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COMPARATIVE PHYTOCHEMICAL ANALYSIS OF FRUIT OF *DRAKSHA* (*VITISVINIFERA* LINN) AND *KAASHMARI* (*GMELINAARBOREA* ROXB)

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ABSTRACT

Purpose

Pratinidhi Dravya- the rational substitution is a concept referred from *Bhavapraksha* written by Bhavamisra of 16th century A.D. The validation of these substitution pairs of drugs using both Ayurvedic principles and modern scientific tools is a necessity in present era as we are facing a tragic scarcity of quality drugs. This study compares one such Ayurvedic substitution pair: *Kaashmariphala* (*Gmelinaarborea*Roxb).which is high yielding and easily propagatable for *Drakshaphala* (*Vitisvinifera*Linn) .

Materials and Methods

Morphological rationale and phytochemical rationale for substitution were analyzed in this study. Methods were adopted according to API standards.

Result

Both shared some similar Morphological characters and phytochemical characters. In HPTLC both showed bands with samerf (retardation factor) values, which indicate the presence of some similar constituents, which further proving similar *karma* (action).

Conclusion

Thus the rationale behind the statement "*Drakshyadhinalabhyethapradheyam Kaashmariphalam*" (fruit of *Gmelinaarborea* Roxb can be used as a substitute for fruit of *Vitisvinifera* Linn) of Bhavaprakasha is prooved.

Keywords: Prathinidhidravya, substitution pairs, *Kaashmari*, *Draksha*, *Vitisvinifera*Linn, *Gmelina arborea* Roxb, Madhura Triphala

ABBREVATION

API- Ayurveda Pharmacopoeia of India

GA-*Gmelinaarborea*Roxb,

HPTLC- High performance Thin Layer Chromatography

RF- Retardation factor

TLC-Thin Layer Chromatography

USDA- United States Department of Agriculture

VV- *Vitisvinifera* Linn

INTRODUCTION

Ayurveda which is a holistic science describes about various concepts. *Prathinidhi (Prathinidheeyathesadrsheekryatheithi)*[1], is one of such unique concept about usage of substitute drug in the absence of an original drug. This view was put forward by Bhavamishra (16th Century A.D). Vagbhataacharya has emphasized that an Ideal drug for medicinal purpose should possess aspects like *bahukalpam*(capable for use in various pharmaceutical modes and dosage forms), *bahugunam* (broad spectrum)*Sampannam* (readily available and potential) and *Yogyam*(Eligible for Therapeutic uses) [2]. When we select a substitute these points should be summoned in mind.

According to WHO, over three quarters of world population relies on herbal products for health care.This study is about comparing Ayurvedic substitution pair: *Kaashmariphala* which is high yielding and easily propagatable[3] for *Drakshaphala*. *Draksha* is having an average temperature for cultivation of about**:** summer average

22 °C (72 °F) and winter average around 3 °C (37 °F)[4] which makes cultivation of grapes in Southern India(average temperature 25°C-31°C)[5] more challengeable. The increasing demand for the fruit has further resulted in increased cost[6]and mistreatment with pesticides and chemicals[7]. Since *Draksha* is said to be "*Phalottama*" best among fruits, its daily consumption is apprehensible. Here comes the need of indigenous fast growing more yielding, climate favoring plant "*Kaashmariphala*". Although the fruit is one among MadhuraTriphala (Three sweet fruits) the consumption of *Kaashmari* among public domain is almost nil.

MATERIALS AND METHODS

The samples of *Gmelinaarborea*Roxb fruit was collected from Ayurveda College Trivandrum and Tripunithura, Kerala and fruit of *Vitisvinifera* from harvest farms, Theni, Tamilnadu. To ascertain the genuineness of samples collected, the quality control parameters like Pharmacognostical and preliminary Physico-chemical parameters as mentioned by API were done. In pharmacognostical study, organoleptic evaluation and microscopy including powder microscopy of both the fruits were done according to the standard procedures mentioned in API, a legally valid Ayurvedic drug document.

