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Original Article

A COMPARATIVE PHYSICO CHEMICAL ANALYSIS OF AMṚTOTTARA KVATHA AND IT'S PRAVAHI KVATHA

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ABSTRACT

Objective: Herbal decoctions are commonly used pharmaceutical dosage form in Ayurveda. The major drawback of decction is short shelf life. To increase the shelf-life preservatives are used. The effectiveness of decoctions added with preservative is not evaluated yet. *Pravāhi kvātha* is fermented decoctions prepared to increase the shelf life as well as the palatability of decoctions. In the present study, an attempt has been made compare the efficacy of freshly prepared herbal decoction, decoction added with preservative and fermented decoction.

Methods: To compare the efficacy, the three samples were subjected to analytical study through organoleptic, physicochemical and other advanced chromatographic and instrumental analysis. For scientifically validating the effectiveness the samples were analysed for Physico chemical parameters, HPTLC, Gallic acid estimation, HR LC-MS and DPPH assay.

Results: After the physico chemical analysis it was found that the parameters of three samples was within permissible limit. The anti-oxidant potential of three samples were proved by the DPPH free radical scavenging activity. The I50 value of three samples shows only less variation and all the sample possess good anti-oxidant potential. Phyto chemical evaluation of the three samples were compared wit HPTLC, Gallic acid estimation and HR LCMS analysis.

Conclusion: The study shows that the modification of *Kaṣāya* to *Pravāhi Kvātha* will not affect its therapeutic efficacy rather it adds more therapeutic value due to the presence of newly synthesized compounds. Addition of Sodium benzoate preservative doesn't alter the therapeutic efficacy of *Amṛtottara Kaṣāya* and it won't produce any toxic chemicals by the interaction with drugs.

Keywords: Decoction, Ayurveda, Amṛtottara Kaṣaya, Pravahi Kvatha, Kaṣaya Kalpana, Preservative, Sodium benzoate

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INTRODUCTION

Kasaya kalpana is the formulation prepared by extracting the active principles of herbal drugs which is also termed as decoction. It is the most significant and widely used dosage form in Ayurvedic pharmaceutics [1]. It has some drawbacks such as very short shelf-life, un pleasant taste and require high dose. For the large-scale production short shelf life is the major drawback. Moreover, in today's chaotic life style, people find it difficult to prepare and administer decoctions daily. To overcome this large pharmaceutical companies are making the *Kasāya* in concentrated form and adding preservatives to increase the shelf life [2].

Also, other modified forms of *Kasāya* like *Kasāya* tablets, Syrup and other flavoured medicaments has led to decline in the use of classically prepared decoctions. The effectiveness and therapeutic potency of these modified dosage forms are not studied properly [3].

Pravāhi kvātha is fermented decoctions which are prepared and practiced as Kāda. In classical texts, direct references are not available regarding this formulation but Ayurveda Sara Sangraha mentioned it as "*Pravahi Kvatha*", which is prepared by fermentation process [4]. It is prepared by fermenting the *Kasāya* by adding sweetening and fermenting agent to increase the shelf life [5]. In the present study, an attempt has been made to lay the Standard Manufacturing Procedure for preparation of this formulation as well as analytically compare it with decoction prepared from same source.

Here, Amṛtottara Kaṣāya yoga has been selected for the preparation of decoction and *Pravāhi Kvātha. Amṛtottara Kaṣāya* is commonly used formulation for treating fever and other inflammatory conditions. It contains only three ingredients namely *Tinospora cordifolia (Guduci), Emblica officinalis (Haritaki),* and *Zingiber officinale (Sunthi).*

Sodium benzoate is the commonly used preservative for Ayurvedic medicine. *Amṛtottara Kaṣāya* added with Sodium benzoate is also subjected for the study to evaluate the efficacy and to check whether the reaction of ingredients with preservative leads to the formation of any toxic chemicals.

