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Comparative Study of *Aragvadha Phalamajja* (*Cassia fistula* Linn.) w.s.r. to its *Sangrahana Vidhi*

Research Article

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Abstract

Ayurved advocates that drug should be collected with proper classical methods. In Ayurvedic texts, specific time, season and procedures or methods have been highlighted for getting better potency, efficacy and therapeutic properties of drug by describing the standard methods of drug collection and preservation in details. Now a day, due to lack of proper traditional knowledge and time very few people bother to follow all these methods of collection given by *Acharya*. Hence, the potency of medicines is of question. *Aragvadha* (*Cassia fistula* Linn.) has been popular as a common drug of choice treatment since ancient time. Classic text like *Charaka Samhita* has quoted the classical and particular method for the collection and preservation of *Aragvadha Phalamajja* (*Cassia fistula* Linn.). *Acharya Charaka* has quoted this collection method of *Aragvadha Phalamajja*(*Cassia fistula* Linn.)as follows- The good qualities of collected pods should be kept covered in river bed sand for seven days. After seven days these pods should be removed and kept in sunlight for some time. And thereafter, fruit pulp (*phalamajja*) should be extracted and stored in a clean vessel. Therefore, study of classically collected *Aragvadha Phalamajja* (*Cassia fistula* Linn.) and Market sample of *Aragvadha Phalamajja* (*Cassia fistula* Linn.) was carried out.

Key Words: *Cassia fistula Linn, Collection method, Aragvadha, Standardization, Sangrahana Vidhi.*

Introduction

In Ayurved quality of drug is quoted as *Bahukalpa* (useful in many forms), *Bahuguna* (having many qualities), *Sampanna* (rich in active ingredients) and *Yogya* (suitable for preparation of medicine).(1) As per *Charakacharya*, standard drug should be examined on the basis of nature (natural composition), properties, action, habitat, time and mode of collection, storage, processing, dosage etc.; any other drug of this type should fulfil the same characters.(2) If drug is not collected with its proper collection methods, there will be increased chances of reduction in its potency and efficacy. Ayurved has described the standard methods of drug collection and preservation in details to enhance their potency, efficacy and therapeutic properties. These methods may also improve shelf-life of that particular dravya or may reduce any harmful properties of that dravya. Moreover, by these means, prevention from mold, other microorganisms and insects; detoxification of toxic ingredients and enhancement in therapeutic actions can be achieved.

Now a day, due to lack of proper traditional knowledge and time very few people bother to follow all these methods of collection given by *Acharya.* Hence, the potency of medicines is of question. *Aragvadha* (*Cassia fistula*Linn.) has been popular as a common drug of choice since ancient time due to its therapeutic properties like laxative, antipyretic etc. useful in the management of diseases like *Hridroga*(cardiac problems), *Kushtha* (skin problems), *Prameha* and many more. As per available sources *Aragvadha* has been screened for its antioxidant activity (3), anti-tumour activity (4), hepatoprotective activity (5), antibacterial activity and antifungal activity (6). *Acharya Charaka* has quoted this collection method of *Aragvadha Phalamajja* as follows-

"Pods of *Aragvadha* should be collected in its proper period of ripening. These good qualities of collected pods should be kept covered in riverbed sand for seven days. After seven days these pods should be removed and kept in sunlight for some time. And thereafter, fruit pulp (*phalamajja*) should be extracted and stored in a clean vessel (7)."

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Aragvadha is very easily available drug but as per available information its *Phalamajja* provided by market sources is not collected by following standard classical method. Market sample and Self-collected sample of *Aragvadha Phalamajja* were studied with the help of standardization methods like organoleptic, Pharmacognostical, physiochemical, chromatographic and physiochemical test.

Aim

To study *Aragvadha Phalamajja* (*Cassia fistula* Linn.) with special reference to its *Sangrahana vidhi* (Collection Method).

Objectives

- To collect the genuine sample of *Aragvadha Phalamajja* (*Cassia fistula* Linn.) from genuine source, as per GACP guidelines and to authenticate the sample.

- To design the model for *Sangrahana* and *Sanskarana* (process) of the genuine sample according to Acharya Charaka (Cha. Ka. 8/6-7). 3. To standardize and compare the authenticated 'Sangrahita and Sanskarita (processed)' i.e. Self- collected sample with the market sample.