RESULTS

I. PHARMACOGNOSTICAL EVALUATION

a) Macroscopic evaluation

The macroscopic characters of samples were evaluated and characters observed were as follow

Table no:1 Macroscopic evaluation of fruit of G.A and V.V

CHARACTERS	*Gmelinaarborea*Roxb	*Vitisvinifera*Linn
Fruit appearance in Rachis	Fruits in clusters with a primary rachis	Fruits in clusters with multiple rachis
Shape	Ovoid	Oblong or Ovoid
Size	1.9-2 cm length, 1.2-1.5 cm width(fresh) 1.0-1.5 cm length, 0.8-1.0 cm width(dry)	1.5-2 cm length, 0.5-1.5 cm(fresh) 0.5-1 cmlength, 0.3-0.8 cm width(dry)

External surface	Thick and fleshy in fresh wrinkled in dry	Thick and fleshy in fresh Irregularly wrinkled forming ridges and furrows on dry
External surface colour	Yellowishgreen,(fresh) Dark brown to black on dry	Purple (fresh) purplish black on dry
Exocarp	Succulent and aromatic	Pulpy
Endocarp	Hard and stony	Hard and stony
Odour	Characteristic, strong	Characteristic
Taste	Sweetish sour	Sweetish and Astringent

b) Microscopic evaluation

Table no:2; Microscopic evaluation of fruit of G.A and VV:

CHARACTERS	GA	VV
Epicarp	Single layered	Single layered
Mesocarp	Multi layered Mesocarp which is fleashy wide zone consisting of isodiametric or oblong thin-walled, parenchymatous cells in the outer portion and thin walled elongated cells in the inner portion.	Multi layered Mesocarp pulpy made up of thin-walled, irregular cells containing prismatic crystals of calcium oxalate
Powder Microscopy	Presence of Calcium oxalate crystal,Blackish-brown content and aleurone grains	Presence of Calcium oxalate crystal, brown content and aleurone grains

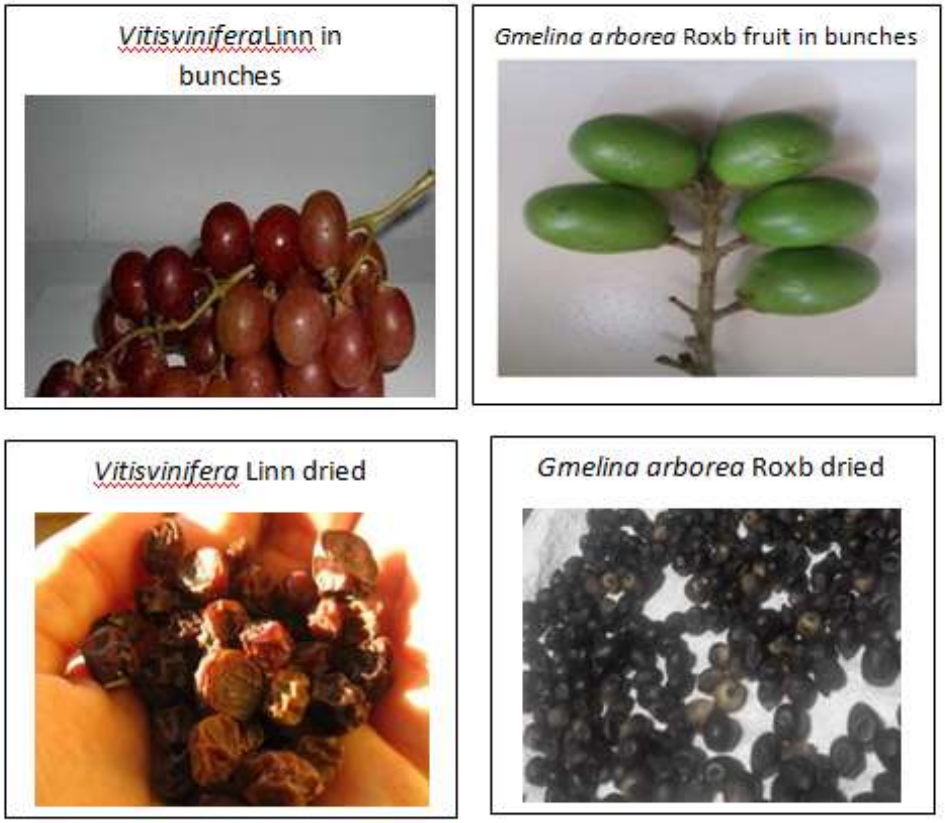


Fig.no: 1 Organoleptic Characters (a)

Fig.no: 2 Organoleptic Characters (b)

