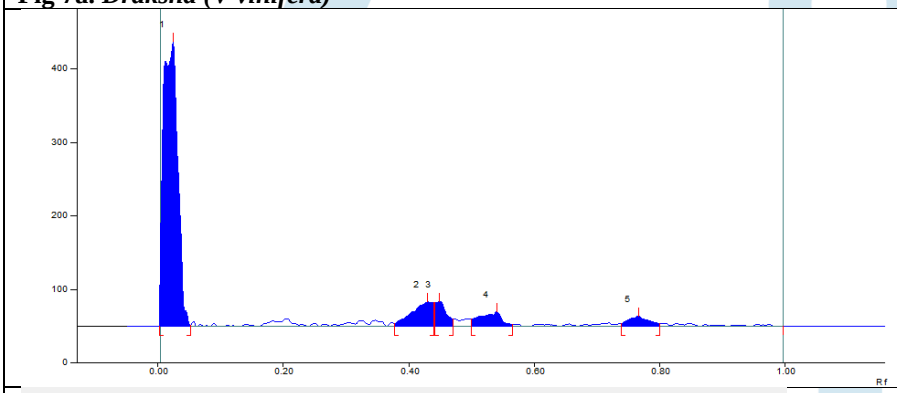
Fig 6b. ***Kashmarya (G arborea)*****, Quercetin at Rf 0.52**



Track 3, ID: Draksha (Vitis vinifera)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	300.4 AU	43.44 %	0.02 Rf	41.5 AU	2438.3 AU	31.03 %
2	0.02 Rf	249.2 AU	0.03 Rf	298.3 AU	43.15 %	0.05 Rf	11.7 AU	2675.4 AU	34.05 %
3	0.06 Rf	0.2 AU	0.15 Rf	22.4 AU	3.24 %	0.17 Rf	20.4 AU	942.3 AU	11.99 %
4	0.19 Rf	20.9 AU	0.20 Rf	23.4 AU	3.39 %	0.25 Rf	3.1 AU	655.6 AU	8.34 %
5	0.35 Rf	4.4 AU	0.37 Rf	17.4 AU	2.51 %	0.37 Rf	14.1 AU	180.8 AU	2.30 %
6	0.38 Rf	15.4 AU	0.41 Rf	29.5 AU	4.27 %	0.46 Rf	3.8 AU	965.1 AU	12.28 %

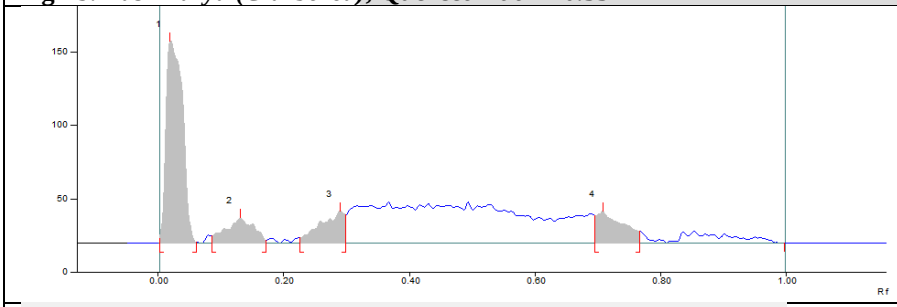
Fig 7a. ***Draksha (V vinifera)***



Track 4, ID: Gambhari (Gmelina arborea)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	387.0 AU	79.31 %	0.05 Rf	1.2 AU	6390.5 AU	75.55 %
2	0.38 Rf	4.0 AU	0.43 Rf	33.2 AU	6.80 %	0.44 Rf	32.5 AU	811.8 AU	9.60 %
3	0.44 Rf	32.6 AU	0.45 Rf	33.9 AU	6.94 %	0.47 Rf	9.6 AU	445.7 AU	5.27 %
4	0.50 Rf	10.1 AU	0.54 Rf	19.9 AU	4.08 %	0.57 Rf	2.0 AU	487.7 AU	5.77 %
5	0.74 Rf	3.5 AU	0.77 Rf	14.0 AU	2.87 %	0.80 Rf	3.4 AU	322.7 AU	3.82 %

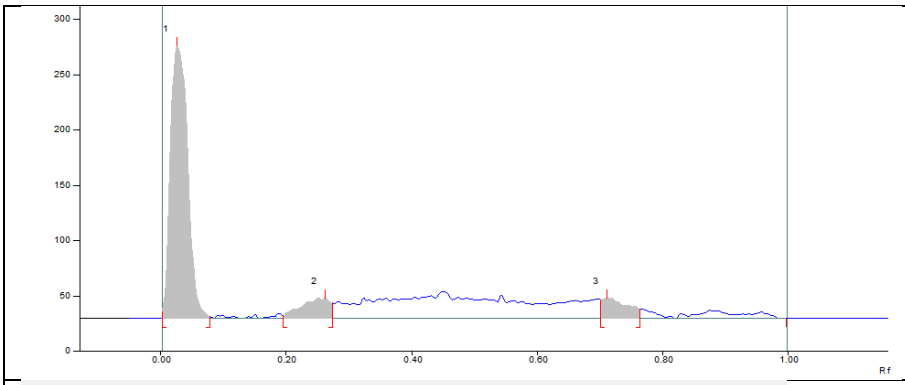
Fig 7b. ***Kashmarya (G arborea)*****, Quercetin at Rf 0.55**