So, to compare the efficacy the three samples are subjected to analytical study through organoleptic, physicochemical and other advanced chromatographic and instrumental analysis.

MATERIALS AND METHODS

Collection of raw material and its authentication

Raw drugs for the preparation were procured from Amrita life, Amrita enterprises Pvt Ltd (GMP certified). All the raw drugs were authenticated and identified by Department of Dravyaguna, Amrita School of Ayurveda. Sodium benzoate (food grade) was procured from Laboratory supplies, Trivandrum.

Pharmaceutical study

Three samples of kvātha were prepared as per the classical reference. It includes:-

Sample 1-*Amṛtottara Kaṣāya* freshly prepared

Sample 2-*Amṛtottara Kaṣaya* added with Sodium benzoate

Sample 3-*Amṛtottara Pravahi kvatha*

Method of preparation of *Amṛtottara Kaṣāya* (fresh decoction)

Table 1: Proportion of ingredients

S. No.	Drugs	As per AFI	Quantity taken
1	Gudūchi	6 parts	90g
2	Harītaki	4 parts	60g
3	Sunthi	2 parts	30g
4	Water	16 parts	28.8L

Procedure

Properly cleaned ingredients such as *Tinospora cordifolia, Emblica officinalis,* and *Zingiber officinale* had been taken and made into small pieces. Water taken in a large vessel and the ingredients added into it (fig. 1). The proportion of ingredients mentioned in table 1.

Fig. 1: Preparation of decoction

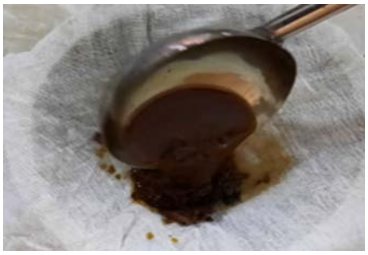

Fig. 2: Filtering of decoction

Fig. 3: Final product

Vessel was kept on *mandāgni* on stove and reduced to 1/8th. The process of heating was continued for 8 h. When *Kasāya* was around 3.6 litres, vessel was taken out from the fire and filtered through a clean and dry cotton cloth (fig. 2). After cooling, it was stored in a clean container (fig. 3).

Method of preparation of *Amṛtottara Kaṣāya* with preservative

Quantity of Sodium benzoate: 2g/l

2 litres of Kasaya were taken in a clean container and Sodium benzoate was added in the ratio 2g per litre. The mixture was stirred well till the Sodium benzoate was completely dissolved.

Table 2: Proportion of ingredients

Ingredients	Quantity
Kasāya	2 Litre
Guda	800g
Dhātaki pushpa	200g

2 L of *Kasāya* was taken and *gaggery* was added into it. The proportion of gaggery and woodfordi fruticose mentioned in table 2. Gaggery should dissolve in *Kasāya* (fig. 4). Again, this solution was filtered to remove physical impurities.

One clean and dried ceramic jar was taken. *Dhūpana (*fumigation) was done with *Sarjarasa, Jatāmāmsi, Guggulu* and *Karpūra*. After *dhūpana* ghee was smeared on the inner surface of ceramic jar.

Fig. 4: Dissolving gaggery in decoction

Fig. 5: Sealing of mud jar for fermentation

Then the mixture of *Kaṣāya* and *Guda* was poured into the jar. The temperature of the mixture was 29 °C. *Dhatāki pushpa* was added into it. The jar was closed and *sandhibandhana* (sealing) was done with mud smeared cloth (fig. 5).

After drying of *sandhibandhana* jar was kept for *sandhāna* (fermentation) in a clean and dry place. The room temperature was 30 °C. The jar was covered with thick sack to maintain optimum temperature for fermentation. After 30 d jar was opened and assessment of *Sandhānasidhhi lakshana* (fermentation tests) was done [9].

Analytical study

The analytical parameters include Organoleptic characters, Total soluble solids, pH, Specific gravity, Total sugar, Acid value, Alcohol percentage were studied.