Fresh fruit and seed

Dried fruit and seed

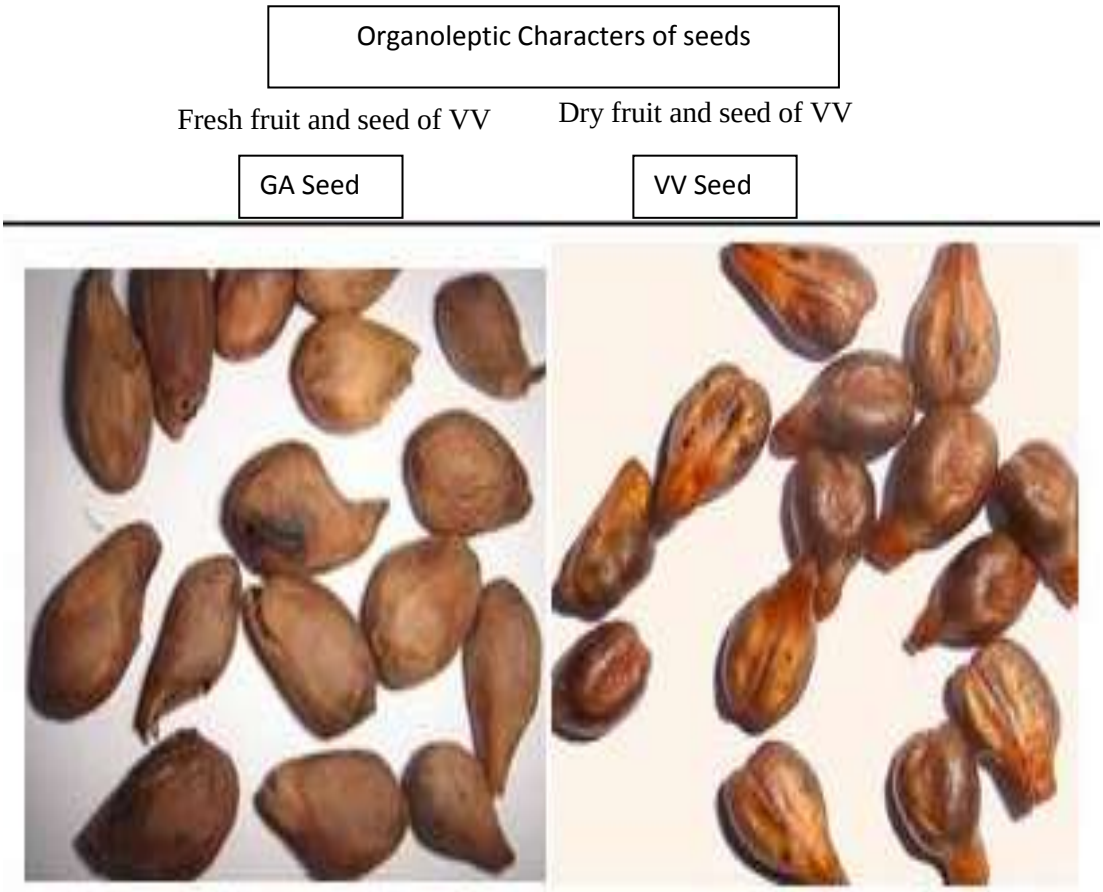


Fig no: 3 Transverse sections of fruit of GA

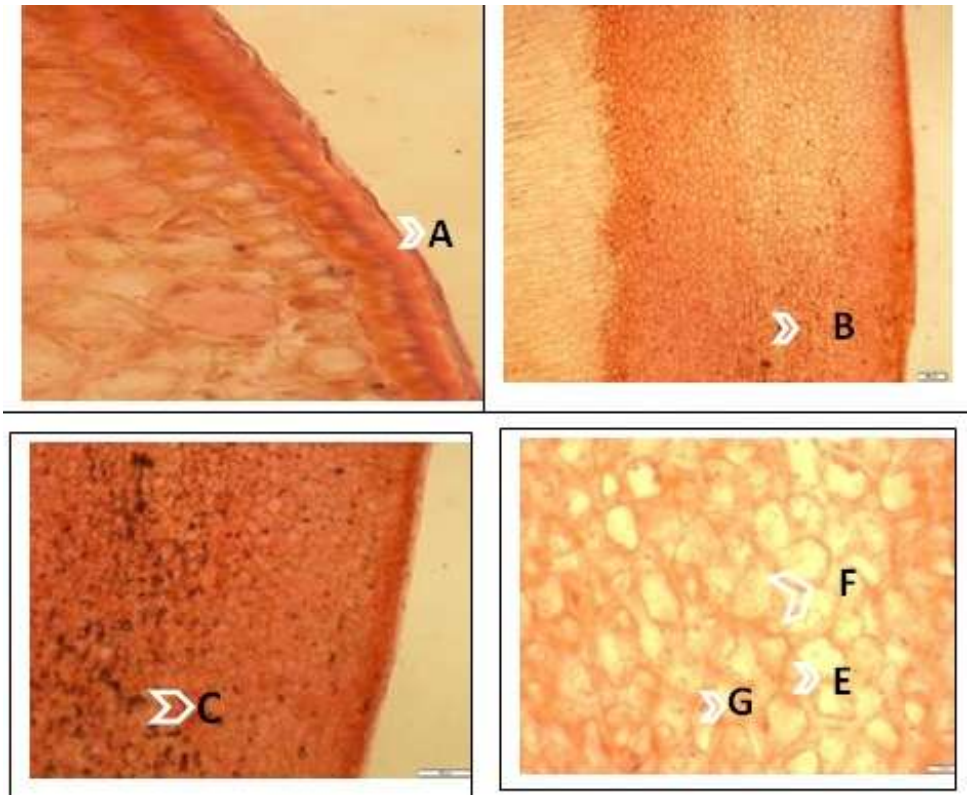


Fig no: 4 Powder microscopy of fruit of GA

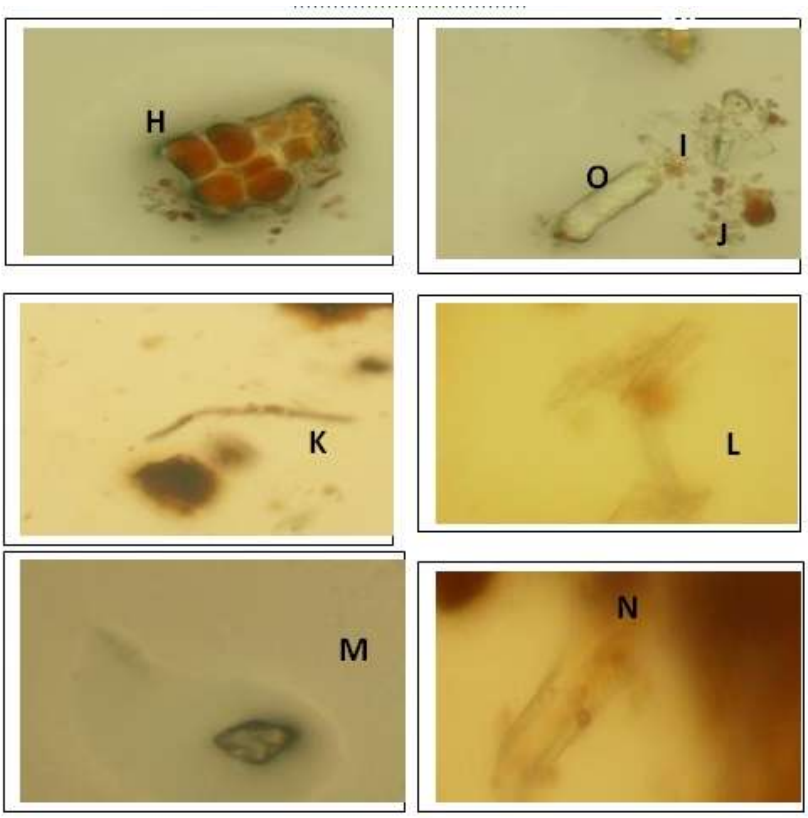


Fig no: *5* Transverse section of fruit of VV

A- Epidermis; B- Double layered mesocarp, C- mesocarp with brown content; E- Starch grains; F- Sclerids G-Aleurone grain, H- Stone cells; I-brown content; J-starch grains; K-Trichome; L- Raphidecrystals;M- Rosette crystals; N-Fibres ,O-Crystals

