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Research Article

COMPARATIVE ANALYTICAL STUDY OF *MADANA SHALATU* (UNRIPEN FRUIT OF RANDIA DUMETORUM LAM) AND *PHALAPIPPALI* (PROCESSED FRUITS OF RANDIA DUMETORUM LAM) - A STUDY OF *ABHAVA PRATINIDHI DRAVYA*

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ABSTRACT

Vamana is the *Agrya karma* for *Kaphaja vikaras*. *Madanaphala* - *Randia dumetorum* Lam is the drug of choice for *Vamana karma* because of its property– *Anapayitwat* (without untoward effect). Specific collection method, season and processing are required to get *Phalapippali* to possess above quality. *Madana shalatu*– Unripen fruits of *Madanaphala* is an *Abhavapratinidhi dravya* mentioned in *Sushrutha Samhita* and *Ashtanga Hrudaya* which does not require this processing- *Samskara*. So, in the present study comparative qualitative and quantitative physicochemical and phytochemical analysis of the *Madana Shalatu*, *Pakwa Madanaphala* Ripen fruit of *Madanaphala* and *Madanaphala Pippali*– Processed fruits of *Madanaphala* has been carried out. **Aim:** 1. Pharmacognostic, Physicochemical and Phytochemical analysis of *Madana Shalatu*, *Pakwa Madanaphala*, *Madanaphala Pippali*. 2. Comparative qualitative and quantitative phytochemical analysis of *Madana Shalatu*, *Pakwa Madanaphala*, *Madanaphala Pippali*. **Method:** 1. Pharmacognostic study – Macroscopic study and Microscopic study. 2. Preliminary physico chemical analysis. 3. Qualitative phyto chemical evaluation of aqueous and alcoholic extracts of the test drugs. 4. Quantitative analysis– UV Spectrophotometry. **Result:** Microscopic study, qualitative phyto chemical evaluation of all test drugs did not showed much differences. In total saponin estimation by UV Spectrophotometry of methanol and water extract of ripen processed fruits of Randia, ripen fruits of Randia and unripen fruits of Randia is found to contain 73.71, 83.23, 71.35 and 72.62, 92.44, 75.25µg DE/mL of Saponin content respectively. **Conclusion:** Quantity of Saponin which is the main active principle in *Randia Dumetorum* responsible for *Vamana Karma* was similar in the *Madana Shalatu* and in *Phala Pippali*, whereas it was highest in *Pakwa Madanaphala*, thus it may be used like *Phala Pippali* without any laborious process of *Samskara*. Further toxicity studies and clinical trials are required to prove its safety and efficacy in humans.

INTRODUCTION

Ayurveda is a system of Indian traditional form of medicine, which depends mainly on medicinal plants. The industrial demand for the medicinal plant resources has been on the rise due to the worldwide buoyancy in the herbal sector engaged in production of herbal health care formulations.

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Hence in the present situation, demand for the herbal raw materials is high but the supply of quality raw drugs is poor because of:

- Diminishing forest area
- Extinction of many species
- Incorrect identification of many plants
- Lack of knowledge related to Sustained harvesting and good agricultural and collection practices etc which may become cause for the adulteration of the raw materials.

Hence, there is a need to search for some alternative source which is equally potent or similar in action to the original drug. "*Abhava Pratinidhi dravya*" is one such concept in Ayurveda which says:

In the absence of a desired first choice medicinal herb, classical Ayurveda recommends use of a functionally similar substitute[1,2]

Panchakarma which is gaining much popularity now a days for its therapeutic value as well as for the preventive aspects through *Rutu shodhana* (seasonal cleansing) people are opting for such therapies voluntarily. *Vamana* is one among *Panchakarmas*. *Madanaphala- Randia dumetorum* Lam is very commonly used *Dravya* for *Vamana karma* because of its *Anapayitwat* (without untoward effect). For this *Vamana karma*, *Samskarita Madana phala pippali* i.e., processed seeds of *Madana phala* is very commonly used. But, the collection of *Madanaphala* and its *Samskara* is a very laborious process and the yield we get after processing is very less.

In *Sushrutha samhita*, *Acharya Sushrutha* has mentioned the use of *Madana shalatu* for *Vamana* in the absence of *Pakwa phala*[3]. Hence, in the present study we are analyzing and comparing the physicochemical and phytochemical properties of *Madanaphala shalatu* (unripen), *Pakwa asamskarita* (ripen unprocessed) and *Pakwasamskarita Madanaphala* (ripen processed *Madanaphala*). If they show similar properties it will be beneficial for the clinicians in regular practice, so that one can reduce the laborious process of *Samskara* of *Madanapahla* and the yield of the drug can be increased.

