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Phytochemical analysis of an indigenous compound Patolamuladi Kashayam

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Abstract
Objectives: *Patolamuladi Kashaya* is an indigenous compound preparation indicated in skin and liver diseases. *Kashaya* (decoction) are a type of *Ayurvedic* formulation prescribed to be used fresh within one day of preparation. But with changing scenario, *Kashaya* are now added with preservatives to extend its shelf life. In order to evaluate the effect of preservatives on the chemical nature of the formulation, the present study was carried out to evaluate the phytochemical and chromatographic profile of freshly prepared *Patolamuladi Kashaya.*
Material and Methods: *Patolamuladi Kashaya* has been tested with various parameters on the day of preparation and at different stages viz., freshly prepared without adding preservatives, after adding preservatives and after completion of six months. The parameters used are preliminary phytochemical analysis, organoleptic, Total Suspended Solids (TSS), Potential of Hydrogen (pH), Specific Gravity, Viscosity and HPTLC (High Performance Thin Layer Chromatography).
Results and Discussion: Study revealed the presence of Carbohydrates, Proteins, Alkaloids, Saponin Glycoside, Flavonoids, Tannins and absence of Steroids and Antheaquinone glycosides. pH values were consistent at all three stages i.e. 3, TSS was 8 without preservatives, 9 with preservatives and become 8 after six months. Specific gravity varied between1.0300 to 1.0165 and Viscosity ranged between 1.0300 to 1.0138. The 5thRf value peak of both 254nm and 366nm gives a further scope of study for phytoconstituents.
Conclusion: The preliminary phytochemical analysis and chromatographic profile of *Patolamuladi Kashaya* reveals that the formulation added with preservative remains chemically stable for six months.

Keywords: *Patolamuladi Kashaya* standardization, HPTLC of Ayurvedic formulation, phytochemical analysis of kashaya

Introduction
Patolamuladi Kashaya is a well-known indigenous preparation used for Skin and liver diseases. In the present study, this *Kashaya* was prepared as per the reference of *Charaka Samhitha kushta* Chikitsa (chapter of skin disdease) by adding *Patola* (*Trichosanthes dicoca* Roxb), *Kutaja* (*Holarrhena antidysenterica* Linn), *Haritaki* (*Terminalia chebula* Retz), *Vibhitaki* (*Terminalia bellarica* (Gaertn) Rob), *Amalaki* (*Phyllanthus emblica* L), *Trayamana* (*Gentiana kurro* Royale), *Katukarohini* (*Picrorhiza kurroa* Royale ex Benth), and *Shunti* (*Zingiber officinale* Roscoe) [1]. This preparation has been explained under *Kwatha Churna* (Decoction powder) preparation in Ayurvedic Formulary of India [2].
Genuinely *Kashaya* should be freshly prepared and used within a day but with changing scenario *Kashaya* are now prepared with preservatives to increase shelf life. Hence there is a need to analyse the difference in the phytochemical constituent of freshly prepared *Kashaya*, *Kashaya* with preservatives and at the end of 6 months.

Material and Methods
Drug collection and preparation of *Kashayam*
The herbs required for *Patolamuladi Kashaya* were procured from different parts of India and were authenticated from Dept of Dravyaguna, Shri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. The details of collected drug with their botanical name and used parts are given in Table 1.

Table 1: Table showing Ingredients of Patolamuladi Kashaya

S. No	Sanskrit name	Botanical name	Family	Parts used	Quantity
1	Patola	Trichosanthes dicoca Roxb	Cucurbitaceae	Root & Panchanga	48gm
2	Kutaja	*Holarrhena antidysenterica* (Linnn.) wall	Apocyanaceae	Twak	48gm
3	Haritaki	Terminalia chebula Retz.	Combretaceae	Fruit rind	48gm
4	Vibhitaki	Terminalia bellarica (Gaertn) Rob	Combretaceae	Fruit rind	48gm
5	Amalaki	Phllanthus emblica L.	Phyllanthaceae	Fruit rind	48gm
6	Trayamana	Gentiana kurro Royale	Gentianaceae	Leaves	12gm
7	Katurohini	Picrohiza kurro Royale ex Benth	Scrophulariaceae	Rhizome	12gm
8	Shunthi	Ziongiber officinale Roscoe	Zingiberaceae	Phizome	6gm
9	Water				2.160 ml

Preparation of Kashaya

The Kashaya was prepared in the teaching pharmacy of department of Rasashastra and Bhaishajya Kalpana, Shri Dharmasthala Manjunatheswara College of Ayurveda and Hospital, Hassan. The above mentioned herbs were coarse powdered, taken in equal quantity. To this mixture of coarse powder, 8 parts of water was added, boiled and reduced to 1/8 th volume. The final product was filtered through a clean cotton cloth and kept in a separate vessel.

