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Research Article

HPTLC ANALYSIS OF *ARAGWADHADI KASHAYA* AND ITS INGREDIENTS

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ABSTRACT

Decoctions, are basically the extraction of medicines into water by means of boiling. In Ayurveda it is known by the name '*Kwatha*', '*Kashaya*', '*Sritha*' and '*Niryooha*'. The term *Kwatha* is derived from word '*Kwathana*' which means boiling. *Aragwadhadhi kashaya* is mentioned in Sharngadhara Samhitha, ingredients of which are *Aragwadha, Kanamoola, Tiktha, Mustha* and *Abhaya*. All the ingredients were checked for its identity, purity and strength and only those satisfying the API parameters were taken for the study. *Kashaya* was prepared as per classical method mentioned in *Sharngadhara Samhitha*. The prepared *Kashaya* was lyophilised and further this extract was used for analysis. The extract of decoction and extracts of individual ingredients were analysed by HPTLC. It was done with two different concentrations of *Kashaya* extract and extract of five individual ingredients in a single Merck, HPTLC Silica gel 60 F254 plate. Quantification was not done in this study, but the results provided valuable information on the presence of various compounds in the decoction. Further research could focus on quantifying these compounds to better understand their concentrations and potential effects. Rf values obtained from two different concentrations of *Kashaya* extract were not exactly similar. There were very few identical Rf values in individual ingredients and *Kashaya* extract.

INTRODUCTION

Ayurveda, the science of traditional medicine, has the possibility of playing a key role in overcoming the health crisis pertaining to this era if formulations in ancient literature are brought to light and investigated. It is a science that views an individual as a whole without merely trying to cure the symptoms and, if administered judiciously, can improve overall health- a feeling of being good with a pleasant mind and body- rather than sticking to a single organ disease cure. Decoctions have always been part of Ayurvedic practice of medicines. *Aragwadhadi kashaya* is a decoction having five ingredients indicated in fever associated with *Vata Kapha dosha* and is mentioned in *Sharngadhara samhitha*. HPTLC is an analytical tool used for the separation and quantification of active ingredients in pharmaceuticals, food, and herbal products.

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In the case of decoctions or complex mixtures, HPTLC offers a rapid and efficient method for analysing multiple compounds simultaneously. It is a cost-effective technique that provides reliable results for quality control and research purposes. By comparing the decoction with its individual ingredients, an attempt was made to identify the specific compounds responsible for the therapeutic effects of the mixture. Also, it helps in identifying any potential interactions or synergistic effects that may contribute to the overall therapeutic properties of the mixture.

After prolonged heating during the preparation of decoction, the changes in chemical composition can be analysed using HPLC, providing insight into how the compounds interact and transform under different conditions. This information is crucial for understanding the bioavailability and efficacy of herbal remedies.

Table 1: Ingredients of *Aragwadhadi kashaya*

S.no	Sanskrit name	Botanical name	Family	Part used	Quantity
1	*Aragwadha*	*Cassia fistula* Linn.	Caesalpinaceae	Stem bark	1 part
2	*Kanamoola*	*Piper longum* Linn.	Piperaceae	Root	1 part
3	*Mustha*	*Cyperus rotundus* Linn.	Cyperaceae	Rhizome	1 part
4	*Tiktha*	*Andrographis paniculata* Wall.	Acanthaceae	Whole plant	1 part
5	*Abhaya*	*Terminalia chebula* Retz.	Combretaceae	Fruit rint	1 part

AIM AND OBJECTIVES

1. To prepare *Aragwadhadi Kashaya* and its extract.
2. To analyse *Aragwadhadi Kashaya* and its ingredients by HPTLC.

MATERIALS AND METHODS

Identity purity and strength of raw materials

To test the genuinity of drugs all the individual drugs were compared with the standards published in Ayurveda Pharmacopiea of India. The collected, washed and shade dried drugs which were stored individually in airtight containers were taken for analysis. The tests done were foreign matter, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive and volatile oil.

Preparation of *Kashaya*

Properly washed *Aragwadha, Kanamoola, Tiktha, Mustha* and *Abhaya* were taken and dried in shade. They were powdered individually to a coarse form. Then it was mixed homogenously taking equal weight of each to prepare *Kashaya choorna*.