Concept of *Abhava Pratinidhi Dravya* (Substitute) in Ayurveda

If any *Dravya* is not available, any other thing which is similar to it in *Rasa* (taste), *Veerya* (potency) and *Vipaka* should be selected by the physician and made use of[1].

Madana phala Pratinidhi

In the same manner, *Madanaphala* recipes (*Madanaphalakalpas*) can be prepared with either the flowers or tender fruits of *Madanaphala* when ripe fruits are not available [4].

Drug Review: *Madanaphala– Randia dumeorum* Lam, is a drug belonging to Rubiaceae family. This is a small but very variable much branched ramous tree 2–4m high. Found throughout India, from Jammu, Sikkim, Sothern India etc [5]. The plant flowers during summer or May–June and fruits ripen during autumn to winter or October to January[6].

Chemical Constituents

- The activity of the drug is attributed to the presence of saponins, which occur to the extent of 2–3% in fresh fruits. 10% in dried whole fruit. The saponins are concentrated mostly in the pulp.
- A mixture of two saponins, viz., Randia or neutral saponins and randia acid or acid saponin has been isolated from the pulp.
- On complete hydrolysis, both the saponins yield oleanolic acid as the sapogenin.
- In a later investigation, a new saponin designated as ursosaponin was isolated from the ethanolic extract of the dried whole fruit.
- Besides saponins, the fruit contains a new triterpene and acid resin and trace of a pale yellow essential oil with characteristic odour of the drug[7].
- The seeds contain oleanlic acid 3 – glucoside[8].
- The bark contains Scopoletin. D– mannitol and a mixture of saponins. The saponins on hydrolysis yield glucose, xylose, rhamnose, and two triterpenic acid sapogenins designated as randialic acid A and randialic acid B[9].

Properties and Actions [5]

Rasa: *Madhura, Tikta*

Guna: *Laghu*, *Ruksha*

Virya: *Ushna*

Vipaka: *Katu*

Dosha Karma: *Kaphahara*

Karma: *Lekhana*, *Vamaka*, *Ropana*

Indication: *Vidradi*, *Pratishyaya*, *Kushta*, *Anaha*, *Shotha*, *Gulma*, *Vrana*

MATERIALS AND METHODS

Procurement of Plant Source

Randia dumetorum – Fruit

- The unripe and ripen fruits of *Madanaphala* were collected from Honnavara, Karnataka, and authenticated at Sushrutha Central Research Facility, SAMC & H, Bengaluru.
- Around 5kg of the unripe fruit and 10kg ripen fruits of *Madanaphala* was collected.
- Unripen fruits were washed thoroughly with water to remove physical impurities like mud and dried under shade till it gives constant weight.
- 100gm of the drugs was kept apart for macroscopic and microsopic studies.
- Ripen fruits were processed as per the classical texts.[10]
- Unripen, ripen and ripen processed fruits were made into coarse powder and kept preserved in air tight container for phyto chemical, physico chemical and chromotographic studies.

Macroscopic and Microscopic Study of Three test Drugs

Randia dumetorum - Fruit

Macroscopic character of the collected fruits were observed and compared with that of given description in classical and standard texts.

Microscopic Examination

Randia dumetorum - Fruit

- Powder microscopy was performed as per the Practical Pharmacognosy, By K.R. Khandelwal, Ed:18, Nirali Prakashan, 2007, PP 15 – 19
- The powder characters were compared with The Ayurvedic Pharmacopoeia of India, Part I, Vol I, Dept of AYUSH, PP 114-115.

Procedure

The sample was grounded to fine powder, stained with phloroglucinol and Conc. hydrochloric acid. Mounted in glycerin and characters were observed under 400X magnification in Metzer Trinocular microscope. Images were captured using capture pro software.

Physico-Chemical Evaluation

Determination of Moisture Content

Materials required: Powdered drugs, digital balance, petridish, desiccator and hot air oven.

Procedure

Accurately weighed 5gm of the coarsely powdered drugs were taken in a dried, weighed petridish. Petridish was taken out, cooled in a desiccator (dessicator was filled with copper sulphate crystals which will absorb the moisture) and weighed. Drying was continued till constant weight was obtained. Percentage of moisture content with reference to the air dried drugs was calculated.

Determination of Ash Value

Determination of Total Ash

Materials Required: Physical balance, porcelain crucible, Muffle- furnace, desiccator, air-dried drug.

Procedure

Clean and dry crucible was taken and weighed. Accurately weighed 2gm of drug was taken. Drug was placed in crucible. Crucible was kept in ignited Muffle-furnace. Temperature was gradually increased upto 450°C until white colored ash was obtained, pending the absence of carbon. Cooled in desiccator and weighed, again kept it in muffle furnace for half an hour. Cooled in desiccator and weighed, there was no difference in the weight of ash content, it was understood that all organic matter has burnt off.