The major analytical techniques include DPPH Assay using Uv-Visible Spectrometer, HPTLC, Estimation of Gallic Acid using HPTLC and HR-LCMS (High Resolution Liquid Chromatography Mass Spectrometer) were also done on three samples of *Kvātha.*

RESULTS AND DISCUSSION

Table 3: Organoleptic characters of the samples

Parameters	AMT *Kaṣāya*	*Kaṣāya* with preservative	AMT *Pravāhi kvātha*
Colour	Dark brown	Dark brown	Brownish black
Odour	Characteristic	Characteristic	Characteristic
State	Liquid	Liquid	Liquid
Taste	Bitter	Bitter	Bitter with slight sweet taste

Table 4: Physico chemical characters of three samples

Parameters	AMT *Kaṣāya*	*Kaṣāya with* preservative	*AMT Pravāhi kvātha*
Brix	11	12	26
Specific gravity	1.44	1.46	1.08
pH	4.29 at 29 °C	4.2 at 29 °C	3.76 at 29 °C
Alcohol content	NA	NA	9%
Acid value	NA	NA	8 mg/g
Total sugar	NA	NA	10%

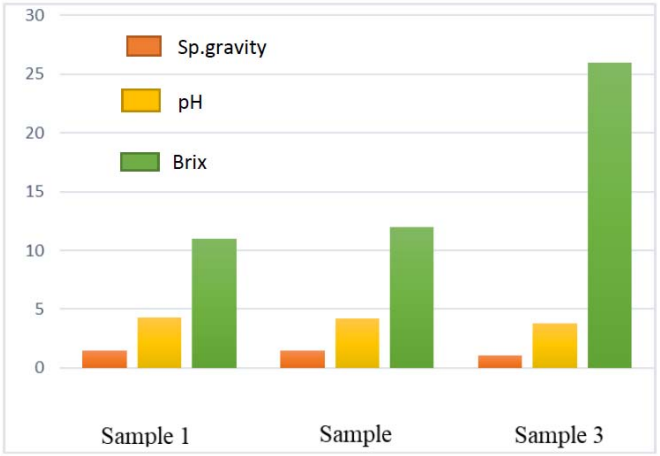

Fig. 6: Physico chemical characters of three samples

The characteristic colour of *Kaṣāya* is due to the extraction of active components from the drugs. *Agnivesa* has mentioned the siddha *lakshana* of *Kaṣāya* as "*Gatarasheshu Aushadheshu*" means the active principle of the drugs should completely extracted into the water [7]. The organoleptic and physico chemical characters shown in table. 3, table 4 and the comparison shown in (fig. 6). Due to the continuous heating the water-soluble principles from the herbs are extracted to the water and makes it dark in colour. The *Kaṣāya* started boiling between 90-100 °C. Eight hours required for the preparation and the average temperature was maintained between 95-110 °C. The characteristic smell of *Kaṣāya* is depending on the ingredient drugs [8]. Here the *Sunti* is aromatic drug so it imparts odour to the Kaṣāya . The characteristic taste of *Kaṣāya* is *Kaṣāya rasa* along with the rasa of ingredient drugs. Here the major ingredient is *Guduci* which makes the *Kaṣāya* more *Tiktha.* The *Pravāḥi kvātha* was prepared following the classical reference and the onset and completion of fermentation were confirmed by classical parameters[6]. *Pravāḥi kvātha* is having alcoholic odour and taste along with slight sweet taste of *Guda*. The characteristic taste of fermented products is due to the presence of flavoring compounds such as carbonyl, ketones and alcohols. TDS of *Kaṣāya* helps to know the complete extraction of water-soluble principles of ingredients drugs into *Kaṣāya* [6]. The Brix value of *Kaṣāya* was within the standard limit. When Sodium benzoate was added Brix was slightly increased. Pravāḥi kvātha showed more Brix as it contains sugar content from *guda.* Specific gravity indicates the presence of solute in solvents. In case of *Amṛtottara Kaṣāya* the solvent is water and the solutes refer to the extracted active principles from the drug. The specific gravity of *Kaṣāya* was with in standard limit. The specific gravity slightly increased after the addition of Sodium benzoate. The Specific gravity of *Pravāḥi kvātha* is less compared to other samples. During fermentation, with the conversion of solute and carbohydrate into alcohol, a slight fall in specific gravity occurs. *Kaṣāya* has acidic pH. When it was converted into *Pravāḥi kvātha* pH value decreased due to the formation of self-generated alcohol. Acid value, total sugar and alcohol content of *Pravāḥi kvātha* was found to be with in standard limit.