Track 3, ID: Draksha (Vitis vinifera) fruit

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	3.3 AU	0.02 Rf	137.1 AU	69.27 %	0.06 Rf	0.3 AU	2589.1 AU	58.99 %
2	0.09 Rf	4.7 AU	0.13 Rf	17.1 AU	8.62 %	0.17 Rf	1.7 AU	548.9 AU	12.51 %
3	0.23 Rf	3.5 AU	0.29 Rf	22.0 AU	11.11 %	0.30 Rf	19.4 AU	594.0 AU	13.53 %
4	0.70 Rf	18.7 AU	0.71 Rf	21.8 AU	11.00 %	0.77 Rf	8.0 AU	656.8 AU	14.97 %

Fig 8a. ***Draksha (V vinifera)***



Track 4, ID: Kashmarya (Gmelina aroborea) fruit

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	5.8 AU	0.03 Rf	246.2 AU	87.07 %	0.08 Rf	1.6 AU	4898.8 AU	80.81 %
2	0.20 Rf	2.3 AU	0.26 Rf	18.4 AU	6.51 %	0.27 Rf	13.7 AU	613.0 AU	10.11 %
3	0.70 Rf	16.9 AU	0.71 Rf	18.2 AU	6.42 %	0.76 Rf	8.2 AU	550.6 AU	9.08 %

Fig 8b. ***Kashmarya (G arborea)***

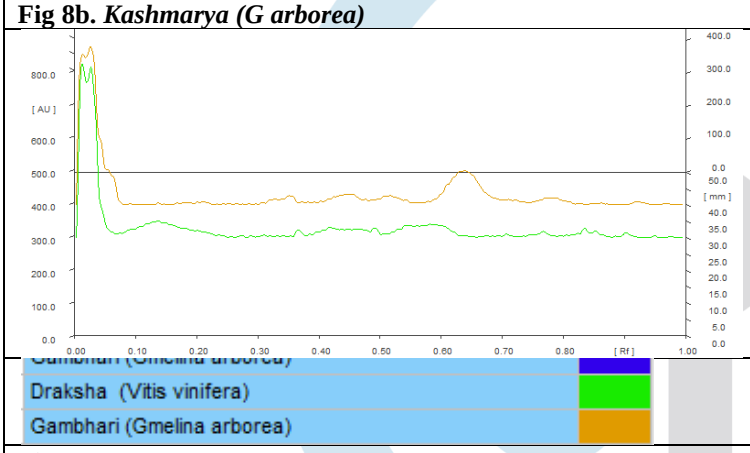


Fig 9a. At 254nm

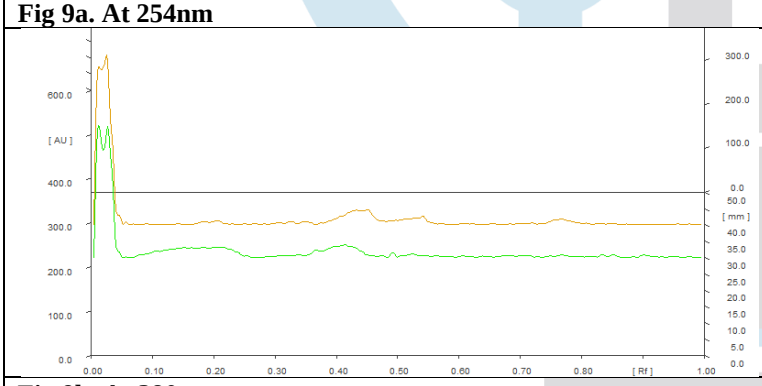


Fig 9b. At 380nm

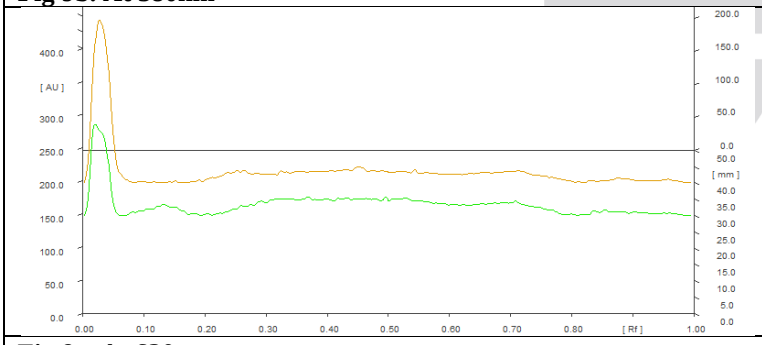


Fig 9c. At 620nm

Discussion

Today world is facing depletion of natural resources, when there is intensive demand on natural healing science like Ayurveda. *Pratinidhi Dravyas* are substitutes mentioned in classics based on similar pharmacological activities. Fruits of *Kashmari(Gmelina arborea* Roxb.) and *Draksha(Vitis vinifera* Linn.) have been advised to take as substitutes since long. Both the fruits find mentioned