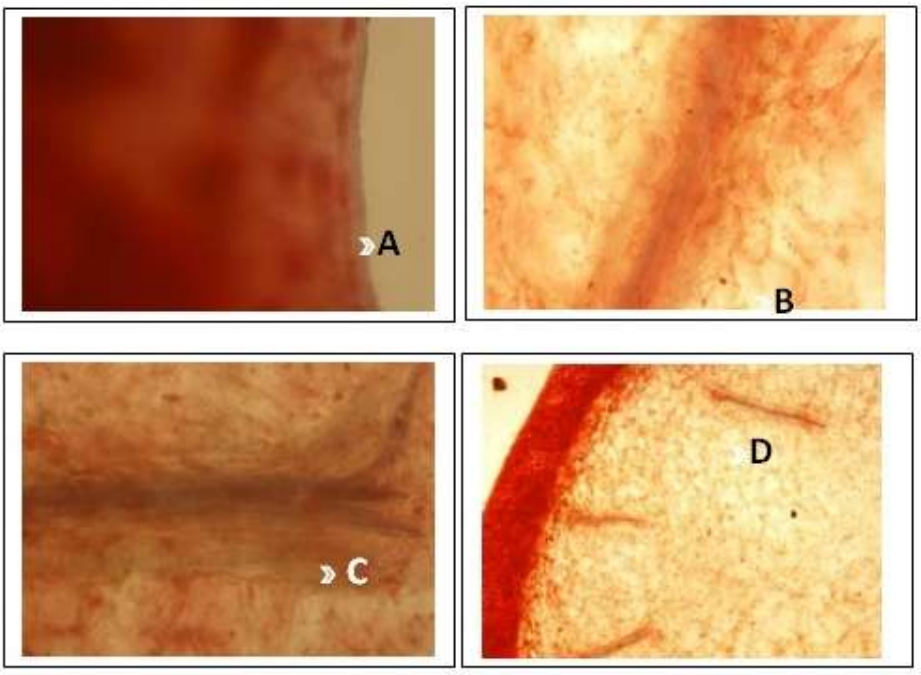


Fig no:6 Powder microscopy of fruit of VV

PRELIMINARY PHYSICOCHEMIAL AND PHYTOCHEMICAL EVALUATION

Table no.:3; Physico chemical evaluation of fruit of GA and VV

Sl.no.	Experiment	G.A	V.V
1	Foreign matter (%)	Nil	Nil
2	Moisture content (%)	5.7	15
3	Volatile oil (%)	Nil	Traces
4	Total ash (%)	5.3	3
5	Acid insoluble ash (%)	0.32	0.2
6	Water soluble extractive (%)	36.4	70
7	Alcohol soluble extractive (%)	26.7	26
8	Fibre content (%)	15.3	3.3
9	Sugar content		
	Reducing sugar (%)	75	70
	Total sugar (%)	0.66	10.2

A- Epidermis; B- Mesocarp,C-Vascular bundles, D-fibres, E- Brown content; F- Spiral Vessels, G-starch grains;H-Crystals

II. QUALITATIVE TESTS DONE IN THE FRUITS

Table no: 4;.qualitative tests done in the fruit of GA & VV

Experiment	V.V			G.A		
	Ethyl acetate	Methanol	Water	Ethyl acetate	Methanol	Water
Steroid	-	-	-	-	-	-
Flavonoid	+	+	+	+	+	+
Phenol	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	-
Tannin	+	+	+	+	+	+
Saponin	-	-	-	-	+	+

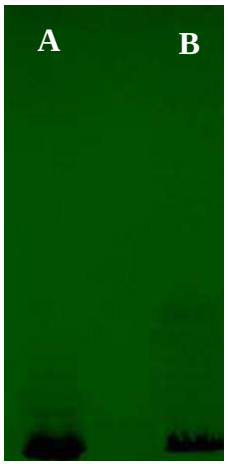
CHROMATOGRAPHY

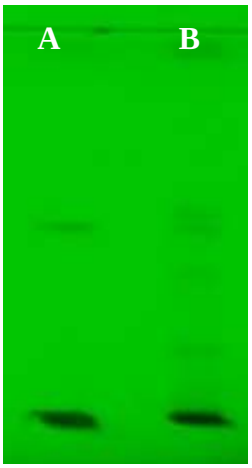
The plate in which best separation achieved was first observed through UV-florescence viewing cabinet (365nm) and the Rf values of the spots were noted. Then the plates were developed in the iodine chamber and in some solvent bands were observed in TLC. Different solvent systems were tested in trial and error method.