HPTLC-high performance thin layer chromatography

HPTLC analysis was done using CAMAG Automatic TLC Sampler 4 (ATS4) "ATS4_170206" S/N 170206 (1.02.13) instrument. The HPTLC analysis of fractions of all the three samples were done using the mobile phase Chloroform: Ethyl acetate: Formic acid (5:4:1) solvent system in Camag Twin trough chamber. Visualization was made at 254 and 366 nm.

The High-end chromatographic technique was used to evaluate the active components of the three samples. *Amṛtottara Kaṣāya* was prepared with three ingredients ie *Guduci, Haritaki* and *Sunthi*, and the formulation contains the phytochemicals from these medicinal plants. In addition to these the second sample contain sodium benzoate.

The HPTLC chromatogram of *Amṛtottara Kaṣāya* showed 7 peaks of unknown compounds and the *Kaṣāya* with sodium benzoate showed 8 peaks of compounds. The additional compounds might be the derivatives of sodium benzoate. Four similar bands were identified in both the samples. Peaks at Rf 0.07, 0.47,0.66,0.72 are common in both samples. Peaks at Rf 0.23 was identified only in second sample. The third sample is *Pravāhi Kvātha*, the fermented sample prepared by adding jaggery and *Woodfordia fruticose (Dhathaki pushpa)*. Hence the sample showed a greater number of compounds. Total 9 peaks were identified and the peaks at Rf 0.07,0.47.0.66 was common in the three samples and peaks at Rf 0.14,0.73, 0.77, 0.86 was identified only in third sample (fig. 7) table 5. The peaks with similar Rf value might be the similar compounds. it can be confirmed only with higher instrumental analysis like LCMS.

Estimation of gallic acid

Gallic acid estimation was done using CAMAG Linomat HPTLC machine. The plate (HPTLC plates silica gel 60 F 254) was developed in Camag Twin through chamber using Chloroform: Ethyl acetate: Formic acid (5:4:1) solvent system in Camag Twin trough chamber was made at 254 and 366 nm.

Table 5: Evaluation sequence of HPTLC

Track	Sample
1	*Amṛtottara Kaṣāya*
2	*Amṛtottara Kaṣāya* with preservative
3	*Amṛtottara Pravāhi Kvātha*

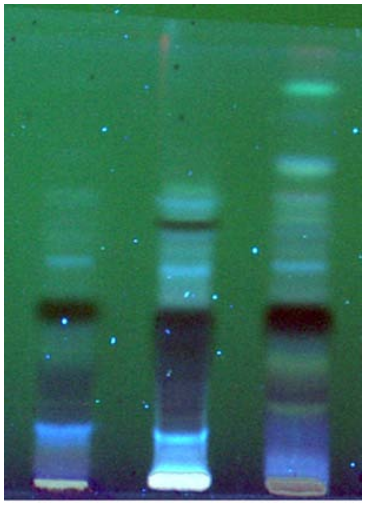

Fig. 7: HPTLC chromatogram

Gallic acid was identified in the three samples by HPTLC analysis. Gallic acid is one of the major compounds in *Amṛtottara Kaṣāya* [10]. Gallic acid (3,4,5-trihydroxybenzoic acid) possesses important medicinal properties. It blocks histamine release and pro-inflammatory cytokine production in mast cells. Gallic acid has anti-inflammatory and anti-pyretic properties. It acts as an antioxidant and helps to protect cells against oxidative damage, cytotoxicity against cancer cells without harming healthy cells. The analysis shows that the addition of preservative or fermentation process doesn't affect the gallic acid content in the formulation.