Calculate the percentage of ash with reference to air-dried drug

$$\text{Ash value in \%} = \frac{\text{Weight of the Residue} \times 100}{2}$$

Determination of Acid Insoluble Ash

Materials required: Digital balance, silica crucible, muffle furnace, desiccator, total ash, ashless filter paper, funnel.

Procedure: To the total ash obtained, 25ml of hydrochloric acid was added and stirred well for 15 minutes and heated for 5 min. It was filtered through an ashless filter paper to separate the insoluble matter. The residue along with the filter paper was taken in pre-heated, weighed silica dish. The dish was transferred to muffle furnace and ignited for half an hour at the temperature not exceeding 450°C. The dish was cooled in a desiccator and weighed again. Heating was continued till constant weight of the dish was obtained. The percentage of acid insoluble ash with reference to the air dried drugs was calculated.

Determination of Water Soluble Ash

Materials Required: Total ash, Digital balance, muffle furnace, desiccator, ashless filter paper, funnel, silica crucible.

Procedure: To the total ash obtained, 25ml of water was added and boiled for 5 minutes. It was filtered through an ashless filter paper to separate the insoluble matter. The residue along with the filter paper was taken in a pre-heated, weighed silica dish. The dish was transferred to muffle furnace and ignited for 15 minutes at the temperature not exceeding 450°C. The dish was cooled in desiccators and weighed again. Heating was continued till constant weight of the dish was obtained. The percentage of water soluble ash with reference to the air-dried drugs was calculated.

Extractive Values

Determination of Alcohol Soluble Extractive Value

Material required: Digital balance, air dried drugs, glass stoppered conical flasks, methyl alcohol, measuring jar, funnel, Whatman filter paper, water bath, hot plate, beaker, desiccator.

Procedure: Accurately weighed 5gm of coarsely powdered air-dried drug was placed in a glass Stoppard conical flask, macerated with 100ml of 90% methyl alcohol. The lid was closed for 24 hours. Frequent agitation was done during first 6 hours and allowed to stand for 18 more hours without shaking. After 24 hours, it was filtered to remove insoluble matter. 25ml of the filtrate was taken in a petridish and kept on water bath for evaporation. The residue was dried in hot air oven at 105°C, cooled and weighed. Drying was continued till constant weight was obtained. Percentage of alcohol soluble extractive value with reference to the air dried drugs was calculated.

Determination of Water Soluble Extractive Value

Material required: Digital balance, air dried drugs, glass stoppard conical flask, distilled water, measuring jar, funnel, whatman filter paper, water bath, hot plate, beaker, desiccator.

Procedure: Accurately weighed 5gm of coarsely powdered air-dried material was placed in a glass-Stoppard conical flask, macerated with 100ml of distilled water. The lid was closed for 24 hours. Frequent agitation was done during first 6 hours

allowed to stand for 18 more hours without shaking. After 24 hours, it was filtered to remove insoluble matter. 25ml of the filtrate was taken in a Petri dish and kept on water bath for evaporation. The residue was dried in hot air oven at 105°C, cooled and weighed. Drying was continued till constant weight was obtained. Percentage of water soluble extractive value with reference to the air dried drugs was calculated.

Fluorescene Analysis by Using UV Chamber

Principle

Many substances in different solutions like dilute sulphuric acid, ethanol, ethanol sodium hydroxide etc, when suitably illuminated, emit light of a different wavelength or color from that which falls on them. This emitted light (fluorescence) ceases when the exciting light is removed. Convenient long and short-wave ultraviolet hand lamps are available for chromatographic observations. Eg;

1. Many alkaloids in the solid state show distinct colours like Aconite (light blue), Berberine (yellow) and Emetine (orange).
2. Pieces of cinchona bark when placed under the lamp show a number of luminous yellow patches and a few light blue ones. If the inner surface of the bark is touched with Dil. Sulphuric acid the spot immediately turns blue.
3. Areca nuts when cut show a light blue endosperm.
4. This method can be used for the detection of Ergot in flour, of cocoa shells in powdered cocoa, and of rumex in powdered Gentian.
5. The location of separated compounds on paper and thin layer chromatograms by the use of ultraviolet light has been extensively employed. With plant extracts it is often worthwhile to examine the chromatogram in ultraviolet light even if the constituent that one is investigating are not themselves fluorescent. In this way the presence of fluorescent impurities may be revealed.

Material required: UV Chamber, petri dish, powdered drugs, chemicals, glass rod.

Procedure: The powdered drugs were taken in a clean petri dish and exposed to ultraviolet rays to a range of long wave 366nm and short wave 254nm along with visible light. The drugs were exposed as such and after treating with ethanol, ethanol sodium hydroxide and with dilute HCl. Various colours observed are reported in the table.