HPTLC study of *draksha (vitisvinifera*linn*) and kaashmari (gmelinaarborearoxb)*

HPTLC analysis of the alcohol extract of *Draksha (Vitisvinifera Linn)* and *Kaashmari (GmelinaarboreaRoxb) Linn*was done with the following solvent system

Toluene : Ethyl Acetate: Formic acid: Methanol (30:10:1:2)	n.butanol: chloroform: acetic acid: ammonia: water (7:7:5:2:1)

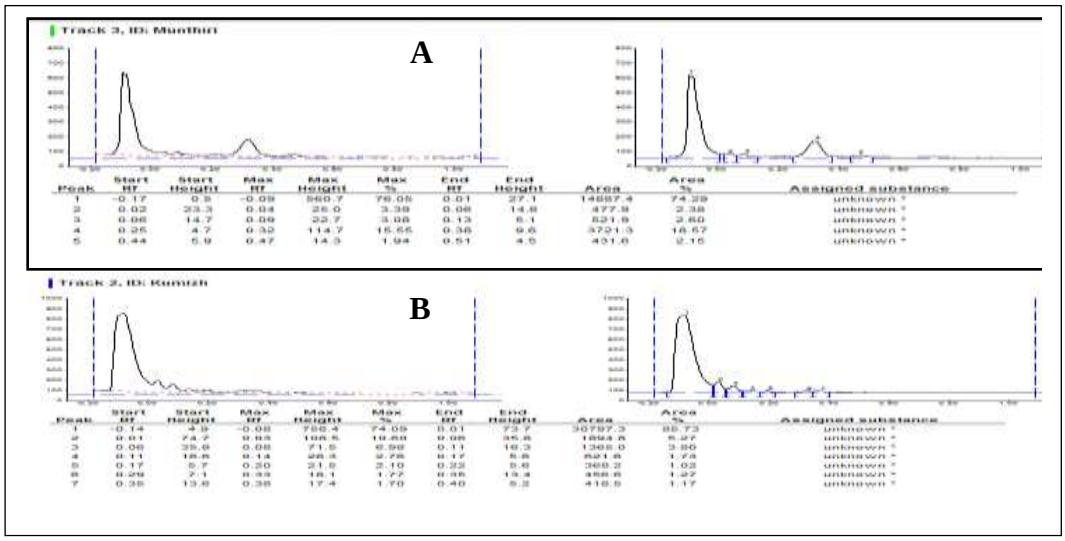




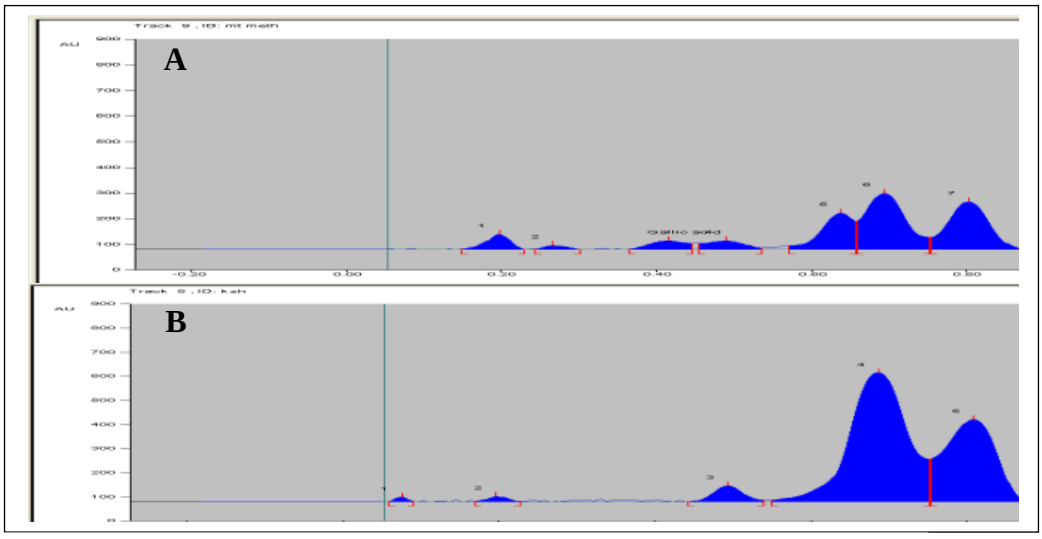
SAMPLE A – VV cold methanol extract

SAMPLE B - GA cold methanol extract

n-hexane: ethyl acetate: formic acid(20:19:1)



HPTLC plate with solvent system n.butanol:chloroform:aceticacid:ammonia:water(7:7:5:2:1)



Solvent system n-hexane: ethyl acetate: formic acid(20:19:1)