DPPH assay using uv-visible spectrometer

The DPPH assay was used for determining the antioxidant capacity of samples by analysing the free radical scavenging activity on DPPH radical. The lower the IC 50 value (the concentration of antioxidant using 50% DPPH scavenging) more will be the antioxidant activity. It was analysed using the instrument UV-Visible spectrophotometer-Varian 50 Bio and 1,1 Diphenyl-2-picryl hydrazyl (DPPH) and Methanol as reagents.

The IC50 Value of *Amṛtottara Kaṣāya* is 25.15 mg/kg and *Kaṣāya* added with preservative is found to be 20.08 mg/kg, whereas the IC 50 value of *Amrtottaram Pravāhi Kvātha* is 26.49 mg/kg. The results shows that all the samples possess good anti-oxidant potential. Only slight variation in IC50 value noted.

HR-LCMS (High resolution liquid chromatography mass spectrometer)

HR-LCMS was done using Q-Exactive plus Biopharma-High Resolution Orbitrap. The analysis was performed using ESI in negative mode. Mobile phase was optimized as methanol (A) and 0.1 % acetic acid in water in a ratio of 60:40 with a flow rate of 0.9 ml/minute [11]. The analysis showed list of compounds identified in each sample.

The properties of each compound identified by literature search. By comparing the compounds identified in each sample the result obtained as follows: -

Total number of compounds in each sample: –

AMT *Kaṣāya*-38

Kaṣāya with preservative-39

Pravāhi Kvātha-44

✔ 26 compounds found common in 3 samples.

✔ 12 compounds common in AMT *Kaṣāya* and AMT with preservative and absent in *Pravāhi Kvātha*

✔ 18 new compounds identified in *Pravāhi Kvātha* sample absent in other samples.

✔ 1 new compound identified in *Kaṣāya* with preservative absent in other samples.

Table 6: Compounds identified in each sample and therapeutic action

Compounds present in 3 samples	
Compound	**Therapeutic action**
Chebulic acid [12]	Anti-inflammatory, anti-oxidant, anti-pyretic
Catechin [13]	Anti-inflammatory, anti-oxidant, anti-pyretic
Gallic acid [14]	Anti-inflammatory, anti-oxidant, anti-pyretic
Shikimic acid [15]	Anti-inflammatory, anti-oxidant, anti-pyretic
Mallic acid [16]	Anti-inflammatory, anti-oxidant, anti-pyretic
Cyclic di-gmp [17]	Anti-microbial
Amritoside [18]	Anti-inflammatory
Aminoglutathimide [19]	Analgesic
Trenbolone [20]	Steroids
Compounds present in sample 1 and2 absent in sample 3	
Cirsimaritin [21]	Flavonoid
Polyporusterone [22]	Triterpene carboxylic acid
Compounds present only in sample 3	
Ketotifen [23]	Anti-histamine, anti-inflammatory
Nifedipine [24]	Calcium channel blocker medication used to manage angina, high blood pressure etc
Luvangetin [25]	Anti-inflammatory

Compounds are highly polar resulting in aqueous extraction during the *Kaṣāya* preparation 15 compounds from the raw materials are found to be still retained as such in the *Kaṣāya* sample 11 number of new compounds are being chemically synthesized[9] during the *Kaṣāya* preparation process due to the synergistic interaction between drugs. The newly developed compounds have medicinal properties. The preservative containing sample showed the presence of Benzoic acid due to the reaction of Sodium benzoate with some of the phenols present in the *Kaṣāya* sample.

