

Physico-Chemical and Phyto-Chemical Screening of Madanaphala Seeds with Reference to its Sangraha and Samskara

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Abstract

Standardization of herbal drugs is the need of the present day, in order to confirm its therapeutic value and to stand out in the global market. Place of collection, season of collection and so on plays an important role to assure the quality of the drug in terms of identity, purity and strength. Samskara is the process that may bring out some radical changes in the substance by replacing their natural properties totally or partially into a new one or by intensifying or moderating their qualities. The present study was planned and carried out to know the significance of Sangraha and Samskara of Madana phala through physico-chemical and phytochemical analysis. Fresh fruits of Madana phala were collected as per the sangraha vidhi and were subjected to samskara by keeping it in the kusha puta and triturating it with ghritha, dadhi, madhu and tila kalka as per the Samskara vidhi mentioned in Charaka samhita. All the three samples i.e, self collected, processed and market samples were analysed through pharmacognostic, physico-chemical and qualitative & quantitative phyto-chemical study. Also analysed through HPTLC by using Oleanolic acid as standard. Quantitative phyto-chemical analysis showed more amount of saponin, tannin, phenol & HPTLC analysis showed more amount of oleanolic acid in self collected sample than the market sample. Whereas there was less amount of saponin, tannin, phenol and Oleanolic acid in processed sample (samskarita) when compared to the self collected sample (asamskarita) of Madana phala. For the better yield of phyto chemicals samples need to be collected as per the classical reference, which may not be followed by the commercial suppliers. Samskara of Madana phala as per Charaka plays an important role in modifying the total saponin concentration which is validated through the qualitative and quantitative analytical tools.

Keywords: Sangraha; Samskara; Madana phala; HPTLC; Oleanolic acid

Introduction

Plants are one of the main sources of medicine since time immemorial. Ayurveda, the science of life emphasize on use of plants as medicine and strongly says that each and every dravya in this universe possesses some medicinal property. Standardization of herbal drug is the

need of the present day, in order to confirm its therapeutic value and to stand out in the global market. Standardization of herbal drug includes authentication, Place of collection, season of collection etc. which plays an important role to assure the quality of the drug. Our acharyas also knew the role of soil, place, season, and storage techniques in procuring a quality crude drug. In

our classics, it is told that, dravyas are capable of producing maximum therapeutic effects when their veerya (active principles) is augmented by collecting the plants in appropriate season (kala sampath), habitat (desha sampath), when they are enriched with excellent attributes (guna sampath), and storing in appropriate container [1].

Acharya Charaka has mentioned the ideal season and features of the fruit to be collected for Madana phala [2]. In spite of standardization, there are some constraints in getting a quality raw drug because herb collectors/suppliers collect the medicinal plants randomly without considering the parameters set for collection of the medicinal plants. According to Acharya Charaka, Samskara is the transformation of inherent qualities of a substance [3]. When this transformation is done in proper way under controlled conditions to get desired effect, it is termed as samskara.

By virtue of appropriate samskara, samyoga, vishlesha and kala, even a small quantity of drug may produce more powerful effects; otherwise even a large quantity of drug may produce very mild effect [4]. Acharya Charaka has explained the Samskara of Madana phala before administering it for vama [3]. Till now, no analytical work has been done on the Sangraha and Samskara of Madana phala. So, this concept of Sangraha & Samskara of Madana phala has to be analysed under the light of modern scientific methodology [4]. Keeping this in mind, the present study was planned and carried out to know the significance of 'Sangraha of Madana phala' by comparing the self collected sample with the market sample through the physico and phytochemical analysis. The significance of 'Samskara of Madana phala' by comparing the changes in Samskarita (processed) Madana phala with that of Asamskarita Madana phala (self collected) through physico and phytochemical analysis. Here after, samskarita Madana phala will be named as processed sample (PS) Asamskarita Madana phala will be named as self collected sample (SCS) and the Market sample will be denoted by MS.

Materials and Methods

The sangraha and samskara vidhi of Madana phala are required to be analyzed under the light of modern scientific methodology. Hence, this analytical study was carried out.