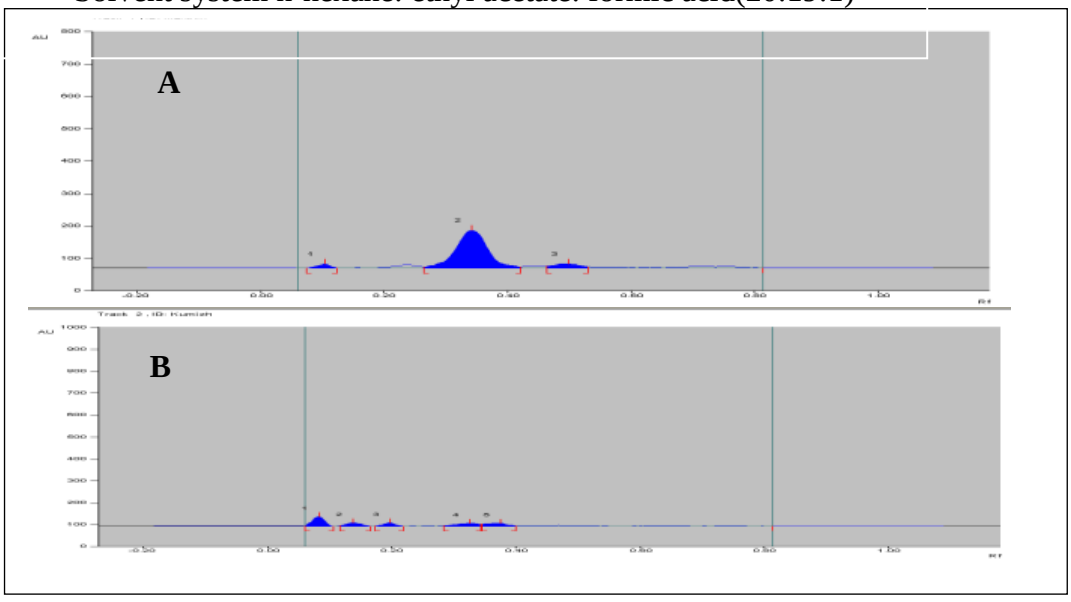


Fig no. 8: HPTLC peaks in various solvent system

Table no:5; HPTLC of samples

Substance	Solvent system with ratio	No. Of spots	Colour of spots	Rf value
VV	Toluene : Ethyl Acetate: Formic acid: Methanol (30:10:1:2)	4	Dark blue	0.04,0.09,0.32,0.47
GA		6	Dark blue	0.03,0.08,0.14,0.20,0.33,0.38
VV	n.hexane, ethyl acetate, formic acid (20:19:1)	6	Dark blue	0.22,0.27,0.34,0.53,0.62,0.78
GA		4	Dark blue	0.23,0.36,0.46,0.59
VV	n.butanol:chloroform: aceticacid:ammonia:water (7:7:5:2:1)	6	Dark blue	0.27,0.42,0.49,0.64,0.69,0.80
GA		4	Dark blue	0.20,0.49,0.69,0.81

Table no: 6; Similarities in HPTLC analysis of both samples

Solvent system with ratio	Substance	Colour of spots	Rf value
Toluene : Ethyl Acetate: Formic acid: Methanol (30:10:1:2)	VV	Dark blue	0.04,0.09,0.32
	GA	Dark blue	0.03,0.08,0.33,0.38
n.hexane, ethyl acetate, formic acid (20:19:1)	VV	Dark blue	0.22,0.27,0.34,0.53
	GA	Dark blue	0.23,0.36,0.59
n.butanol:chloroform:aceticacid:ammonia:water (7:7:5:2:1)	VV	Dark blue	0.27,0.42,0.49,0.64, 0.69,0.80
	GA	Dark blue	0.20,0.49,0.69,0.81

III. ATOMIC ABSORPTION SPECTROSCOPY (in parts per million-ppm)

Heavy metal content was screened in the samples of fruit of *Vitisvinifera Linn* and *GmelinaarboreaRoxb.* The results observed were as follows.

Table.No:7;Atomic absorption spectroscopy

Heavy metal concentarion in ppm	V.V	G.A	Maximum Permissible Limits
LEAD	0.3439	0.1808	10
CADMIUM	0.0311	0.0264	0.3
IRON	9.96096	4.3706	27.4
ZINC	2.2606	0.8670	20(for edible herbs)

DISCUSSION

Ayurveda, since ancient times have suggested the use of appropriate locally available plants which are abundant and easy to obtain instead of plants which are having practical difficulty in its availability. The most essential criteria for substitution is its pharmacological activity. The validation of these substitution pairs of drugs using both Ayurvedic principles and modern scientific tools is a necessity in this present era to overcome problems like non-availability of expensive drugs, inferior quality of available drugs, rare or difficult to obtain drugs, there by solve the crisis of scarcity and indirectly help in its conservation and sustainability.

