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Comparison of the two extraction methods of fruit pulp of *Aragvadha* (*Cassia fistula Linn.*) by HPTLC

Research Article

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Abstract

Background: The existence of significant bioactive compounds in herbs makes them pharmacologically treasured. *Aragvadha (Cassia fistula Linn.*), generally known as 'golden shower tree' is popular for its medicinal properties in Ayurveda. The fruit pulp of it owns medicinal values. Each plant has certain bioactive important compounds. For the extraction of different vital compounds, there is a need of specific method of extraction, which are known as '*panchavidha kashaya kalpana*' (five varieties of extraction) in Ayurveda. These are - *Svarasa* (expressed juice), *Kalka* (paste), *Kvatha* (decoction), *Hima* (cold infusion) and *Phanta* (hot infusion). From centuries, Ayurvedic physicians are using extraction method, called as '*phanta*' (hot infusion) for the fruit pulp of *Aragvadha* as a conventional technique; Ayurvedic scriptures also recommend the *'kvatha'* (decoction) extraction process. To assess the superiority of these two extraction procedures, the present work used high-performance thin layer chromatography (HPTLC) analysis of *Aragvadha* (*Cassia fistula Linn.*) fruit pulp methanolic extract for phytochemical profiling. Results: The HPTLC analysis of *Aragvadha (Cassia fistula Linn.*) fruit pulp methanolic extract was carried out using winCATS Planar Chromatography Manager system, and the outcomes, which were obtained as chromatograms (scanned at the wavelength of 254 nm and 366 nm) representing multiple peaks. The plant's phytochemical profile of both samples were established, and tables indicating the total count of peaks with the height, area, percent area of peaks, and Rf values. Conclusion: The study revealed that the *'kadha'* (decoction) of *Aragvadha (Cassia fistula Linn.*) fruit pulp methanolic extract contains a rich variety of phytochemicals as compare to '*phanta*' (hot infusion) which might be accountable for its therapeutic value and thus justifies superiority of the '*kadha*' (decoction) extraction method.

Keywords: *Aragvadha, Cassia fistula Linn, Analytical study, Medicinal plant, Extraction, HPTLC.*

Introduction

The use of products made from medicinal plants has raised extremely over the earlier three decades with more than 80% of persons worldwide trusting on them for healthcare. (1) The scientific community has noticed the Indian medicinal plants as a rich source of several pharmacological principles and compounds that are widely utilised as over-the-counter treatments for a variety of illnesses. (2)

With the emerging globe-wide interest in adopting and studying conventional systems and exploiting their prospective based on different health care systems, the evaluation of the rich legacy of conventional medicine is vital. (3) Ayurveda has well defined to extract the medicinal plants before using it for the treatment. Ayurvedic classics have advocated suitable '*panchavidha kashaya kalpana*' (five varieties of extraction) of plant material to utilize. (4) These are the basic preparations of Ayurvedic pharmaceutical science, which are accepted and followed. These are - *svarasa* (expressed juice), *kalka* (paste), *kvatha/ kadha* (decoction), *hima* (cold infusion) and *phanta* (hot infusion). (5) To make any classical or self-invented preparation of Ayurvedic drug or drugs, one has to first prepare basic form from five basic fundamental forms.

Aragvadha (Cassia fistula Linn.) is an important medicinal plant used in many traditional medicinal systems including Ayurveda and Chinese Traditional Medicine. It occurs all over India. It is useful as oral medicine as well external applications. The majority of the active phytochemicals are present in all plant parts, including leaves, stems, roots, flowers, and fruits. (6) Herbalists employ the majority of this plant's species extensively in traditional medicine. Most Cassia plant species are found hepato-protective, anti-inflammatory, antibacterial, antitussive, antifungal, and wound healing qualities, according to herbalists. Numerous compounds, including tannins, flavonoids, glycosides, carbohydrates, stearic acids, oleic, oxalic, linoleic, oxyanthraquinones, and anthraquinones derivatives, are abundant in the majority of these species. (7)