Preparation of Extracts

In certain cases extraction of the drug is by maceration, in others by a continuous extraction process. For the latter the Soxhlet extractor is particularly useful and has been in use for many years, not only for determination of extractives but also for small scale isolation. In this apparatus extraction is by boiling solvent followed by percolation; finally, evaporation yields the extract.

The extraction was carried out by soxhlation technique.

Materials Required: Coarsely powdered drugs, solvent, soxhlet apparatus, glass beaker, hot plate.

The drugs were extracted with water and alcohol (methyl alcohol) in the round bottom flask. In order to avoid the bumping of the solvent while boiling 3-4 pieces of small porcelain chips were kept inside the round bottom flask. Soxhlet apparatus was set-up. Care was taken for continuous water flow to the condenser.

Procedure: 50gm of dried, coarsely powdered drugs were kept on a piece of cotton in the extractor. Extraction was carried out at 100°C for water extract and 45-50°C for alcohol till extraction was complete. Complete extraction was confirmed when the extract in the siphon tube was colorless liquid. It was transferred into a beaker kept over hot plate and evaporated to obtain the extract. The weight of the residue was noted. Percentages of the extract with reference to the air dried drugs were calculated. The physical characters of the extracts were noted and were preserved in air tight containers for further analytical studies.

Preliminary Phytochemical Study

Preliminary Qualitative Phytochemical Screening

Test for alkaloids (Wagner's reagent), test for carbohydrates (Molisch's test), test for cardiac glycosides (Keller Kelliani's test), test for flavonoids (alkaline reagent test), test for phenols (ferric chloride test), test for phlobatannins (precipitate test), test for amino acids and proteins (1% ninhydrin solution in acetone), test for saponins (foam test), test for sterols (Liebermann-Burchard test), test for tannins (Braymer's test), test for terpenoids (Salkowki's test).

Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or colouration).

Test for Oxalate

To 3ml portion of extract, added a few drops of ethanolic acid glacial. A greenish black colouration indicates presence of oxalates.

TLC

10mg/ml samples were prepared 2.5µl of samples were spotted on TLC plate and allowed to dry. A TLC plate is made up of a thin layer of Silica gel 0.25mm with fluorescent indicator F_{254} with Solvent system Chloroform: ethanol (9.5:0.5) was used for TLC analysis. The strip or plate is then placed with this end dipping in to the solvent mixture, taking care that the sample spot/zone is not immersed in the solvent. As the solvent moves towards the other end of the strip, the test mixture separates into various components.

This is called as the development of TLC plates. The separation depends on several factors, the plate is removed after an optimal development time and dried and the spots/zones are detected using UV chamber and Rf value is calculated using

Rf = Distance moved by compound/distance moved by solvent.

Quantitative Phytochemical Study

Total Saponin Quantification- Spectrophotometry

MATERIALS AND METHODS

- Vanilin or Anisaldehyde
- 72% sulphuric acid solution
- Diosgenin

Procedure

- Test extract was dissolved in 100% methanol.
- Add 2ml of vanillin in ethanol and mixed well.
- Add 2ml of 72% sulphuric acid solution and mixed well.
- The test solution was heated in a water bath maintained at 60°C for 10 minutes with occasional shaking.
- Absorbance at 544nm was recorded against the reagent blank.
- A standard calibration plot was generated at 544nm using known standard Diosgenin
- The concentrations of saponin in the test sample were calculated from the calibration point and expressed Diosgenin mg equivalent per gram of sample

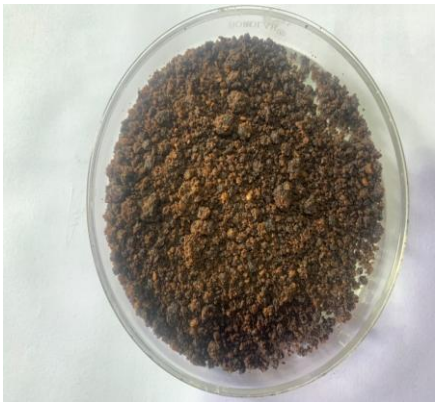
OBSERVATIONS AND RESULTS

Observation during collection of sample

All the samples in fresh state were collected. Unripen fruits were dark green in colour. Ripen fruits were yellowing green in colour.