Material and Method

Collection of Drug

The pods of *Aragvadha* (*Cassia fistula* Linn) were collected in first week of March from Akurdi Campus, Pune. It has been described in Charaka Samhita that fruits of a plant should be collected in the fruiting season of that plant (8). Moreover, in Medicinal Plants text (9), the ripening period of *Aragvadha* is given in February and March month. Therefore, pods were collected in the first week of March. Collection and storage of sample was done as per norms of GACP guideline.

Authentication of genuine sample

The collected genuine sample was subjected to Agharakar Research Institute, Pune for Authentication.

Method of Preparation of Model for *Sangrahana Vidhi*:

Selection of Pods

Pods of *Aragvadha* having average length of 60-65 cm respectively, externally deep brownish black coloured, well- ripened (matured), undamaged, unaffected by insects, unbroken and having good qualities were selected.

Selection of Area for preparation of Pit

- Area was selected which was devoid of drainage, waste materials etc.
- Area was having plenty source of sunlight in open area was selected.

Preparation of Pit for *SangrahanaVidhi* of *Aragvadha Phalamajja*

- Dimensions of the Pit were made according to sizes of pods i.e. on the basis of their length.
- Pit having size of 2.5' length, 2.5' breadth and 2' depth were prepared in an open area.
-

Image 1: Collection of Drug

Image 2: Selection of Pods

Image 3: Selection of area

Image 4: Preparation of pit

Image 5: Storage of Pods in pit

Image 6: Extraction of Fruit pulp *(Phalamajja)*

Image 7: *Sangrahan Vidhi* of *Aragvadha Phalamajja (Cassia fistula* fruit pulp)

Storage of Pods in the pit

- After preparation of pit, selected pods were buried in the pit with sand collected from riverbed.
- Thus, pods were covered beneath of sand for seven days in sunlight.

Extraction and Collection of *Aragvadha Phalamajja-*

- After seventh night, on the next morning pods were removed from sand and from the pit.
- Pods were kept in the Sunlight for few hours.
- Thereafter, two ends of pods were cut with sharp knife; pods were separated into two parts vertically for easy removal of the *Phalamajja*.
- *Phalamajja* was taken out carefully and stored in clean, airtight jar.

Standardization of Drug

Both Sample were studied for:

- Organoleptic study (*Shabda, Sparsha, Roopa, Rasa, Gandha*)
- Pharmacognostic Study (Macroscopic examination, Microscopic examination)
- Physiochemical Study (Foreign matter, Ash Value- Total Ash & Acid insoluble ash, Alcohol soluble extractive value, Water soluble extractive value and Total solids dissolved
- Chromatographic Study (HPTLC)
- Phytochemical Study (Alkaloids, Carbohydrates, Sterols, Glycosides, Tannins, Proteins, Gums & Mucilage, Amino acids, Flavonoids)

Observations and Results

Organoleptic Study

Table 1: Organoleptic observations of both samples

	Market Sample	Self-collected Sample
***Shabda* (Sound)**	*Abhangura* (Non-breakable)	*Abhangura* (Non-breakable)
***Sparsha* (Touch)**	*Snigdha* (Unctuous), *Mrudu* (Soft), *Sheeta* (Cold), *Shlakshna* (Smooth)	*Snigdha* (Unctuous), *Mrudu* (Soft), *Sheeta* (Cold), *Shlakshna* (Smooth)
***Roopa* (Appearance)**	*Brownish black, Irregular, Ardra* (Moist)	*Black, Round, semicircular, Alpa Ardra* (Less moist)
***Rasa* (Taste)**	*Madhura* (Sweet), *Tikta* (Bitter)	*Madhura* (Sweet), *Tikta* (Bitter)
***Gandha* (Odour)**	*Ugra* (Strong), *Sugandha* (Aromatic), *Madhuragandhi* (Sweet in odour)	*Manda* (Mild), *Sugandha* (Aromatic), *Madhuragandhi* (Sweet in odour)

Pharmacognostic - Microscopic Study

Table 2: Microscopic observations of both samples

Contents	Market Sample	Self-collected Sample
Epidermal tissue	Present	Present
Fibro-vascular Bundles with Xylem and Phloem	Present	Present
Parenchymatous Cells	Present	Present

Physiochemical Study(10)

Determination of Foreign Matter

The crude drug taken for study are either the part of the plant or whole plant. It should be completely free from other foreign material which is not at all part of plant (insects or moulds, dust, visible and excreta contaminant, chemical residues, harmful and poisonous foreign matter). This determines foreign material present in crude drug (Khandelwal K R (2007).

