International Journal of Scientific Research and Engineering Development-– Volume 7 Issue 5, Sep-Oct 2024
Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

Phytochemical Analysis of Vasadrakshadi Kashaya

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ABSTRACT

Vasadrakshadi Kashaya is a Classical formulation mentioned in the text book Sharangadara samhita Kashaya Kalpana.[1], commonly used for the management of Shwasa and Raktapitta. Vasa [2](Adathoda vasica) Draksha[3] (Vitis vinifera) Haritaki [4](Terminalia chebula Retz.) are the contents. Inadequate standardization of polyherbal formulations causes difficulty in validation of the efficacy and maintaining quality of the product. Hence an attempt has been made to standardize the quality parameters of Vasadrakshadi Kashaya by determining total solids, Ph, loss of drying at 105 degree celcious, specific gravity at 25 degree celcious, identification of sugar, identification of alcohol, heavy metal limit test, microiological assay, HPTLC of haritaki was done.The data obtained in the present study will help as a reference document to maintain the quality standards of the formulation.

Keywords: Vasadrakshadi Kashaya, Vasa, Draksha, Abahya, phytochemical analysis

INTRODUCTION

Ayurveda aims to maintain the health of healthy individuals and restore health of the diseased by eradicating the root cause of disease using a blend of medicines and prevent the recurrence of imbalance by modifying diet and regimens. Ayurvedic classics like Sharngadhara Samhita highlights various herbal formulations in a particular proportion to augment therapeutic efficacy and lessen toxicity. **Kashaya:** Generally, kashaya is referred to kashaya rasa. **Kwatha:** The word Kwatha refers to decoction, where in the drug is boiled in water and reduced to a specific quantity. The liquid prepared by simmering 1 part of herbs in 16 parts of water and reducing it to 1/8th part of the water is known as a decoction. It is also called Kwatha and Kashaya in Ayurveda.[5]

An integral part of traditional medicine is quality assurance[6]. For delivery of good quantity of medicament physical evaluation of drugs and its comparison with standard values guarantees authenticate top quality of drugs, free from adulterations.[7] Physicochemical parameters, microbiological features and HPTLC fingerprint profile may be used as marker parameters for quality evaluation and standardization of polyherbal formulations[8].

MATERIALS AND METHODS

Collection of Raw Material

The raw drugs required for preparation of Kashaya were collected from Rasashastra and baishajaya kalapana lab of Karnataka Ayurveda Medical College. The ingredients and parts used in the preparation of Kashaya are listed in (Table 1).

Preparation of Kashaya (Decoction)

Vasadrakshadi Kashayam was prepared with the ratio mentioned in Table-1 in Rasashastra and Baishajaya

kalapana lab of Karnataka Ayurveda Medical College. These raw drugs were repeatedly washed in running water, so as to remove physical impurities. It was later dried in shade. Then Abhaya phala, Vasa patra and moola were made into coarse powder separately in a khalva yantra. Draksha was crushed, equal quantity of these drugs where measured (48 gm (one pala) of disintegrated drugs) and added to sufficient amount of water 768 ml of water (16 parts) which was finally reduced to 1/8th part (96 ml (1/8th) of the original quantity.). Kashaya after cooling was filtered through a fine cloth and measured to know the quantity obtained. This Kashaya was separated to 100ml bottles for supply to patients.

Table 1 List of herbal raw materials

SL.No	SANSKRIT NAME	BOTANICAL NAME	PARTS USED	RATIO
1	VASA	ADATHODA VASICA	LEAF/ROOT	1 PART
2	DRAKSHA	VITIS VINIFERA	DRY FRUITS	1 PART
3	ABAHYA	TERMINALIA CHEBULA RETZ	DRY FRUITS	1 PART

Organoleptic evaluation

Organoleptic evaluation is a scientific method that uses the five senses to study the quality of a product, such as food, beverages, or drugs:

- Taste: Assesses the flavour profile
- Smell: Focuses on the aroma
- Sight: Evaluates the appearance
- Touch: Measures the texture, including factors like crunchiness

Organoleptic characters like colour, odour, taste and consistency of the Kashaya were evaluated

Table 2 Organoleptic evaluation

SL. No	PARAMETER	OBSERVATION
1	COLOUR	BROWN
2	ODOUR	CHARACTERISTIC SMELL OF VASA & DRAKSHA
3	TOUCH	COOL
4	PHYSICAL APPERANCE	LIQUID
5	TASTE	ASTRINGENT

Physicochemical Analysis of Raw Materials

Analysis like loss of drying at 105^{0}C, specific gravity at 25^{0}C were carried out as per standard procedure mentioned in Ayurvedic Pharmacopeia of India.

Table 3 phytochemical analysis

SL. No	PARAMETER	OBSERVATION
1	Ph	5.12
2	TOTAL SOLIDS	2.1%
3	LOSS OD DRYING AT 105^0 C	97.9%
4	SPECEFIC GRAVITY AT 25^0 C	0.9851

Heavy metal analysis

Heavy metal analysis of the formulation revealed that the formulation lacked heavy metals or if present their concentration was below the permissible limits.