*Draksha*is a medicinal plant which is extensively used in Ayurvedic formulations and in API is identified as dried mature fruits of *Vitisvinifera* Linn of Family Vitaceae. The increase in demand for the fruit has further resulted in increased costand mistreatment with pesticides and chemicals. Fruit of *Kaashmari*is the textual substitute, botanically identified as *Gmelinaarborea*Roxb.*Draksha* and *Kaashmariphala* both are the components of *phala -varga*(fruit group) and are constituted under some of similar groups like*madhura-triphala*(three sweet fruit),*hrswatriphala*(three small fruits), *virechanopaga*(that which aid purgation) etc.

Morphological rationale for substitution

Considering its morphological characters; *Gmelinaarborea*Roxb is anunarmedtree while *Vitisvinifera* Linn is a deciduous climber and both are having extremely different

families, Verbenaceae (GA) and Vitaceae (VV). Both thefruits set up as bunches or clusters (fig no:1)**,** the dried fruits are having similar organoleptic features(table no:1) (fig no :2); Dried fruits of both the fruits were having greater structural similarity, even there seeds shares similar organoleptic features. On microscopy also both possessed similar properties to an extent (as show in the table no:2). This structural similarity along with similar *Rasadiguna* (properties starts with Rasa) ie, *Draksha* is having *madhura Rasa(sweet taste), guru snigdhaguna (heavy and oily in nature), madhuravipaka (sweet after completion of digestion)* and *Sheetaveerya (cold potency)* ,which seems to be similar to that of *Kaashmariphala*[8] may be the reason for considering GA as the substitute of VV in classics.

Phytochemical rationale for substitution .

Physico chemical characters like foreign matter, total ash, acid insoluble ash, water insoluble ash, , water and alcohol soluble extractive (as shown in table.no:3) were checked based on the procedures in *Ayurvedic Pharmacopoeia of India* (API), and results were in compliance with the standard values which prove the genuinity of the drug . Fiber content, sugar content, moisture content, volatile oil content etc. were in compliance with the literature JN Ingweyeetal[9] for GA and USDA values for VV.

Moisture content of VV was found to be greater than GA (as shown in table.no:3). During drying GA was cut into pieces and seeds were taken out and for VV it was not done, that may be the reason for this difference in moisture content. Traces of volatile oil was present in VV and it may be due to the presence of seed kernel in VV. Percentage amount of fiber was more in GA than VV, Which further propose its action as a *Virechanopagadravya*, along with the presence of tartaric acid in both the fruit.

Qualitative analysis of extracts of both the fruits revealed the presence of alkaloids, phenol, flavonoid and tannins (as shown in table.no:4). GA fruit showed the presence of saponin in methanol and water extract. The presence of saponins in the GA fruit may pave the way for the preparations like face wash as an anti-aging formulation[10] since they may act as very good antioxidant and cleanser[11].Atomic absorption spectroscopy was done for 4 heavy metals namely lead, cadmium, zinc and iron, all were found to be under the permissible limits (as shown in table 7). TLC of the alcohol extract of both the fruits and good separation was achieved using n.butanol: chloroform: acetic acid: ammonia: water (7:7:5:2:1). After TLC, HPTLC of the study drug was conducted and

several peaks were obtained for both fruits (as shown in figure). We got similar rf in both fruit in some of the solvent system (as shown in the table no 5,6) which further shows the presence of similar constituents in both fruits, lighting the knowledge of reason for considering an entirely different plant as a substitute.

Not only *kaashmari* can be used as a substitute as for *draksha*, we can make use of it as good fruit source, among Dry fruits. The enriched nutrients in the*kaashmari* have to make enlightment in all that, the tag name *Dasamoola*(ten plants with root as useful part)does not indicate use only its *moola*(root). Remember and make use of other useful parts of a plant.

CONCLUSION

Thus, this study reveals that, both the fruits of Kaashmari *(Gmelinaarborea Linn)* and *Draksha* (*Vitisvinifera* Linn) possess almost similar properties and *Kaashmari*can be used as the substitute of *Draksha***.**

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CONFLICT OF INTEREST- NO