Unripen Fruits of *Madanaphala*

Ripen Fruits of *Madanaphala*



Processed Fruit of *Madanaphala*

Loss on Drying

S.No	Name of the Sample	Weight in fresh state – In kg	Weight after drying – In kg	Loss on Drying in kg	Loss of Weight in %
1	*Madana Shalatu*	5	1.65	3.35	67
2	*Pakwa Madana Phala*	3	1.2	1.8	60

Pharmacognostic Study

Macroscopic Study

Name of the sample	Colour	Texture	Size	Odour	Taste	Transverse section
Madana Shalatu	Dark Green	Smooth	3.5 - 3.8cm	Sweet odour	Slight bitter	Slimy pulp with seeds embedded
Pakwa Madana Phala	Yellowish Green	Smooth	4.0 – 4.3cm	Strong Sweet	Bitter	Slimy pulp with seeds embedded
Pakwa Samskarita Madana Phala	Brownish black	Sticky powder	-	Strong odour	Bitter	

Microscopic study

Madana Shalatu: Observed characters are

- Large irregular reddish brown cells.
- Sclerides (stone cells) of various size (pink coloured).
- Pieces of xylem vessels with annular and with reticulate thickening.
- Thin walled parenchyma cells.
- Yellow – Orange pieces of seed coat.
- Unicellular, covering trichomes

Pakwa Madanaphala* and *Pakwa Samskarita Madanaphala: Observed characters are

- Reddish brown content
- Pippted parenchyma
- Trichome
- Stone Cells
- Sclereids from endocarp
- Xylem elements

Flourescence analysis using UV Chamber

Drug as such

Name of the Sample	Visible light	At 366 nm	At 254 nm
Madana Shalatu	Brown & cream coloured particles	Brown	Light brown
Pakwa Madana Phala	Cream colur with brown particles	Brown and cream particles	Cream colour
Samskarita Madanaphala	Snuff brown powder with white particles	Brown & white particles	Black & white particles

Drug with Ethanol

Name of the Sample	Visible light	At 366 nm	At 254 nm
Madana Shalatu	Brown & cream coloured particles	Cream & white	Light brown & white particles
Pakwa Madana Phala	Light brown & cream	Dark brown & cream	Light brown & cream
Samskarita Madanaphala	Brownish grey with white particles	Creamish brown & white particles	Grey particles

Drug with Dil. Hcl

Name of the Sample	Visible light	At 366 nm	At 254 nm
Madana Shalatu	Cream & white coloured particles	Dark brown & white particles	Cream & white coloured particles
Pakwa Madana Phala	Cream & brown coloured particles	Dark brown/snuff & Cream coloured particles	Brown & cream coloured particles
Samskarita Madanaphala	Brownish grey	Brownish snuff	Greyish black

Ash Values

S.No	Ash	*Madana Shalatu*	*Pakwa Madana Phala*	*Samskarita Madanaphala*
1	Total Ash	4.1%	3.45%	3%
2	Acid Insoluble Ash	0.65%	0.3 %	0.6
3	Water Soluble Ash	2%	1.25%	1.9

Extractive Value

Extract	*Madana Shalatu*	*Pakwa Madana Phala*	*Samskarita Madanaphala*
% of Alcohol soluble extractive for 100ml	16.64	14	45.92
% of Water soluble extractive for 100ml	19.28	22	26.08

Preliminary Qualitative Phytochemical Analysis

Tests	*Shalatu*		*Pakwa Phala*		*Samskarita Madanaphala*	
	Alcoholic extract	Aqueous extract	Alcoholic extract	Aqueous extract	Aqueous extract	Alcoholic extract
Alkaloids	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+

Phlobatannins	+	+	+	+	+	+
Aminoacids and proteins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Sterols	-	-	-	-	-	-
Tannins	+	+	+	+	+	+
Terpenoids	-	-	-	-	+	+
Quinones	+	+	+	+	+	+
Oxalate	-	-	-	-	-	-

Thin Layer Chromatography

1. Methanol Extract

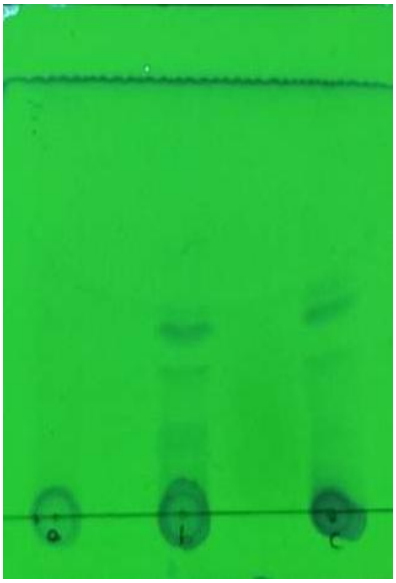


Fig 1: TLC chromatogram at 254nm wave length crude of Methanol extract, Clane 1- Ripen processed fruits of randia, lane 2- Ripen fruits of randia, lane 3- Unripen fruits of randia)

Fig 2: TLC chromatogram at 366nm wave length crude of Methanol extract, Clane 1- Ripen processed fruits of randia, lane 2- Ripen fruits of randia, lane 3- Unripen fruits of randia)

Fig 3: TLC chromatogram at visible light on crude of Methanol extract, Clane 1- Ripen processed fruits of randia, lane 2- Ripen fruits of randia, lane 3- Unripen fruits of randia)