Sangraha Vidhi of Madana Phala

The sangraha vidhi of Madana phala explained by Charaka includes kaala sampath (collecting in appropriate

season) and guna sampath (enriched with excellent attributes/proper maturity).

Procurement of Plant Material: Madana phala fruits (Randia dumetorum Lamk)

Fruits were collected from the forest areas of Chikkamagalur district in the month of april (vasantha greeshmayor anthare) as mentioned in Charaka kalpa sthana.

Samskara Vidhi of Madana Phala

Samskara vidhi of Madana phala explained by Charaka includes:

Kaala samskara: Among 3 kgs of collected fruits, immature, over-ripened, foul smelling and infected fruits were removed and only 1.5 kgs were taken for the study. 3 kusha puta were prepared. Each puta consisting of 500gms of Madana phala. Over this kusha puta gomaya lepa was done. This gomaya lepitha puta is dried for 3 days and later kept inside the heap of kulattha for 8 days. After 8 days when madhu gandha (smell of honey) or ishta gandha (pleasant odour) is emitted from fruits, they were removed from kusha puta and dried under sunlight for 20 days. Later, phala pippali is separated from fruits and kept for drying under shades for about 3 days and made into churna.

Mardana samskara: Phala pippali churna is triturated with ghritha, dadhi, madhu and tila kalka one after the other. The amount of drava dravya taken is till the churna is completely immersed in it and should be sufficient to mix the powder to be triturated. The duration of Mardana samskara to Madana phala is till it gets converted into sticky paste form and is soft in nature. The analytical procedures involved in these studies are in accordance with Protocol For Testing Ayurvedic, Siddha & Unani Medicines, Ayurvedic pharmacopoeia of India and WHO guidelines.

Physico – Chemical Study

Foreign matter, Loss on drying, total ash, Acid insoluble ash, Water soluble ash [5-7]. Alcohol soluble extractive value, Water soluble extractive value, PH

Phyto – Chemical Study

Preparation of extract: Preparation of aqueous and alcoholic extract of MS, SCS, PS of adanaphala by cold maceration method.

Preliminary phytochemical analysis: A systematic and complete study of crude drugs should include a complete investigation of both primary and secondary metabolites

derived from plant metabolism [5,8,9]. The different qualitative chemical tests are to be performed for establishing profiles of the given extracts for their nature of chemical composition [10].

Phyto - Chemical Study

- Qualitative phyto- chemical analysis [10]
- Quantitative phyto-chemical analysis.
- Qualitative & Quantitative chromatographic analysis by TLC and HPTLC.

Results

Sangraha & Samskara of Madanaphala

Sl.no	Parameters	Quantity
1	Freshly collected fruits	3kgs
2	Mature ,non-infected fruits taken for study	1.5kgs
3	Fruits after drying	1kg
4	Yield of phala pippali	250gms
5	Yield of phala pippali after drying	150gms
6	Yield of Phala pippalai churna	125gms
7	Yield of Phala pippali churna after mardanawith ghritha, dadhi, madhu and tila kalka	200gms

Table 1: Yield of Madana Phala after Sangraha and Samskara.

	Colour	Odour	Taste	Form
Market sample of Madana Phala				
Aqueous extract	Dark brown	Agreeable	No taste	Powder
Alcoholic extract	Cream	Agreeable	-	powder
Self collected sample of Madana Phala				
Aqueous extract	Light brown	Agreeable	-	Powder
Aqueous extract	Blackish Brown	Agreeable	-	Semisolid
Processed sample of Madana Phala				
Aqueous extract	Brown	Agreeable - fruity odour	-	Semisolid
Alcoholic extract	Golden brown	Agreeable	-	Semisolid

Table 2: Organoleptic study of Extracts.

Powder Microscopy of Randia Dumetorum Lamk

S.No	Diagnostic features	Marketed sample	Self collected samples	Processed Samples
1	Cells of mesocarp containing tannin	+	+	+
2	Endosperm containing aleurone grains and oil globules	+	+	+
3	Fibre	+	+	+
4	Rosett crystals of calcium oxalate	+	+	+
5	Stone cells	+	+	+
6	Testa in surface view	+	+	+
7	Tannin cells	+	+	+

Table 3: Powder Microscopy.