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Anthraquinones, flavonoids, and derivatives of flavan-3-ol are some of the strongest phenolic antioxidants found in cassia fistula. (8) It can be characterised as a stimulant laxative because anthraquinone is the cause of its laxative effect. (9) Ayurvedic physicians are practicing *phanta* (hot infusion) kind of extraction method for *Aragvadha* fruit pulp as a conventional technique from several years. However, we found several references from classical texts of Ayurveda, to make decoction of it. *Charaka* has described that according to necessity, physician can prepare medicine by boiling a drug on fire is called *'kadha'* or '*shruta*' (decoction). (10)

For the qualitative and quantitative evaluation of phytochemicals found in plants, various techniques are available. Now, it is possible to identify the active components and isolated with the help of technological improvements. High-performance thin layer chromatography (HPTLC) is a more sophisticated version of TLC since it produces data with a high level of accuracy and precision. (11) On a global scale, it is one of the most potent analytical methods for biomedical and phytochemical analysis. The pharmaceutical industry uses it the most because it is an affordable, easy, and quick approach for estimating the chemical components present in test samples for fresh drug discovery as well comparative study. The present study was performed for the comparison of two extraction methods of Ayurveda regarding *Aragvadha* fruit pulp, viz. *kadha* (decoction) and *phanta* (hot infusion), to decide the superiority of the extraction process.

Aim

To evaluate the superiority of extraction method of *Aragvadha phala majja* (*Cassia fistula Linn.*fruit pulp) by comparing it with that of two pharmaceutical methods.

Materials and Methods

Collection and authentication of plant material

The mature pods of the plant *Aragvadha* (*Cassia fistula Linn.*) collected from a nearby forest in Pune (India). A botanist and a specialist in Ayurveda both confirmed it. The sticky, brown pulp inside the pods possess high medicinal values. The authentication of *Aragvadha phala majja* (*Cassia fistula Linn.* fruit pulp) carried out with the specialized Government recognised lab and it has given the detailed assay report as follows; which has proved the originality of fruit pulp of *Aragvadha.*

Description

- Crushed fruit, chocolate brown coloured sticky mass, with seeds, mucilage and pieces of fruit wall,
- Odour-characteristic.
- Loss on drying - 27.32%
- Total Ash - 7.36%
- Acid insoluble ash - 2.65%
- Alcohol soluble extractive - 20.15%
- Water soluble extractive - 37.18 %
- TLC - Adsorbent Used Silica gel G60F254
- Methanolic extract
- Solvent system - Ethyl acetate: Methanol: Water (100: 13.5: 10)
- Detection - Visible light - One spot, Rf - 0.45 (Yellow)
- UV 254 nm - Nine spots, Rf - 0.11, 0.19, 0.23, 0.40, 0.45, 0.50, 0.67 (Blue, green), 0.75, 0.81 (Blue).
- UV 365 nm - Thirteen spots, Rf - 0.11, 0.13, 0.19, 0.23, 0.40 (Flu.Blue), 0.45.0.50 (Red), 0.54, 0.67, 0.75, 0.81, 0.90 (Flu. Blue), 0.93 (Red).
- Spray reagent - Nine spots -10% ethanolic Potassium Hydroxide, Rf - 0.04, 0.11, 0.13, 0.19, 0.23 0.40, 0.45 (Brown), 0.50, 0.54 (Pink)

Extraction

Cassia fistula Linn. fruit pulp cleaned before being dried in the shade for a week Figure 1 and 2). Then the extracts made by two pharmaceutical processes, viz. '*phanta*' (hot infusion) and *'kadha'* (decoction) as described in Ayurvedic texts. The preparation of Sample A (*phanta*/ hot infusion) (12) involved mixing 10 grams of *Aragvadha* fruit pulp with 40 ml of boiling water, and filtering the mixture once it had cooled to room temperature. 12.5 grams of *Aragvadha* fruit pulp and 200 ml of water were boiled on low heat to create Sample B (*kadha*/ decoction), (13) and the cooking was stopped when the water level was 1/4th full (50 m.l.). The methods used to extract Samples A and B were the same. 20 ml of either the Sample A or Sample B pharmaceutical formula have taken and mixed with 20 ml of distilled water. Concentrated hydrochloric acid (0.5 ml) then added. After 5 minutes of heating on the water bath, it allowed to cool. After that, 20 ml of ethyl acetate added, and the mixture was thoroughly agitated. Ethyl acetate layer has since accumulated. Ethyl acetate extract has gathered after the identical procedure carried out again, and it has since dried up by evaporation. In 4 ml of methanol, the above residue has liquefied (Figure 3).