A steel vessel with wide mouth was taken to which 250g of *Kashaya choorna* and 500ml of water was added, the level of water was assessed using a measuring scale. Then 3500 ml of water was added. And the vessel was kept in an ignited stove in mild fire. The mouth of the vessel was kept open and the heating was continued until the level reached 500ml. The *Kashaya* obtained was filtered through a double layered cloth and collected. It was stored in sterile airtight container.

Preparation of *Kashaya* Extract

The extract of the *Kashaya* was prepared by lyophilisation to obtain a dry porous mass. This was further converted into methanolic extract by hot reflux extraction process. 3gm of lyophilised dry porous mass of *Kashaya* was added to 50ml of methanol and refluxed for 24 hours and methanolic extract was obtained. Further analyses were done using this extract.

Preparation of Extract of Individual Ingredients

Fine powder of each of the ingredients were taken, 3gm of each were subjected individually to hot reflux extraction process in 30ml of methanol. The extracts of individual ingredients thus obtained were stored in sterile glass containers.

HPTLC

Software: Server DESKTOP-60R1I2G, version 3.1.21109.3

Stationary phase: Merck, HPTLC Silica gel 60 F254

Application 1 - Linomat 5 (S/N: 241506)

Mobile phase: Chloroform:Methanol:Formic acid (8.75:1:0.25)

Sample solvent type: Methanol

HPTLC was done with the *Kashaya* as well as their individual ingredients. The seven samples used for HPTLC analysis are listed below

1. AK1-Methanolic extract of Lyophilised product of *Kashaya* at concentration of 1µl
2. AK1-Methanolic extract of Lyophilised product of *Kashaya* at concentration of 2µl
3. CF-Methanolic extract of Cassia fistula stem bark
4. PL-Methanolic extract of Piper longum root
5. CR-Methanolic extract of Cyperus rotundus rhizome
6. AP-Methanolic extract of Andrographis paniculate whole plant.
7. TC-Methanolic extract of Terminalia chebula fruit rind.

Initially HPTLC was done directly trying to dissolve the lyophilised product of *Kashaya* in methanol, but the result obtained was with very faint spots which were not clearly distinguishable. Then the analysis was repeated by taking methanolic extract of lyophilised product and the spots were clearly visible. The individual ingredients of *Kashaya* and the *Kashaya* at two concentrations were spotted in the same plate for comparing the constituents present in them.

Figure 1: Raw drugs of *Kashaya*, A-*Aragwadha*, B-*Pippali moola*, C-*Musta*, D-*Kalmegha*, E-*Hareethaki*