Sl.no	Physico-Chemical Tests	Market sample	Self Collected sample	Processed sample
1	Determination of foreign matter	0.56%	0.47%	0.49%
2	Total ash	4.25%	2.75%	2.80%
3	Acid insoluble ash	0.35%	0.25%	0.37%
4	Water soluble ash	2.82%	2.27%	2.40%
5	Alcohol soluble extractive	24.78%	27.54%	28.60%
6	Water soluble extractive	18.76%	26.27%	27.19%
7	Loss on drying	8.47%	5.52%	7.53%
8	P _H	6.51	5.67	5.95

Table 4: Comparative Physico-Chemical Constants.

Qualitative Phyto-Chemical Study

Phytoconstituents	Marketed sample		Self collected sample		Processed sample	
	Aq	Al	Aq	Al	Aq	Al
Glycosides	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-
Triterpenoids	-	+	-	+	-	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Alkaloids	-	-	-	+	-	-
Saponins	+	+	+	+	+	+
Steroids	-	+	-	+	-	+
Proteins	-	-	+	+	+	+
Carbohydrates	+	-	+	-	+	-
Resins	-	-	+	+	+	+
Mucilage	-	-	+	+	+	+

Table 5: Comparison of Phyto-Chemical Results.

Foaming Index

Sl.no	Sample	Foaming index
1	Market sample	166.6
2	Self collected sample	250
3	Processed sample	142.8

Table 6: Comparison of Foaming Index.

Quantitative Phyto-Chemical Study

Sl no	Sample	Total % of saponin	Total % of tannins	Total % of total phenols
1	Market sample	22.53%	0.62%	0.63%
2	Self collected sample	34.97%	1.12%	1.11%
3	Processed sample	12.43%	1.05%	0.55%

Table 7: Total saponin, tannins and phenol content.

Qualitative Hptlc Analysis

Qualitative HPTLC analysis of alcoholic extract of Market, Self Collected and Processed Sample of Madana Phala

Extracts: Methanolic extract of market, self collected and processed sample of Madana phala.

Solvent System: toluene: ethyl acetate: formic acid (7:3:0.3v/v)

Spray agent: Anisaldehyde sulphuric acid

Solvent Front: 8.8 cm

Volume applied: Standard Oleanolic acid: 4 µl; Sample or test solution: 4 µl.

Sl. No.	Market Colour	Self collected		Processed		
		Rf value	Colour	Rf value	Colour	Rf value
1	Pinkish purple	0.03	Pinkish purple	0.03	Light bluish	0.22
2	Pinkish purple	0.1	Light blue	0.07	Purple	0.295
3	Light blue	0.17	Light bluish	0.136	Violet	0.55
4	Purple	0.295	Bluish	0.22	Purplish	0.57
5	Blue	0.44	Purplish	0.295	Light purple	0.63
6	Violet	0.55	Violet	0.55	Light bluish	0.82
7	Purple	0.57	Purplish	0.57		
8	Light purple	0.63	Light purple	0.63		
9	Purplish	0.82	Bluish	0.82		

Table 8: R_f values of HPTLC profile.

- Standard oleanolic acid :1 spot observed @ R_f 0.55
- All the samples showed the presence of oleanolic acid at R_f 0.55
- All spots were visible only after spraying anisaldehyde reagent on it.

Quantification of Oleanolic Acid by Hptlc Analysis

Sl. no	Samples	% of Oleanolic acid
1	Market sample	0.34%
2	Self collected sample	0.56%
3	Processed sample	0.34%

Table 9: Quantification of oleanolic acid.