2. Water Extract

Fig 4: TLC chromatogram at 254nm wave length crude of Water extract, Clane 1- Ripen processed fruits of randia, lane 2- Ripen fruits of randia, lane 3- Unripen fruits of randia)

Fig 5: TLC chromatogram at 366nm wave length crude of Methanol extract, Clane 1- Ripen processed fruits of randia, lane 2- Ripen fruits of randia, lane 3- Unripen fruits of randia)

Fig 6: TLC chromatogram at visible light on crude of Water extract, Clane 1- Ripen processed fruits of randia, lane 2- Ripen fruits of randia, lane 3- Unripen fruits of randia

TLC Characteristic for Given Methanol Extract Crude

Solvent	Solvent	TLC Band at 254 nm	Retention Factor	TLC Profile characteristics		
				254 nm	366nm	Visible light
Methanol	Ripen processed fruits of randia	2	0.21	light green	-	Brown
			0.61	light green	Light blue	Brown
			1	Blue	light blue	Brown
	Ripen fruits of randia	5	0.18	Blue	-	Brown
			0.23	light green	-	Brown
			0.32	light green	-	Yellow
			0.44	light green	-	Brown
			0.62	Blue	light blue	Brown
			1	Blue	light blue	Brown
	Unripen fruits of randia	3	0.16	light green	light blue	Brown
			0.24	light green	-	Yellow
			0.57	light green	light blue	Brown
			1	Blue	light blue	Brown

TLC characteristic for given water Extract Crude

Solvent	Solvent	TLC Band at 254 nm	Retention Factor	TLC Profile characteristics		
				254 nm	366nm	Visible light
Water	Ripen processed fruits of randia	1	0.11	light green	-	Brown
			1	Blue	light blue	Brown
	Ripen fruits of randia	2	0.111	light green	-	Brown
			0.62	Blue	light blue	Brown
			1	Blue	light blue	Brown
	Unripen fruits of randia	1	0.14	light green	light blue	Brown
			1	Blue	light blue	Brown

Quantitative Phytochemical Study

Total Saponin Quantification

Table 1: Standard Diosgenin

Sample	Conc	Abs
Control	0	0.032
Standard (Diosgenin)	100	0.059
	200	0.097
	300	0.152
	400	0.199
	500	0.263

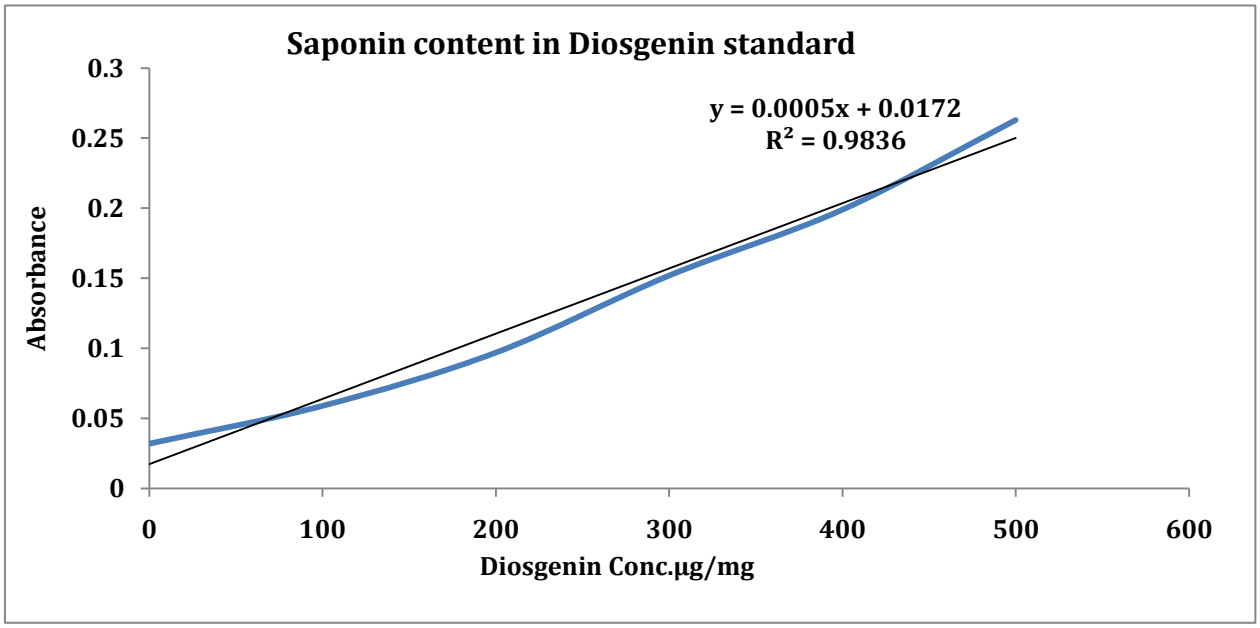


Figure 1: Standard Saponin graph

Table 2: Saponin content in the samples (Methanol Extract)