Determination of Ash value

In present study, we have calculated total ash and acid insoluble ash. The total ash is carried out to calculate the total amount of crude drug remaining after ignition. Whereas, acid-insoluble ash is residue acquire after boiling dilute hydrochloric acid with the total ash (Khandelwal K R (2007).

Determination of Extractive value

This determines the amount of active constituents were extracted with the selected solvents from a given amount of crude drug. This also gives the understanding about nature of the chemical constituents present in the crude drug. In present study, we have calculated water soluble extractive value and alcohol soluble extractive value (Khandelwal K R (2007).

Determination of Total solid content

A drop of prepared Phanta of sample was putted on the cleaned surface of inner side of refractometer's prism with the help of glass rod. Line of refraction was observed through the eye piece. Readings were noted which was the percentage of solid dissolved in that solution.

Table 3: Physiochemical observations of both samples

Samples	Market Sample	Self-collected Sample	API Values	QSMP Values
Foreign matter	28.56%	Nil	Not more than 2%	-
Total Ash %	5.28%	10.64%	Not more than 6%	Not more than 18%
Acid insoluble ash %	0.48	1.08	Not more than 1%	Not more than 1.3%
Alcohol Extractive %	68.8%	92.6%	Not less than 15%	Not less than 8%
Water Extractive %	60.6%	78.5%	Not less than 46%	Not less than 41%
Total Solids Dissolved	15%	20%	-	-

Chromatographic Study (11)

- Sample solvent type- Methanol
- Reagent- 10% Methanolic Sulphuric Acid
- Solvent System (Mobile Phase) - Toluene: chloroform: ethanol (4:4:1)
- Stationary Phase- Merck, TLC glass plates silica gel 60 F 254 aluminium supported sheet 10×10 cm
- Instruments- CAMAG Linomat 5 (S/N: 080222), Visualizer (S/N:150503), Scanner 4 (S/N: 170422), Development Chamber

Method & Procedure- 1gm of market sample and Self-collected sample were taken in conical flasks and 10 ml of methanol was added in each. Sonication method was applied for extraction. The extracts were filtered through filter papers. Then filtrates were used for applications. 2 µl and 5 µl each of test sample were

applied on a pre- coated silica gel 60 F 254 TLC plate (10×10 cm) of uniform thickness at uniform distance. The plate was developed in the solvent system in developing chamber. Maximum separation was observed at 366 nm in every band as compared to other wavelengths. So evalutions of tracks were done at 366 nm wavelength.

Before Derivatization

Image 8: High Performance Thin Layer Chromatography (HPTLC) analysis before derivatization

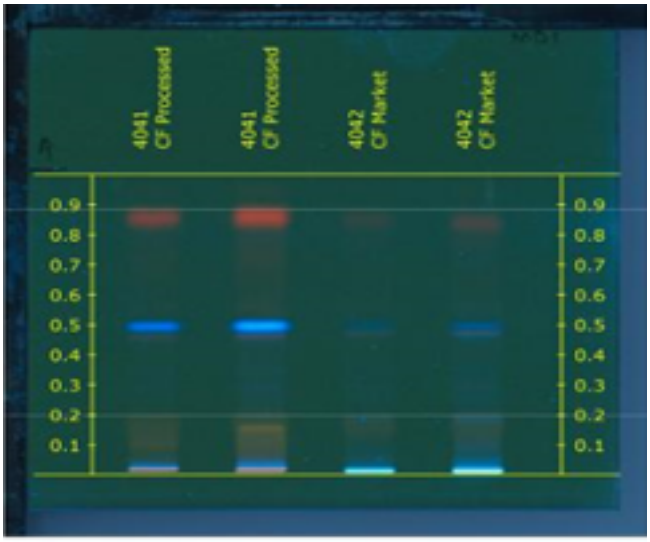

Table 4: HPTLC before derivatization

Market Sample				Self-collected Sample			
Track- 3 (2 µl)		Track- 4 (5 µl)		Track- 1 (2 µl)		Track- 2 (5 µl)	
Max. Rf	Area %	Max. Rf	Area %	Max. Rf	Area %	Max. Rf	Area %
0.015	96.32	0.013	99.02	0.023	34.98	0.019	23.84
0.185	3.68	0.739	0.98	0.497	40.91	0.497	51.05
				0.858	24.11	0.863	25.11