All the newly formed compounds are retained in preservative containing Sample. It suggests no appreciable variation in medicinal efficacy of the preservative containing sample. The *Pravāhi Kvātha* sample retained 18 compounds from *Kaṣāya* and some new compounds synthesized during the fermentation process. Among the new compounds, two compounds were derived from *Woodfordia fruticosa*. Some compounds present in *Kaṣāya* not retained after fermentation due to the effect of enzymatic decomposition. Most of these compounds have no relevance in therapeutic action [26]. Structurally they are identified as ester, N-H and COOH groups containing compounds. Most of the compounds identified in three samples possesses anti-inflammatory, anti-pyretic and anti-oxidant potential validates the

therapeutic potency of *Amṛtottara Kaṣāya* and its *Pravāhi Kvātha*. In addition to that, the compounds identified only in *Pravāhi Kvātha* possess anti-hypertensive, Analgesic and anti-histamine properties table 6 (fig. 8).

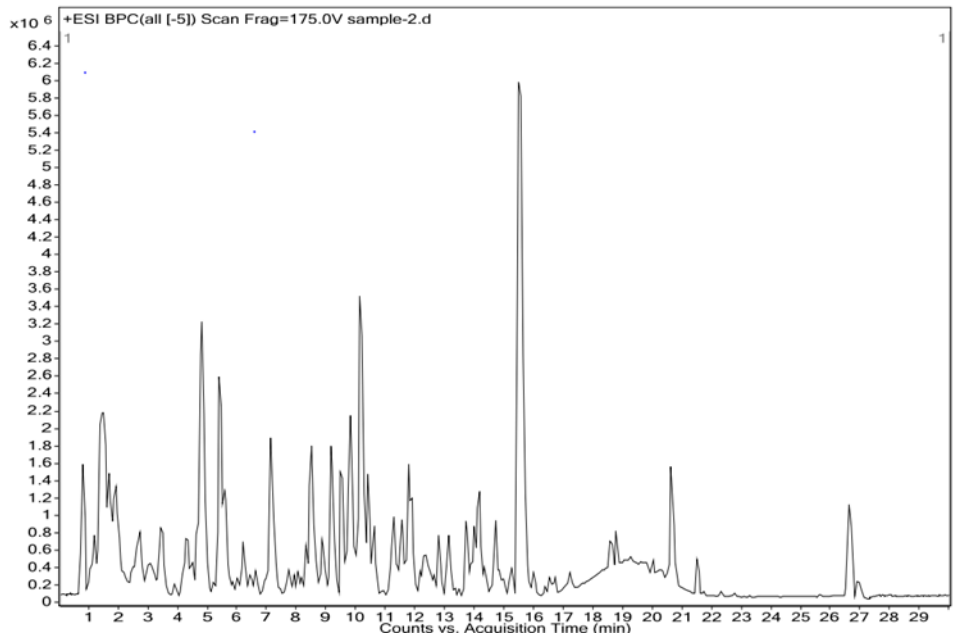

Fig. 8: HR-LCMS chromatogram

CONCLUSION

By combining the results of HPTLC and HR LCMS it can be assumed that by modifying *Kaṣāya* to *Pravāhi Kvātha* the compounds capable of therapeutic action are retained. Sodium benzoate preservative doesn't produce any toxic chemicals by the interaction with drugs. So, it can be assumed that within the normal limit it won't produce any harmful effect. The study shows that the modification of *Kaṣāya* to *Pravāhi Kvātha* will not affect its therapeutic efficacy rather it adds more therapeutic value due to the presence of newly synthesized compounds. Since this preparation has good palatability and nutritional value it can be used in pediatric cases, due to the presence *tīkshanadi gunas* rendered by *Sandhāna* it can stimulate the activity of digestive enzyme, the self-generated alcohol present in it acts as preservative which increase the shelf life. Hence, *Amṛtottara Pravāhi Kvātha* can be given as an alternative to *Amṛtottara Kaṣāya*.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors do not have any conflicts of interest.

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