Table 4 Heavy metal analysis

SL. No	PARAMETER	OBSERVATION
1	Test for arsenic	BOQ<0.3ppm
2	Test for lead	BOQ< 0.1ppm

Microbial load

Test for specific organisms revealed that pathogenic bacteria like Escherichia coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa were absent.

Table 5 Microbial study

SL. No	PARAMETER	OBSERVATION
1	TOTAL BACTERIAL COUNT	2 X 10^4 cfu/gm
2	TOTAL FUNGAL COUNT	3 X 10^2 cfu/g
3	E. COLI	ABSCENT
4	SALMONELLA sp/g	ABSCENT
5	STAPHYLOCOCCUS AUREUS/g	ABSCENT
6	PSEUDOMONAS AERUGINOSA/g	ABSCENT

Identification for sugar & alcohol

The sample doesn't answer the identification test for the sugar and alcohol.

HPTLC Analysis

An attempt has been made to develop simple, precise and accurate HPTLC method by estimating Gallic acid as a marker compound. The detection and quantification of Gallic acid were performed at 280 nm respectively. A linear regression model is often applied to the data from the calibration curve. This helps determine the relationship between the peak area (or height) and the concentration of the analyte. Here the regression mode was linear-2

Range Deviation refers to the variability in results when analyzing samples at different concentrations within the calibration range. It assesses how consistently the method can quantify compounds over this range. Here Range deviation was 5.00 %,

single assignment mode with no Limit of Detection (LOD) and Limit of Quantitation (LOQ) for gallic acid.

The **calibration function** establishes a relationship between the concentration of a substance and its corresponding response, typically measured as the peak area or height on the chromatogram. A linear regression analysis is performed to create a calibration curve, usually represented as: y = mx+by where:

- y= response (peak area or height)
- m= slope of the line
- x = concentration
- b = y-intercept

Calibration function was y =1.731 X 10^{-11} x +1.695 X 10^{-3}

Coefficient of Variation (CV)

The CV is defined as the ratio of the standard deviation (SD) to the mean (average) of a dataset, expressed as a percentage. It provides a standardized measure of dispersion relative to the mean, making it useful for

comparing variability across different datasets or conditions.

CV=(SD/Mean)×100
Coefficient of variation (CV) was 5.19%
A lower CV indicates higher precision,

Correlation coefficient

The correlation coefficient, typically denoted as R, ranges from -1 to 1:

- R=1: Perfect positive correlation.
- R=−1: Perfect negative correlation.
- R=0: No correlation.

Here, R = 0.991937,

Retention factor

- Rf = Distance travelled by the compound / Distance travelled by the solvent front.
- The Rf (retention factor) value of sample is 0.392 at beginning, Maximum 0.413 at maximum, 0.440 at end

Peak Intensities:

- Higher peak intensities generally indicate higher concentrations of the compounds. Higher peak intensity of the sample is 0.0100

Area:

In an HPTLC report, the area under the peaks is crucial for quantitative analysis. It represents the concentration of the compounds in your sample: larger areas typically indicate higher amounts. Area of the sample is 0.00025

Chromatography

Plate layout:
Stationary phase-Merck, HPTLC Silica gel 60 F254
Plate format-200 x 100 mm
Application type-Band
Application- Position Y: 8.0 mm, length: 8.0 mm, width: 0 mn
Track-First position X: 20.0 mm, distance: 11.4 mm
Solvent front position-70 mm