After Derivatization

Image 9: High Performance Thin Layer Chromatography (HPTLC) after derivatization

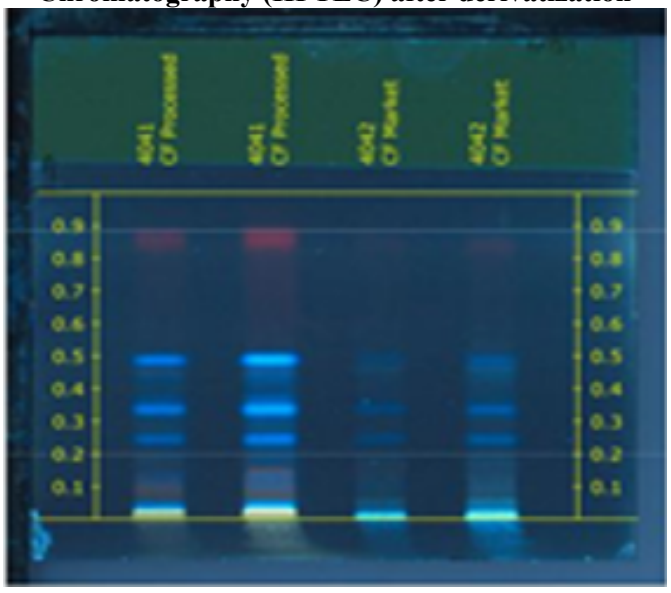

Table No. 5 HPTLC after derivatization

Market Sample				Self-collected Sample			
Track- 3 (2 µl)		Track- 4 (5 µl)		Track- 1 (2 µl)		Track- 2 (5 µl)	
Max. Rf	Area %	Max. Rf	Area %	Max. Rf	Area %	Max. Rf	Area %
0.16	20.83	**0.003**	2.32	**0.22**	9.24	**0.003**	1.05
0.23	28.42	0.034	3.65	**0.31**	28.50	0.11	3.56
0.34	50.75	0.16	3.82	0.46	43.86	**0.22**	8.55
		0.23	12.77	0.83	18.41	**0.31**	25.94
		0.32	18.82			**0.47**	48.88
		0.47	36.61			0.84	12.02
		0.82	22.01				

Table No. 6: Comparison of Equal or relative Rf with different concentration (area %) after Derivatization

Track- 3 Market Sample (2 µl)	Track- 1 Self-collected Sample (2 µl)	Track- 4 Market Sample (5 µl)	Track- 2 Self-collected Sample (5 µl)
Rf (Area %)	Rf (Area %)	Rf (Area %)	Rf (Area %)
0.23 (28.42)	0.22 (9.24)	0.003 (2.32)	0.003 (1.05)
		0.23 (12.77)	0.22 (8.55)
		0.32 (18.82)	0.31 (25.94)
		0.47 (36.61)	0.47 (48.88)

Phytochemical Study (12)

Table No. 7: Qualitative chemical evaluation of Ethanol & Water Extracts of Market and Self-collected Samples of *Aragvadha Phalamajja*- ('+' present and '-' absent)

Sr no	Test	Market sample		Self-collected sample	
		Ethanol extract	Water extract	Ethanol extract	Water extract
1	**Alkaloids-**				
	Mayer's reagent	+	+	+	+
	Dragendroff's reagent	+	+	+	+
	Hager's reagent	+	+	+	+
	Wagner's reagent	+	+	+	+
2	**Carbohydrate-**				
	Molisch reagent	+	+	+	+
	Fehling reagent	+	+	+	+
3	**Sterols-**				
	Libermann Burchard reaction	+	+	+	+
4	**Glycoside-**				
	Borntrager's reaction	+	+	+	+
	Saponins	+	+	+	+
5	**Tannins-**				
	$FeCl_3$ solution	+	+	+	+
	Lead Acetate	-	-	-	-
6	**Proteins-**	-	+	-	+
	Millon's test	-	+	-	+
7	**Gums and Mucilage-**				
	Alcoholic precipitation	-	+	-	+
	Molisch's test				
8	**Amino acids**	+	+	+	+
9	**Flavonoids**	+	+	+	+