Adding of Preservatives

Sodium benzoate in a ratio of 0.05% per litre was added to the hot Kashaya then allowed to cool overnight followed by packing and labelling [3].

Further the freshly prepared Patolamuladi Kashaya and preservative added Kashaya samples were subjected to different types of physical parameters and quality assessment parameters on the same day and once again after six month.

1. Organoleptic Assessment

It is the preliminary physical assessment by using own sense organs with coming contact. In this study except sound test all other tests has been performed like Rupa (colour), Rasa (taste), Gandha (smell), Sparsha (touch) at each interval.

2. Qualitative Parameters for assessment of Kashaya [4]

pH– The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in grammas. It determines potentiometrically by means of a glass electrode and a suitable pH meter. In this study pH was determined using a calibrated pH meter.

TSS-it is a water quality parameter that is defined as the quantity of material suspended in a known volume of water that is trappable in a filter. This was determined by taking 10 ml of the formulation in porcelain evaporating dish and heating it on an electric water bath at 60 – 70˚C and then in an oven at 105˚C until constant weight of residue was obtained.

Specific Gravity – The specific gravity of a liquid is the weight of given volume of the liquid at specific temperature compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air. In this study 10 ml of Patolamuladi Kashaya measured by Weight of 10 ml of liquid/ 10

Weight of 10 ml of water/10 formulas.

Viscosity-The internal friction of liquids, due to intermolecular attractions is known as Viscosity. In a flowing liquid each layer of molecules exerts a drag on the next and, to cause the liquid flow, work must be done to push the layers past one another. In this study the capillary viscometer (Poiseuille's Law) has been used to determine the viscosity of Patolamuladi Kashaya.

3. Preliminary Phytochemical analysis

For the determination of organic phytochemical constituents of Patolamuladi Kashaya, it was accessed through various tests by taking references of K.R Khandelwal and Vrunda Sethi practical guideline book [5].

Table 2: Determination for presence or absence of Preliminary Phytochemicals

Organic Phytochemical constituents	Name of the test	Chemicals used
1. Carbohydrates	Fehling's test	Fehling's solutions (A &B
	Benedict's test	Benedict's solution
2. Proteins	Xanthoprotein test	Conc. H_2SO_4, NH_4OH
	Precipitation test	5% $CuSO_4$, 5% lead acetate
3. Steroids	Salkowski reaction	Chloroform & Conc.H_2SO_4
4	Liebermann-Burchard reaction	Chloroform, Acetate anhydride Conc.H_2SO_4
5. Saponin	Foam test	By shakeing of the drug
6. Glycoside	Legal's test	Pyridine & Sodium Nitroprusside (1ml each)
	Liebermann's test	3ml of Acetic anhydride
7. Flavonoids	Shinoda test	5ml of 95%ethanol, conc HCL & 0.5 g magnesium
8. Tannins	Bromine water test	Few drops of Bromine
9. Anthraquinone Glycosides C-glycosides	Borntrager's test	Diluted H_2SO_4 &Chloroform
	Modified Borntrager's test	5ml 5% $FeCl_3$ &5ml diluted HCL, adding of benzene

4. Determination of water soluble extractive value [6]

Process: Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of chloroform-water in a closed flask for twenty-four hours, shaking frequently during six hours and allow to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105*, to constant weight and weigh. Calculate the percentage of chloroform-water soluble extractive with reference to the air dried drug.

5. Methods of HPTLC Analysis

10ml of Patolamuladi Kashaya samples was partitioned with 20 ml butanol in a separating funnel and kept for 24hr. The butanol fraction was collected and filtered. The butanol was then made to evaporate on a water bath and it is dissolved in 10.0ml of methanol. 4, 8 and 12µl of the above samples were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate: Acetic acid: Methanol (3.0:3.0:0.8:0.2). The developed plates were visualized under short UV, long visualization with vanillin sulphuric acid spraying reagent, and scanned under UV 254nm, 366nm, and then derivative with vanillin sulphuric acid reagent and scanned under white light at 620 nm. Rf, colour of the spots and densitometry scan were recorded.