in *madhura skanda, phala varga, virechanopaga dashemani* and they also form a part of the trio - '*Madhura Triphala'*. Both these fruits possess Madhura rasa, Sheeta veerya and rejuvenating, beneficial in blood disorders. Though any standard modern parameters are not yet established to study Abhava pratinidhi drugs, drug standardization parameters are taken here to compare basic macro-microscopic, phytochemical similarity of these drugs.

The fresh samples of fruits of *Kashmarya phala* were drupe, 2-2.5 cm long, ovoid or pyriform, smooth, green when raw, orange-yellow when ripe whereas *Draksha* (*Vitis vinifera* Linn.) were globose, succulent berry arising in clusters, green when raw, greenish yellow or reddish black colour when ripe depending on the variety.

Transverse section of fruit of *Gmelina arborea* Roxb. shows pericarp differentiated into epicarp and mesocarp. The epicarp has few layers of thin-walled longitudinal cells. Pigment cells are seen frequently in between cells at the outer layers with occasional presence of sclereids and stone cells. The mesocarp has large, thin-walled, multi-layered iso-diametric parenchymatous cells with supply of vascular strands. Endocarp is not appreciated well. Powder microscopy of fruit shows multi-layered epicarp with sclereids underneath the thick layers of straight-walled epidermis. Mesocarp shows scattered cells of vessels, sclerenchyma and trichomes. Stone cells and cells containing starch are seen with pigmented layers

Microscopy of fruit of *Vitis vinifera* Linn. shows pericarp differentiated into a single-layered epicarp and a multi-layered mesocarp. Epicarp consists of thin-walled irregular cells with frequent occurrence of pigment cells stone cells and prismatic crystals of calcium oxalate. The mesocarp is pulpy with large cells and abundant vascular strands. Powder microscopy shows epicarp with pigment cells and remains of pollen grains. Mesocarp shows presence of stone cells. Stone cells were derived from endocarp of *Vitis vinifera* Linn. when it was macerated with glycerine.

The physicochemical tests are used to ensure the safety, efficacy and quality parameters of the drugs. The moisture content in dried fruit of *Gmelina arborea* was found to be 8.26% whereas dried *Vitis vinifera* Linn showed 26.89%. Ash value was 4.28% and 2.78% of Kashmari and Draksha. Acid insoluble ash of both fruits were 0.17% and 0.09%. Water soluble extractive which indicates carbonaceous matter was 4.11% and 2.67% among Kashmari and Draksha. Aqueous extract both fruits have shown similar values, whereas alchoholic extract of fruits have shown huge difference.

Preliminary phytochemical analysis of *kashayas* of both *Draksha* and *Kashmari phala* were done and compared. It indicated the presence of alkaloids, steroids, carbohydrates, carboxylic acids and resins in both the drugs. Additionally, *Kashmari phala* showed presence of tannins, whereas *Draksha* showed flavonoids, triterpenoids and amino acids.

HPTLC of fruits showed numerous peaks among which R_f 0.55 ± 0.02 is the important phytochemical constituent quercetin. Quercetin is responsible for anti-oxidant activity, which acts as scavenger of free- radicals developed during cell-death and eventually being used as anti-cancer cell-protective and can be used in degenerative disorders.

Phenolic compounds and flavonoids which are rich sources of anti-oxidants are also found to be present in *Gmelina arborea* and *Vitis vinifera*. Densitometric scan at UV 254 nm showed 11 bands for *Draksha* and 6 bands for *Kashmarya*. In both samples a band at 0.55± 0.03 was observed in *Draksha* (1.59%) and *Kashmarya phala* (3.62%) respectively. Densitometric scan at UV 380 nm was evident with 6 bands for each samples among which 0.55 ± 0.02 (5.77%) was observed only in *Kashmarya* but not observed in *Draksha*. Densitometric scan at 620 nm under visible light after derivatisation with vanillin sulphuric acid showed 4 bands for *Draksha* and 3 bands for *Kashmarya* and none of them among these were for Quercetin in both.

CONCLUSION

Pratinidhi dravyas are the substances which have similar pharmacological properties are also called as substitutes. Ayurveda science gives liberty to use a set of medicinal plants in the non-availability of original drug. Fruits of *Draksha(Vitis vinifera)* are advised to take in the scarcity of Kashmari(*Gmelina arborea* Roxb.). Pharmacognostic test have been conducted to study similarity of these two drugs, form as reference standard.

CONFLICTS OF INTEREST

The corresponding author declares no conflicts of interest.

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