Instrumentation

We employed a winCATS Planar Chromatography Manager system with CAMAG5 TLC scanner, Linomat5_080222, applicator fitted with 100µl syringe, illumination types of 366 nm and 540 nm, and winCATS software.

Figure 1: Aragvadha pod or fruit

Figure 2: Aragvadha fruit pulp

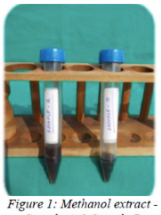

Figure 1: Methanol extract - Sample A & Sample B

Solvents and chemicals

All of the chemicals employed were of the analytical reagent grade, and all of the solvents were of the chromatography grade. For the present study, Linomat 5 applications parameters were of inert spray gas, Methanol solvent, dosage speed 150 nl/s with predosage volume 0.2 ul.

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Sample preparation

Samples A and B's dried extracts (10 g each) diluted in separate batches of 100 ml HPTLC grade methanol before filtered. For the HPTLC study, these solutions served as a test solution (Figure 3).

Conditions for chromatography

A pre-coated silica gel 60 F 254 HPTLC plate of 7.0 x 10.0 cm used for the HPTLC procedure. There was no prior modification or cleaning of the plate. The sample solution put over the plate in bands by using a CAMAG Linomat applicator. It was 150 nl/s for the steady application rate. The mobile phase, composed of chloroform, ethyl acetate, and formic acid (5:4:1 v/v/v), was used to keep the sample-loaded plate in the automatic development chamber. The CAMAG TLC scanner-4 with winCATS software used for densitometric scanning. The photos were taken in white light, 366 nm (short UV), and 540 nm (long UV) wavelengths, and the bands were seen using CAMAG visualizer. UV-active substances suffer fluorescence quenching at a wavelength of 366 nm, which causes them to become visible as black spots against a bright background. On the other hand, substances that absorb 540 nm UV light manifest as bright spots on a dark background.

Results

As shown in the figures below, the HPTLC examination of *Aragvadha* (*Cassia fistula Linn.*) revealed the presence of many phytochemicals. The three dimensional overlay of chromatograms of all tracks is demonstrated in figure 4. After scanning at UV 254 nm and 366 nm, the chromatograms (Figures 5 to 10) and produced and peak tables were created. The Rf values, peak height, peak area, and percent area of the phytochemicals are shown in the figures.

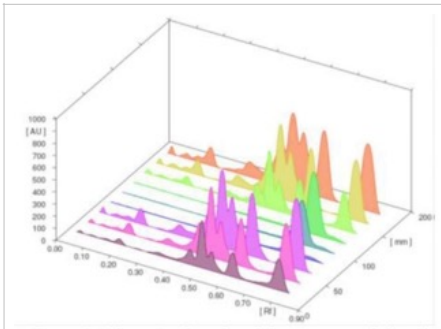
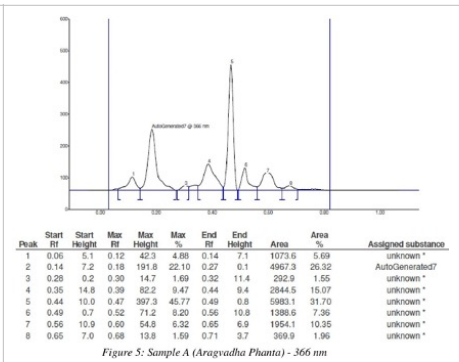
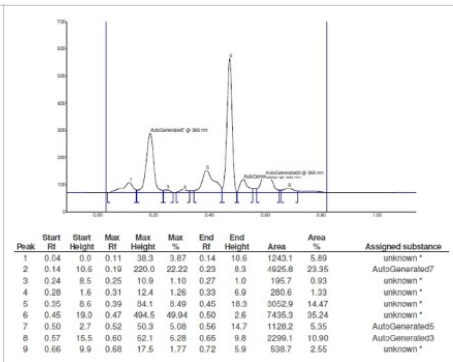