Results

Table 3: Organoleptic Parameters of Patolamuladi Kashaya

S. No	Parameters	Before adding Preservatives	With Preservatives	After six months
1	Color	Blackish Dark Brown	(Milk tea) Brown	(Milk tea) Brown
2	Taste	Bitter and Pungent	Sour Bitter &Pungent	Little sour, bitter & Pungent
3	Smell	Characteristic	with added smell of Preservatives	Characteristic
4	Form	Liquid base	Liquid base	Liquid base

Table 4: Physio Chemical Parameters analysis at different stages

S. No	Parameters	Before Adding Preservatives	With Preservatives	After six months
1	(pH) Potentia l of Hydrogen	3	3	3
2	TSS (Total Suspended Solids)	8	9	8
3	Specific Gravity	1.0300	1.0320	1.0165
4	Viscosity	1.0496	1.0263	1.0138

Table 5: Qualitative parameters of Patolamuladi Kashaya

S. No	Parameters	Present(+) /Absent(-)
1	Carbohydrates	+
2	Proteins	+
3	Steroids	-
4	Alkaloids	+
5	Saponin	+
6	Glycoside	+
7	Flavonoids	+
8	Tannins	+
9	Anthraquinone glycosides and C- Glycosides	-

Water soluble extractive value

In this study the obtained water soluble extractive value for Patolamuladi Kashaya is 9.06%.

HPTLC Scan of Patolamuladi Kashaya at 254nm

Under the short UV 254nm and the Rf value were recorded. 5th peak found the highest area percentage 45.37%. Details are given in the Table 6.

Table 6

Peak	Position	Area	Area %
1	0.04Rf	33449.5AU	21.897%
2	0.15Rf	14370.0AU	9.40%
3	0.21Rf	24908.1AU	16.29%
4	0.40Rf	5801.5AU	3.79%
5	0.76Rf	69373.9AU	45.37%
6	0.86Rf	1239.6AU	0.81%
7	0.95Rf	3774.8AU	2.47%

Densitometric scan after derivatisation at 254nm

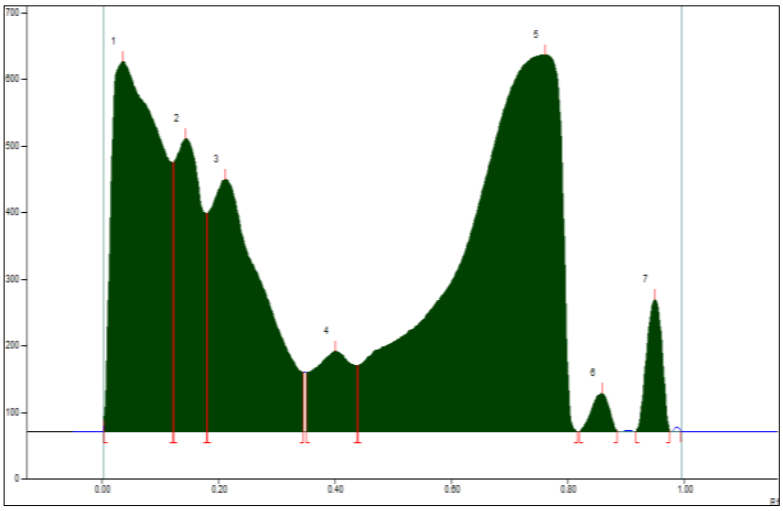

Fig 1

HPTLC Scan of Patolamuladi Kashaya at 366nm

At the long UV 366nm and the Rf value were recorded. Theth peak found as the highest area percentage 22.94%. The details are given in the table 7.

Table 7

Peak	Position	Area	Area%
1	0.03Rf	23567.3AU	19.15%
2	0.13Rf	13557.1AU	11.01%
3	0.20Rf	24286.7AU	19.74%
4	0.57Rf	19808.2AU	16.10%
5	0.77Rf	28242.4AU	22.94%
6	0.86Rf	7357.3AU	5.98%
7	0.95Rf	6259.2AU	5.09%

Densitometric scan after derivatisation at 366nm

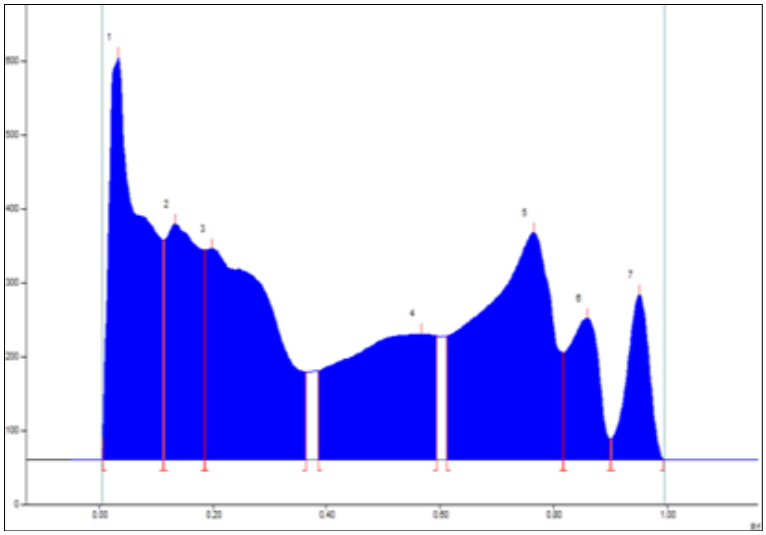

Fig 2

HPTLC Scan of Patolamuladi Kashaya at 620nm

The sample was mixed with vanillin sulphuric acid reagent and scanned under white light at 620 nm the Rf value were recorded. The 6th peak was found having highest area percentage 57.31% details are given in Table 8.