Discussion

Madanaphala is a drug known from samhitha period itself. It is a drug of choice in vamana karma, which has been mentioned in agrya dravyas. All the authors have considered that Madana phala is a Shreshta Vamaka but in Arka prakasha it has been quoted that Arka of Madana is Chardighna [11]. The term phala specifically indicates Madana phala even though there are several other fruits which are used in medicine. The fruits contains the phyto constituents like phenols, triterpenoids, steroids, tannins and more amount of saponins. In our classics it is told that vamana and virechana dravyas acts because of Prabhava [12]. The active principle responsible for the emetic action of the drug is not yet known. Apart from being used in vamana, it is also used in other Panchakarma therapies such as basti, virechana and nasyakama. There is a specific sangraha & samskara vidhi of madanaphala in all the brihat traysis. So the present study was carried out in order to know the significance of Sangraha and Samskara vidhi of Madana phala analytically.

Concept of Dravya Sangraha

Environmental conditions such as sunlight, rainfall, altitude, temperature, soil, storage conditions as well as different harvesting procedures, time and method of collection can create substantial variability in product quality and in the concentration of plant chemicals within different products. The therapeutic or toxic components of plant vary depending on the part of the plant used as well as stages of ripeness [13]. The quantity of active principles is believed to be responsible for the rate of drug action [14].

All the classical texts have mentioned the collection of fruits according to the fruiting season (yathaa rutu). But in the sangraha of Madana phala it is told to collect in vasantha greeshmayoranthare (in the middle of summer and spring season) which is a fruiting season for Madana phala. The maturity of the plants is also important factor to be kept in mind while collecting the drug. This has been explained very well by Charakacharya as –the drugs which have grown in their proposed time, with all its proposed morphological features and which has properly matured in its pramana, rasa, veerya, gandha should be collected in order to get the maximum therapeutic effects. Hence in this work, the fruits collected according to the Sangraha vidhi as mentioned in the Charaka samhitha were compared with the market sample of Madana phala to know the significance of Sangraha.

Concept of Samskara

Samskara means the transformation of inherent

attributes. Qualitative alteration done for improvement, enhancement, modification, lowering bad effect or any such procedure, pharmaceutical treatment etc. have been denoted by a single term Samskara. By this process, one can modify the dravya to meet his requirements. The drugs available can be utilized multi dimensionally only with the help of Samskara. The present study includes kaala and mardana samskara, where samskarita Madana phala was compared with the asamskarita (self collected) to know the significance of Samskara.

Physico and Phytochemical Investigations

By following this Sangraha vidhi of Madana phala there may be increase in the number & amount of phyto constituents which is to be known through qualitative and quantitative phytochemical investigations by comparing with the market samples which are usually collected randomly by the herb collectors who are unaware of the medicinal plants and its collection. Physico chemical investigations are of less significant in knowing the number and amount of the phyto constituents, but are important in knowing the purity of the drug. As we are comparing this with the market sample, in order to check the genuinely and purity of market sample physico chemical analysis like total ash, loss on drying were done for both the samples. Collected fruits were kept in kusha puta and gomaya lepa was done over it in order to prevent the air entry by which fast ripening of fruits occurs (presence of ethylene¹⁵). Reduction in the weight of the fruit from 1.5 kg to 1 kg is due to drying it under sunlight. From 1.5 kgs of Madana phala, we obtained only 125gms of Madana phala pippali churna. This shows that the yield of churna is less compared to the quantity of fruits taken. More amount of dadhi was required for triturating as it became thick in consistency and difficult in grinding. Yield of phala pippali churna after mardana increased by 37.8% which is due to the addition of 4 dravyas for mardana.

Qualitative Hptlc of Aqueous Extract of Ms, Scs and Ps of Madana Phala

Oleanolic acid was not seen under visible light/366nm/254nm. Because the aqueous extract was not hydrolysed with 2N methanolic hydrochloric acid. Only on hydrolysis, saponin (oleanolic acid) will be separated from the saponins. Only few spots were visualized under visible light. This indicates that the solvent system used is not suitable for the water soluble phytoconstituents. Presence of only 3 spots in MS, 4 spots in SCS and 1 spot in PS @ 366 & @ 254nm indicates that water soluble phyto-chemicals are less in the samples and were visible only under UV light.