Figure 4: 3-dimensional overlay of chromatogram of all tracks



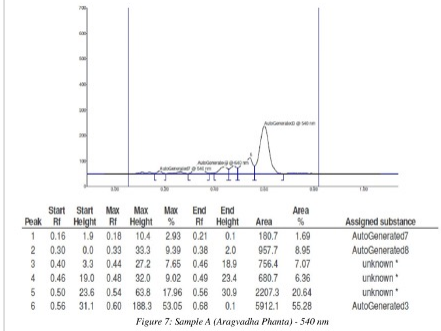
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	5.1	0.12	42.3	4.88	0.14	7.1	1073.6	5.69	unknown *
2	0.14	7.2	0.18	191.8	22.10	0.27	0.1	4967.3	26.32	AutoGenerated7
3	0.28	0.2	0.30	14.7	1.69	0.32	11.4	292.9	1.55	unknown *
4	0.35	14.8	0.39	82.2	9.47	0.44	9.4	2844.5	15.07	unknown *
5	0.44	10.0	0.47	397.3	45.77	0.49	0.8	5983.1	31.70	unknown *
6	0.49	0.7	0.52	71.2	8.20	0.56	10.8	1388.6	7.36	unknown *
7	0.56	10.9	0.60	54.8	6.32	0.65	6.9	1954.1	10.35	unknown *
8	0.65	7.0	0.68	13.8	1.69	0.71	3.7	369.9	1.96	unknown *

Figure 5: Sample A (Aragvadha Phanta) - 366 nm



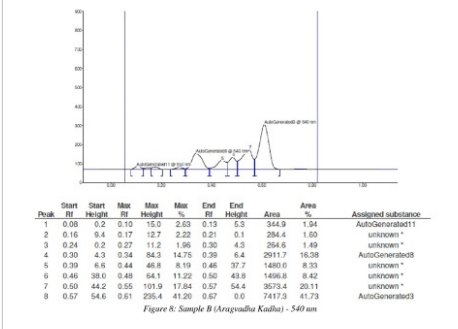
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	0.0	0.11	38.3	3.87	0.14	10.6	1243.1	5.89	unknown *
2	0.14	10.6	0.19	220.0	22.22	0.23	8.3	4925.8	23.35	AutoGenerated7
3	0.24	8.5	0.25	10.9	1.10	0.27	1.0	195.7	0.93	unknown *
4	0.28	1.6	0.31	12.4	1.26	0.33	6.9	280.6	1.33	unknown *
5	0.35	8.6	0.39	84.1	8.49	0.45	18.3	3052.9	14.47	unknown *
6	0.45	19.0	0.47	494.5	49.94	0.50	2.6	7435.3	35.24	unknown *
7	0.50	2.7	0.52	50.3	5.08	0.56	14.7	1128.2	5.35	AutoGenerated5
8	0.57	15.5	0.60	62.1	6.28	0.65	9.8	2299.1	10.90	AutoGenerated3
9	0.66	9.9	0.68	17.5	1.77	0.72	5.9	538.7	2.55	unknown *

Figure 6: Sample B (Aragvadha Kadha) - 366 nm



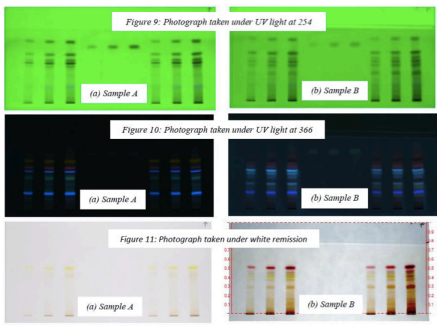
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.16	1.9	0.18	10.4	2.93	0.21	0.1	180.7	1.69	AutoGenerated7
2	0.30	0.0	0.33	33.3	9.39	0.38	2.0	957.7	8.95	AutoGenerated8
3	0.40	3.3	0.44	27.2	7.65	0.46	18.9	756.4	7.07	unknown *
4	0.46	19.0	0.48	32.0	9.02	0.49	23.4	680.7	6.36	unknown *
5	0.50	23.6	0.54	63.8	17.96	0.56	30.9	2207.3	20.64	unknown *
6	0.56	31.1	0.60	188.3	53.05	0.68	0.1	5912.1	55.28	AutoGenerated3