Table 8

Peak	Position	Area	Area %
1	0.02Rf	1206.7AU	7.98%
2	0.13Rf	741.0AU	4.90%
3	0.29Rf	222.7AU	1.47%
4	0.34Rf	497.3AU	3.29%
5	0.55Rf	1878.1AU	12.42%
6	0.73Rf	8666.8AU	57.31%
7	0.96Rf	1910.4AU	12.63%

Densitometric scan after derivatisation at 620nm

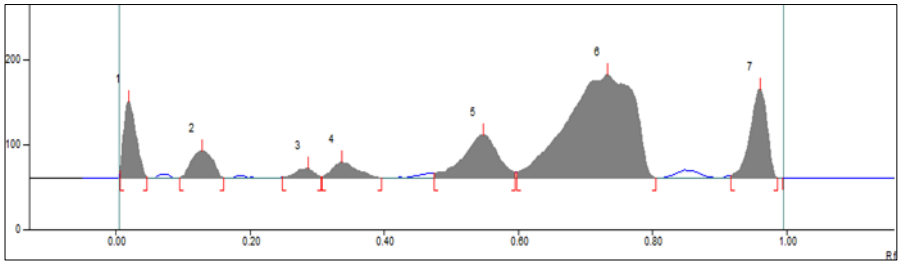

Fig 3

Discussion

As per literature and Ayurveda formulary of India *Kasaya Kaplana* (Decoction procedure) are defined as filtered liquid obtained by boiling coarse powd*er of* drug(s) in proportion of 4, 8 and 16 parts of water based on *Mrudu* (*Soft*), *Madhayama* (Medium), *Kathina* (Hard) substance respectively and reduced to one fourth [7]. Standardisations of liquid preparation like Asava/Arista (Alcohol based formulations) is available, but not for Kashaya Kalpana. Previous works on certain *Kashaya Kalpana* are available which are used as reference standards for the present work [8, 9]. However, this

work seems to be the first attempt in characterisation of *Patolamuladi Kashaya.*

Based on preliminary organoleptic study carried out on the day of preparation change was observed in colour from brown to milky brown, taste from bitter to mild sourness, and odour of characteristic to preservative smell makes a physical difference which could be due to adding preservative. Further These Characteristics Remained unchanged till the end of 6 month period which reveals the stability throughout. In this study pH, TSS, Specific Gravity and Viscosity tests were performed to access basic qualitative parameters the of *Kashaya* during freshly prepared without adding preservatives, immediately after adding preservatives and after six months. pH of *Kashaya* maintained as (3-3-3) in all stages. It reveals that *Kashaya* is acidic in nature till the end of six months. TSS maintained in between (8-9-8) respectively which shows that the suspended solid particles remained same and did not under go any change with the passage of time. Specific gravity of *Patolamuladi Kashaya* was maintained in between1.0300 ± .0165thisreveals that the density was maintained from the day of preparation to till six months. Viscosity is the internal friction of liquids, due to intermolecular attractions. In the present study, it varied in between 1.0496 to 1.0138 from the day of preparation to after six months respectively. Obtained results revealed that there was no much difference of values found through pH, TSS, Specific Gravity and Viscosity before and after which shows that the Kashaya added with preservative remained near stable to the freshly prepared Kashaya. The 5thRf value peak of both scan at short UV 254nm and scan at long UV 366nm gives a further scope of study to elicit the phyto constituents. This study was limited to preliminary organic phytochemical constituents presence of Carbohydrates, Proteins, Alkaloids, Saponin Glycoside, Flavonoids, Tannins and absence of Steroids and Anthraquinon glycosides. No vitamins, minerals and heavy metal tests were conducted which can be taken as further scope of study.

Conclusion

The study revealed that there was no significant change in the parameters, like pH, TSS, Specific Gravity and Viscosity which shows that the *Kashaya* added with preservative remained near stable to the freshly prepared *Kashaya*. Further qualitative & quantitative phytochemical analysis and higher chromatographic techniques would reveal the minute changes found in the study. The present study may be used as a reference standard for characterisation of *Patolamuladi Kashaya*.

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