Sample name	Conc. (ug/ml)	Absorbance at 544nm	Conc. Saponin (ug DE/100ul sample)
Ripen processed fruits of Randia	500	1.268	73.71
Ripen fruits of Randia		1.432	83.23
Unripen fruits of Randia		1.227	71.35

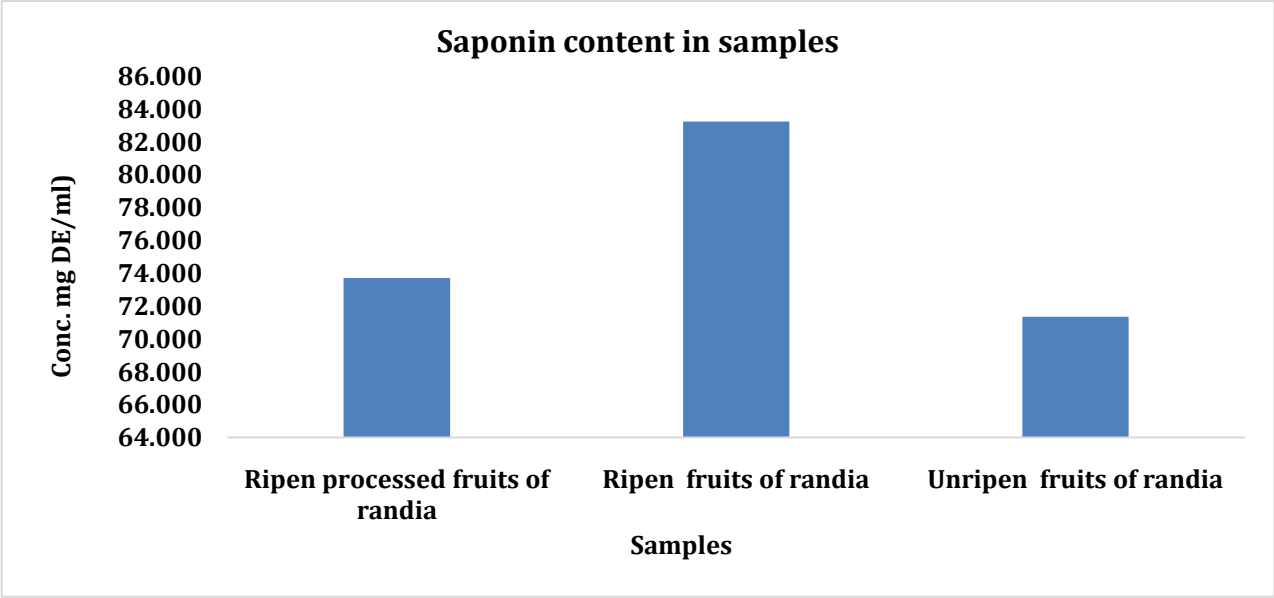


Figure 2: Saponin content in samples

Table 3: Saponin content in the samples (Aqueous extract)

Sample name	Conc. (ug/ml)	Absorbance at 544nm	Conc. Saponin (ug DE/100ul sample)
Ripen processed fruits of Randia	500	1.5906	72.62
Ripen fruits of Randia		1.2497	92.44
Unripen fruits of Randia		1.2948	75.25