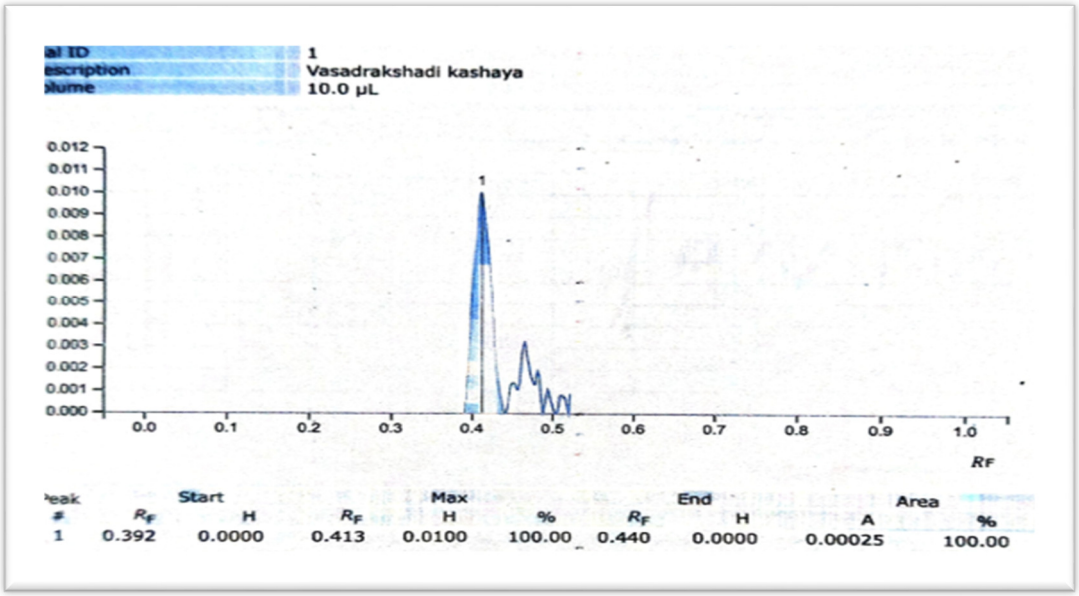

Fig 1- HPTLC Chromatogram standard gallic acid -sample of vasadrakshadi kashaya

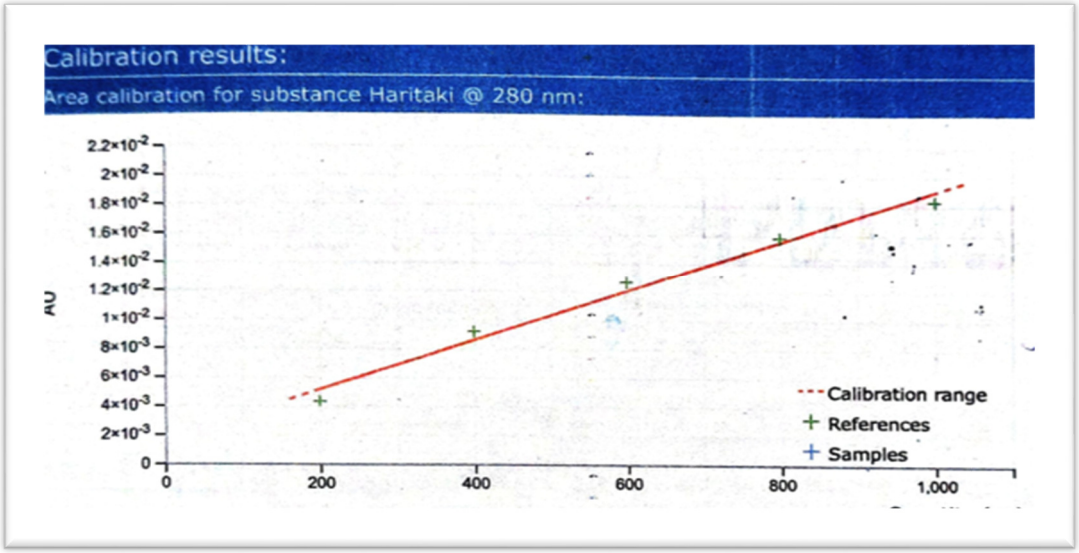

Fig2-Calibration result- area calibration for substance haritaki @280 nm

DISSCUSSION

Organoleptic analysis is perceived by sense organs and helps in the preliminary quality evaluation. Hence the organoleptic parameters of of Vasadrakshadi kashaya were analysed as preliminary quality check which revealed the characteristic of Vasadrakshadi kashaya.

Established physicochemical standards provides information for further investigation and facilitate identification of formulations. Hence the specific gravity, pH, total solids, loss of drying at 105^0 C, specific gravity at 25^0C, which will serve as reference for future analysis.

Heavy metals are toxic to humans and other living organisms at certain levels of exposure. The toxicity of Heavy metals depends on the specific metal, the dose and the route of exposure (viz. inhalation or skin contact). Heavy metals such as Lead (Pb), Mercury (Hg), Cadmium (Cd) and Arsenic (As) are particularly toxic and cause a no. of health problems The permissible limit of Heavy metals as per the Ministry of AYUSH is as follows: Lead: 10 ppm, Mercury: 1.0 ppm, Cadmium: 0.3 ppm., Arsenic: 3.0 ppm.

Contamination of formulation with microorganisms from soil, air or water may occur which can lead to health problems to their consumers. This may also affect the therapeutic quality of herbal products. Hence

the finished product must be free from microbial contamination.

Quality checks and standardized manufacturing practices for finished products can be ensured by HPTLC with suitable markers. HPTLC method has been used for the estimation of markers and standardization of different formulations. The Rf (retention factor) value indicates how far a compound travels on the plate relative to the solvent front. Compare Rf values of your sample with those of standards for identification. Rf = Distance travelled by the compound / Distance travelled by the solvent front. Higher peak intensities generally indicate higher concentrations of the compounds. Compare intensities across samples to assess relative quantities. The correlation coefficient is a crucial metric in HPTLC for assessing the linearity and reliability of calibration curves. By ensuring a high correlation coefficient, analysts can validate their methods and ensure accurate quantification of compounds

CONCLUSION

Vasadrakshadi Kashaya was standardized with respect to organoleptic, phytochemical, microbial, heavy metal anlaysis. A HPTLC method for quantitative determination of gallic acid present in Abhaya of Vasadrakshadi Kashaya was successfully developed and validated. Standardisation protocols using non-conventional analytical practices are therefore required for the authentication of herbal/polyherbal Ayurvedic formulations.

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