Discussion

Pods and riverbed sand were kept in that pit for seven days. After seven days pods were removed from the pit and dried in sunlight for few hours. During removal of *Phalamajja* it was noticed that *Phalamajja* had become loosened and was easily removed. This collected *Phalamajja* was then stored in airtight glass containers. Market sample was collected from standardized and authentic local area source. In market sample it was observed that pods were crushed and then provided to the customer. This sample was containing seeds and few traces of shell of pods. Both the samples were having *madhuragandhatva* (Sweet odour); but market sample was with disagreeable strong (*ugra*) odour and Self-collected sample was having mild (*Manda)* odour. This difference may be due to *Sangrahana Vidhi* of Self-collected sample.

Market sample was containing extraneous matter like seeds, few traces of shell. In 100 gm of market sample there was 28.56% of extraneous matter. As *phalamajja* was sticky it was adhered to seeds and other parts, separation of *phalamajja* and seeds were little bit difficult. Few wastage of *phalamajja* was observed in market sample (means we didn't get exact 100 gm of *phalamajja* while purchasing 100 gm of *Aragvadha Phalamajja* from market). After removal of this extraneous part (Seeds) market sample was used for further studies.

It was observed that extract of *Aragvadha Phalamajja* is higher in alcohol solvent than that of water solvent. Self-collected sample showed markedly higher range of extractive values in both the alcohol and water solvents than market sample. It was observed that Self-collected sample having higher value of total solids dissolved (20%) than that off market sample (15%).

HPTLC showed the difference in numbers of the peaks belonging to both samples varying in each track. This may be due to absence of few constituents or their low amount in the sample. Both the samples showed some similar bands of Rf 0.003, Rf 0.23, Rf 0.32 and Rf 47 at different concentrations. This may indicate chemical constituents in both the samples are similar but having different concentrations. Two bands were seen only in Self-collected sample with low volume (2µl) but same volume of market sample didn't show any band at that Rf. These two bands were of Rf 0.46 and 0.83 having maximum concentration (Area %) with 43.86% and 18.41% respectively. Rf 0.003 (Track 4) and Rf 0.23 (Track 3) showed maximum concentration (Area %) 2.32% and 28.42% respectively in Market sample, but in Self-collected sample similar Rf 0.003 (Track 2) and Rf 0.22 (Track 1) showed less concentration (Area %) 1.05% and 9.24% respectively. This may indicate decrease concentration of certain chemical constituents in Self-collected sample as compared to Market sample. This may be due to few remnant traces of extraneous material. Rf 0.31 (Track 2) and Rf 0.47 (Track 2) showed maximum concentration (Area %) 25.94% and 48.88% respectively in Self-collected sample, but in Market sample similar Rf 0.32 (Track 4) and Rf 0.47 (Track 4) showed less concentration (Area %) 18.82% and 36.61% respectively. This may indicate increase concentration of certain chemical constituents in Self-collected sample than that of market sample. This may be due to the procedure of burring of pods (*sangrahana vidhi*).

While Phytochemical study showed Alkaloids, carbohydrates, glycosides, tannins, sterols amino acids and flavonoids were present in both the extracts (ethanol and water) of market and Self-collected samples of *Aragvadha Phalamajja.* It was found that proteins, gums and mucilage were present only in water extracts of the samples. However the exact identification of components, their quantitative estimation was not possible because it requires higher techniques.

Conclusion

- The yield of *phalamajja* in Self-collected sample is more.
- The *phalamajja* is easily extracted from pods in Self-collected sample.
- Physiochemical study reveals that maximum water and alcohol extractive values of *phalamajja* in Self-collected Sample than marketed sample.
- *Aragvadha* (*Cassia fistula* Linn.) pods should be kept covered with sand for seven days for easy removal and for more yield of *phalamajja*.

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