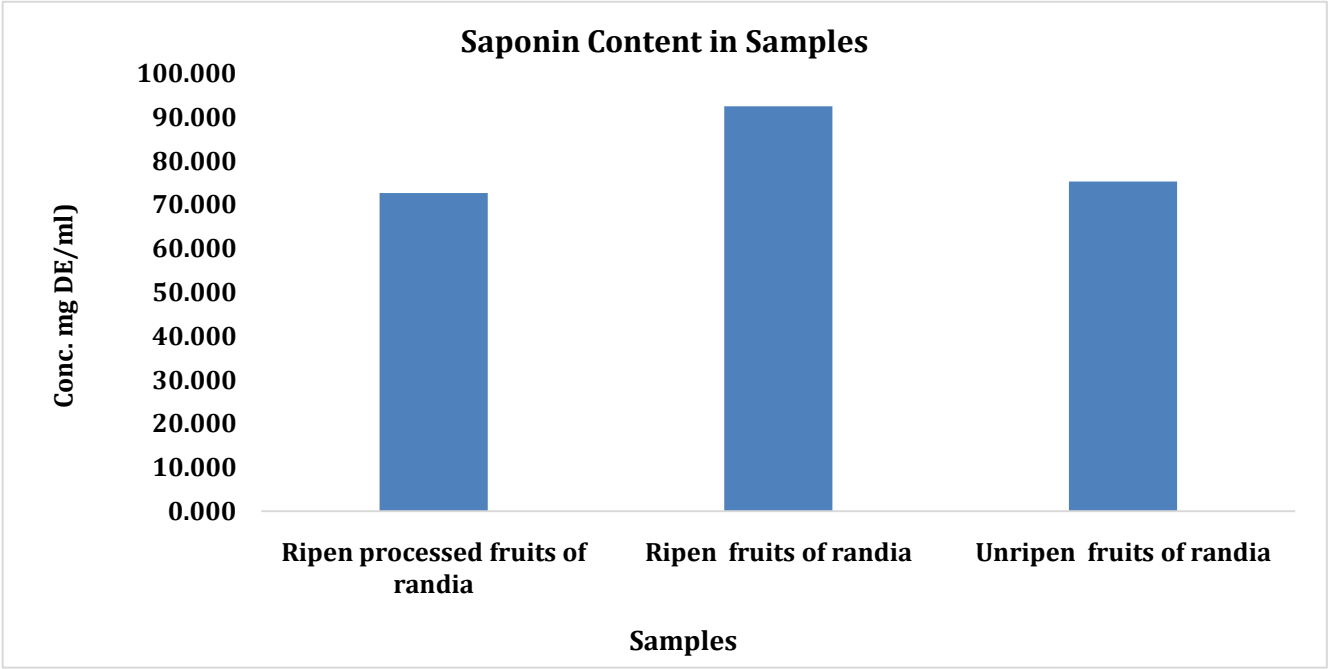


Figure 3: Saponin content in samples

In saponin estimation, methanol and water extract of ripen processed fruits of Radia, ripen fruits of Randia and unripen fruits of Randia is found to contain 73.71, 83.23, 71.35 and, 72.62, 92.44, 75.25µg DE/mL of Saponin content respectively.

DISCUSSION

The microscopic study of all the three samples did not showed significant changes in the powder microscopy. They showed the presence of reddish brown cells, sclereids, xylem, parenchyma, trichomes, yellow orange pieces of seed coats, trichome etc. Ash values of *Shalatu*, *Pakwa Phala* and *Samskarita Madanaphala* are 4.1%, 3.45%, 3%, in all the three samples its under API limit of not more than 6% which is mentioned for the dried fruits of Randia. Alcohol Soluble Extractive Values of all the 3 samples are *Shalatu*- 16.64%, *Pakwa Phala* 14%, *Pakwa Samskarita*- 45.92%. Water Soluble Extractive Values of *Shalatu*- 19.28, *Pakwa phala*- 22, *Samskarita Madanaphala*- 26.08. In both aqueous and alcohol soluble extracts, the extractive value is highest in *Samskarita Madanaphala* which may be because of the addition of other ingredients like *Tila* (sesame), honey etc during the time of processing. The preliminary qualitative phytochemical analysis of the aqueous and alcoholic extracts of all the three samples showed the same phytoconstituents except the presence of Terpenoids in the extracts of *Samskarita Madanaphala* which is absent in *Shalatu* and *Pakwa Madanaphala*. TLC of the methanolic extract of unripen fruit, Ripen fruit and ripen processed fruit showed 3, 5, 2 bands at 254nm respectively. Aqueous extract of unripen fruit, Ripen fruit and ripen processed fruit showed 1, 2, 1 bands at 254nm. Saponin is one of the main active principles responsible for the emetic action in *Randia dumetorum*. Total Saponin estimation was done by UV spectrophotometry. Methanol and water extract of ripen processed fruits of randia, ripen fruits of randia and unripen fruits of randia is found to contain 73.71, 83.23, 71.35 and 72.62, 92.44, 75.25µg DE/mL of Saponin content respectively, which suggests the quantity of Saponin one of the main constituent responsible for emesis is in same range in unripe and processed fruits of Randia whereas it is in highest in ripen fruits of Randia. May be because of this highest quantity of Saponins in ripen fruits of Randia, these fruits are not advised directly for therapeutic purpose to induce emesis without *Samskara* (processing). After *Samskara* (processing) the quantity of saponins are reduced which may be adequate to induce emesis and stops on its own. As the quantity of saponin in *Shalatu* (unripe fruit) is also in same range as in processed fruit, it may be because of this it is advised as an *Abhava Pratinidhi* (substitute) for the *Samskarita Madanaphala* in classical textbooks.

CONCLUSION

The powder microscopic study, preliminary qualitative phytochemical analysis and quantitative analysis of saponin show similarity between the *Shalatu* (unripe fruit) and the processed fruits of *Madanaphala*. Thus, concluding the rationality of using the *Shalatu* as substitute for *Phala Pippali*. Further the preclinical experimental and toxicity studies should be conducted to prove the safety and efficacy of the drugs.

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