Figure 7: Sample A (Aragvadha Phanta) - 540 nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	0.2	0.10	15.0	2.63	0.13	5.3	344.9	1.94	AutoGenerated11
2	0.16	9.4	0.17	12.7	2.22	0.21	0.1	284.4	1.60	unknown *
3	0.24	0.2	0.27	11.2	1.96	0.30	4.3	264.6	1.49	unknown *
4	0.30	4.3	0.34	84.3	14.75	0.39	6.4	2911.7	16.38	AutoGenerated8
5	0.39	6.6	0.44	46.8	8.19	0.46	37.7	1480.0	8.33	unknown *
6	0.46	38.0	0.48	64.1	11.22	0.50	43.8	1496.8	8.42	unknown *
7	0.50	44.2	0.55	101.9	17.84	0.57	54.4	3573.4	20.11	unknown *
8	0.57	54.6	0.61	235.4	41.20	0.67	0.0	7417.3	41.73	AutoGenerated3

Figure 8: Sample B (Aragvadha Kadha) - 540 nm



Discussion

The chromatogram scanned at UV wavelength 366 nm, for sample A (*phanta*/ hot infusion) has shown 8 peaks in figures 5; while figure 6 which is of sample B (*kadha*/decoction) revealed 9 peaks. It has clarified that the sample B has 1 more peak than sample A, which has demonstrated that the extraction method of making sample B has extracted more phyto-constituents as compare to extraction method of sample A. As well the peak areas of sample A was recorded 18874 (figure 5); whereas of sample B was 21099.4 (figure 6). The difference between these both numbers was 2225.4. The comparison of the numbers of peak areas has shown that sample B contains a greater amount of phyto-constituents than sample A. For the phyto-constituents contained in the tested samples, the Rf values, peak area values, and compound concentrations listed in tables of figures 5 to 8.

Similar to this, figure 7 of the chromatogram for sample A (*phanta*/hot infusion) displayed 6 peaks when scanned at UV wavelength 540 nm, while figure 8 of sample B (*kadha*/decoction) revealed 8 peaks. It has been made clear that sample B had two more peaks than sample A, proving that sample B was made using a process of extraction method that extracted more phyto-constituents than sample A did. Additionally, the peak areas of sample A were 10694.90 (figure 7), while sample B's peak areas were 13774.1. (Figure 8). 3079.2 was the difference between these two figures. The comparison of the peak area counts has revealed that sample B contains more phyto-constituents than sample A does.

The winCATS data has shown that chromatogram obtained at UV wavelength 366 nm, for sample A (*phanta*/ hot infusion), the peak no. 5 was the most intense peak and was possessing height of 397.3 (figure 5); while for sample B (*kadha*/decoction), the most intense peak was of number 6 which was owing height of 494.5 (figure 6). This has validated that sample B was showing more intense peaks than sample A, obviously confirming the superiority of extraction method which was used for sample B.

WinCATS data also revealed that peak number 6 on the chromatogram obtained at UV wavelength 540 nm for sample A (*phanta*/hot infusion) had the highest intensity and had a height of 188.3 (figure 5); meanwhile, peak number 8 on the chromatogram for sample B (*kadha*/decoction) had the highest intensity and had a height of 235.4. (Figure 6). This proved that sample B was producing more powerful peaks than sample A, demonstrating the superiority of the extraction technique utilised for sample B.

The photographing evidence of the bands of separated compounds observed (Figures 9 to 11) on the TLC plates visualized under white light and UV of wavelengths 254 nm and 366 nm of sample B (*kadha*/ decoction) and visible region also more intense than sample A (*phanta*/hot infusion); which again demonstrate the superiority of the extraction method of sample B.

Conclusion

The superiority of the decoction (*kvatha*) extraction method has been revealed by a High-performance thin layer chromatography (HPTLC) study conducted for two Ayurvedic pharmaceutical procedures for fruit pulp of *Aragvadha* (*Cassia fistula Linn.*). The current study has also shown the necessity to test the traditional methodologies described in classical texts using current scientific technology from scientific validation of Ayurveda.

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