RESULTS AND DISCISSION

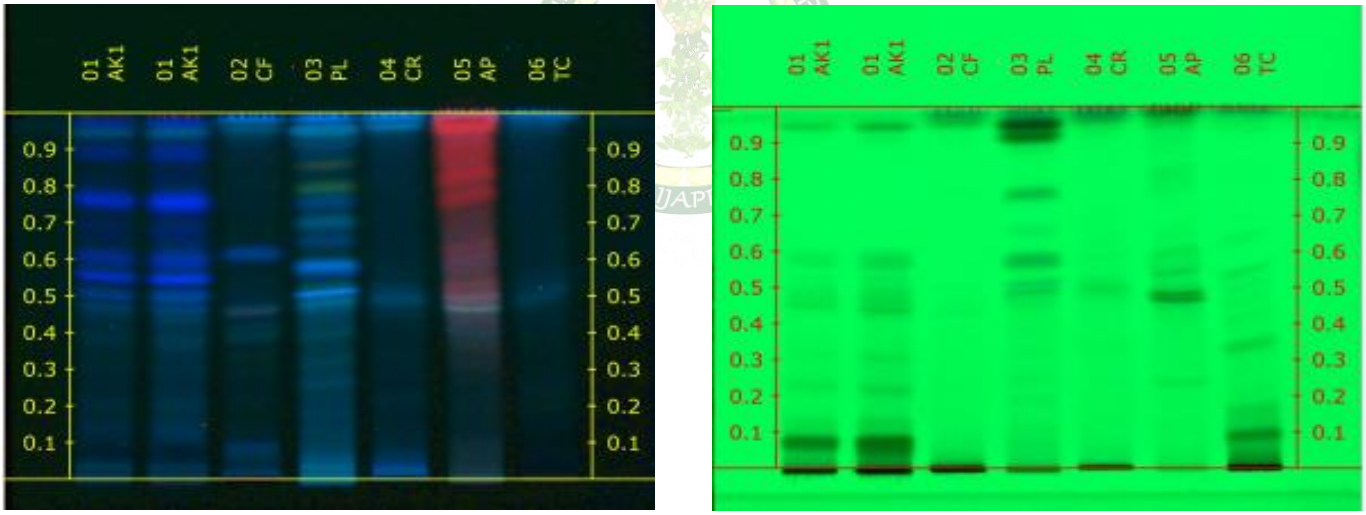

Figure 2: HPTLC image at 366nm and 254nm

Table 2: Rf values of HPTLC obtained at 366nm

S. no	AK1(1µl)	AK1(2µl)	CF(2µl)	PL(2µl)	CR(2µl)	AP(2µl)	TC(2µl)
1	0.003	0.005	0.006	0.005	0.011	0.006	0.10
2	0.024	0.079	0.042	0.487	0.094	0.127	0.106
3	0.084	0.177	0.079	0.582	0.397	0.495	0.169
4	0.181	0.298	0.381	0.923	0.498	0.781	0.218
5	0.315	0.461	0.468	0.953	0.985	0.832	0.355
6	0.473	0.950	0.502	-	-	0.903	0.427
7	0.950	-	0.981	-	-	0.982	0.511
8	-	-	-	-	-	-	0.990

Table 3: Rf values of HPTLC obtained at 254 nm

S.no	AK1(1µl)	AK1(2µl)	CF(2µl)	PL(2µl)	CR(2µl)	AP(2µl)	TC(2µl)
1	0.005	0.006	0.010	0.005	0.011	0.006	0.011
2	0.081	0.082	0.434	0.071	0.053	0.119	0.029
3	0.132	0.135	0.469	0.113	0.248	0.242	0.098
4	0.234	0.221	0.505	0.190	0.342	0.292	0.171
5	0.323	0.310	0.766	0.248	0.505	0.469	0.347
6	0.453	0.440	0.981	0.329	0.589	0.547	0.427
7	0.473	0.460	-	0.492	0.982	0.602	0.553
8	0.531	0.527	-	0.515	-	0.805	0.647
9	0.579	0.571	-	0.577	-	0.989	0.840
10	0.950	0.950	-	0.661	-	-	0.990
11	-	-	-	0.763	-	-	-
12	-	-	-	0.921	-	-	-
13	-	-	-	0.950	-	-	-

At 366nm, while analysing the results of HPTLC it was noticed that there is difference in Rf values of *Kashaya* at 2 different concentrations. Only one Rf value obtained was as similar in both concentrations (0.950). Only one Rf value each of *Piper longum* extract and *Cassia fistula* extract was seen as common in *Kashaya*.

At 254nm, for the two different concentrations of *Kashaya*, 10 Rf values were obtained and were almost similar. 4 spots of Cassia fistula, 7 spots of *Piper longum*, 1 spot of *Cyperus rotundus*, 4 spots of *Andrographis paniculata*, and 2 spots of *Terminalia chebula* were seen, similar to the Rf values obtained with the *Kashaya*. The spots of the individual plant extract that match the Rf values obtained with the *Kashaya* indicate similar chemical components. The reason for variation in different concentrations of the same extract needs to be evaluated by further investigation. It is possible that different concentrations of the plant extract may contain varying amounts of specific chemical compounds, leading to differences in Rf values.

While analysing the results, it is seen that all the constituents present in individual ingredients are not present in decoction; only some of the chemical components are transferred during the extraction process. This could be due to differences in the solubility or stability of certain compounds in the decoction. Another important finding is the presence of new entities other than those present in individual ingredients. This highlights the complexity of the chemical reactions that occur during the decoction process. In a polyherbal compound, there are multiple interactions between different chemical constituents that can result in unique properties and effects.

CONCLUSION

Kwatha Kalpana- aqueous extraction of a group of herbs- is one of the most widely used traditional dosage forms in which the medicinal properties of the group of botanicals are extracted into water using heat. Being one of the most important and effective dosage forms in Ayurvedic pharmaceutics *Kwatha Kalpana* needs to be explored in the aspect of isolation of active compounds and their pharmacological activities. Further research in this area could lead to significant advancements in Ayurvedic medicine and treatment options. This could potentially revolutionize the way certain diseases and conditions are treated.

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