Quantification of Oleanolic Acid by HPTLC

Comparison between Market & Self Collected Sample of Madana Phala

Oleanolic acid % in the MS (0.34%) was less compared to the SCS (0.561%) i.e, Oleanolic acid in MS is less by 38.93% when compared to amount of Oleanolic acid in the SCS. Isolated Oleanolic acid has been proved experimentally as analgesic and anti-inflammatory [14]. So more amount of oleanolic acid in self collected sample of Madana phala may be responsible for its increased rate of action like analgesic & anti-inflammatory activity in Madana phala.

Comparison between Market & Self Collected Sample of Madana Phala

Oleanolic acid % in PS (0.33%) was less compared to the SCS (0.561%). This shows that after samskara, the amount of Oleanolic acid was reduced by 41.07% when compared to the amount of oleanolic acid in SCS. Isolated Oleanolic acid (aglycone part of saponin/sapogenin of triterpene saponin), may have gastric irritant activity in a lower degree or may be absent when compared to the corresponding saponin [16]. The HPTLC finger print of self-collected Madana phala can be used as standard or reference material as it was collected considering the season and maturity of fruits as mentioned in our classics.

Significance of Increase in the Phyto-Constituents of Self Collected Sample of Madana Phala

In Ayurveda, Madana phala has been widely used for vamana karma. But it has got various actions like shotha hara, vedanasthapaka, kapha nissaraka, krimighna, arthava janaka, lekhana and vishagna. It has also got its prayoga in various diseases like amavatha, vata vyadhi, vidradhi, vrana etc.

It has been proved experimentally also for its pharmacological activities like anti-inflammatory, analgesic, anti-oxidant, immunomodulatory, anti-diabetic and so on. So, more amount of saponins (oleanolic acid), tannins, phenols in the self collected sample of Madana phala, may be responsible for increased rate of action of pharmacological activities mentioned above and from this maximum therapeutic effect can be achieved.

Madana phala is used as an ingredient in many formulations like brihat saindhavadi taila, lavanagaasava and so on. So instead of purchasing it from market, if collected acc to its sangraha vidhi maximum therapeutic efficacy of the formulation can be achieved.

For vamana karma also, Madana phala has to be collected acc to its sangraha vidhi, and later has to be

subjected to its samskara vidhi as the from this study we came to know that market samples are not genuine in terms of purity.

Probable Reason behind Reduction in the Phyto-Constituents Content after Samskara

Only probable reason behind the reduction in the saponin content of processed sample can be known, but reduction in tannin and phenol could not be illicited.

The complex structure of saponins may undergo chemical transformations during storage or processing which in turn may modify their properties/activity¹⁶. Trituration of Madana phala with 4 different media might have caused some interaction of sterols and proteins of lipids with the saponin and would have resulted in the modification of the physicochemical properties of these saponin¹⁷. Enzymes present in the dadhi (rennin) and Madhu (diastase, invertase, catalase, glucose oxidase) might have caused hydrolysis of the glycosidic bond (between the sugar chain and the aglycone), and the interglycosidic bonds between the sugar residues of saponins and resulted in the formation of aglycones, prosapogenins, sugar residues or monosaccharides. When total saponin estimation was done for processed sample the amount of saponin was reduced as most of the saponins have got hydrolysed.

So, The amount of saponin in the processed one has reduced by 3 times when compared to the total saponin content in the unprocessed one which shows that the reduction of saponin is not by dilution of the concentration by addition of double quantity of media, but due to some chemical changes of saponin occurred by enzymatic reaction.

Significance of Samskara of Madana Phala

It was already proved clinically that unprocessed Madana phala resulted in pratiloma pravrutti (diarrhoea) when given for vamana, whereas processed did not show any pratiloma pravrutti and there was controlled way of initiation and completion.

No where the active principle or the bio-marker responsible for the emetic action of Madana phala is mentioned. But in one reference we found that saponins on contact produce a generalised irritation of the mucous membranes producing sneezing, vomiting and irritation. So only based on this reference we may infer that reduction in the saponin content after samskara might have resulted in the controlled way of initiation and completion of vamana and absence of pratiloma pravrutti in the clinical study conducted previously.

The physico & phyto-chemical results and HPTLC fingerprint of processed Madana phala may be helpful in authenticating the classically samskarita Madana phala and will also serve as a reference material (standard) for further studies.

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