Pharmacognostical And Pharmaceutical Analysis of *Triphaladi Kashaya* -An Ayurvedic Herbal Formulation for Diabetes Mellitus

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ABSTRACT

**Background:** *Triphaladi Kashaya* is indicated as one of the drug management of Prameha, so for assurance of quality of polyherbal compounds pharmacognostical and pharmaceutical analysis should be done. **Methods:** *Triphaladi Kashaya* was subjected to microscopic evaluation like macroscopic /organoleptic study, Powder microscopy, Foreign matter, Loss on drying at 105°C, Total ash, Acid insoluble ash, Alcohol-soluble extract, Water-soluble extract, TLC Analysis solvent systems: Toluene: Ethyl acetate: Formic acid (5:4:1) Stationary phase: Pre coated silica gel 60 F254 plates. **Results:** Pharmacognostical study showed the presence of certain identifying characters of all the ingredients of *Triphaladi Kashaya* which are Haritaki, Bibhitaki, Amalaki, Vishala, Devdaru, Mustaka. In pharmaceutical study, preliminary physiochemical analysis showed loss on drying 3.80% w/w, total ash 2.22% w/w, acid insoluble ash 0.18% w/w, alcohol soluble extract 2.49% w/w, water soluble extract 2.00%w/w, TLC analysis showed 6 spots in 254 nm and 4 spots in 366 nm. **Conclusion:** Pharmacognostical and physiochemical observation revealed the specific characters of all active constituents of *Triphaladi Kashaya* and confirm purity and actualities of the drug.

**KEY WORDS:** *Triphaladi Kashaya*, Diabetes mellitus, Pharmacognosy, Pharmaceutical analysis

INTRODUCTION:
Acharya Sushruta has described *Triphaladi Kashaya* for the treatment of Sarvaprameha¹, in which Haritaki, Bibhitaki, Amalaki, Vishala, Devdaru and Mustaka are combined. In this, all the constituents have been taken in equal amount. *Triphala* is a drug widely used in many disorders due to its various pharmacological activities. It has been described in the ancient Ayurvedic text as a *Tridoshic Rasayana*, a therapeutic agent with balancing and rejuvenating effects on the three humours or constitutional elements in Ayurveda Vata, Pitta and Kapha. *Triphala*, being a combination of all three, is therefore balanced, making it useful as an internal cleansing, detoxifying formula. It is regarded as an important Rasayana and good purgative in Ayurvedic medicine. Recipe for this traditional herbal supplement is
described in the traditional Indian texts, Charaka and Sushruta Samhita. *Triphala* has been reported to be a rich source of Vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, B-sitosterol, ascorbic acid and Spectroscopic techniques including mass spectroscopy, flavonoids- nuclear magnetic resonance and Infrared spectroscopy showed gallic acid as the major component. *Triphala* also contains about 20% tannins of both condensed and hydrolysable type. Other constituents identified in the fruit include lipids, sitosterol, saponins, cardiac glycoside and various carbohydrates. Therefore, for the internal use of any drug, it should be safe, effective and free from adulteration. It is difficult to identify the individual herbal drug in dry or powdered form, hence the need of the hour is to determine the criteria required for standardization of herbal drug. Pharmacognostical study reveals the identity of the plant, because at present, the variety of many drugs is found in doubtful form, therefore it becomes necessary to standardize the herbal drugs. Generally, the physiochemical analytical study helps to explain pharmacognostical and pharmacodynamic of drug. With the help of physiochemical analytical studies, it is possible to standardize the drug and differentiate the adulterants. Thin layer chromatography is a method used in the analysis of secondary metabolites originating from plants. It is the necessity of time in the field of Ayurveda to go for quality control of the raw drugs as well as final products using modern parameters which provides credibility to Ayurvedic medicines and also helps in the globalization of Ayurveda. Hence to evaluate the authenticity of *Triphaladi Kashaya* through various pharmacognostical procedures, and to develop the pharmacognostical and phyto-chemical profile of *Triphaladi Kashaya*, the present study was carried out.

**METHODS:** Collection, identification and authentication of raw drugs.

The raw materials were purchased from Surajdeen Harbhukhan Pasari Ayurved Pharmacy, Rewa and the raw drugs were identified and authenticated in Government Drug testing laboratory, Amkho, Lashkar, Gwalior (M.P.). The ingredients and part used of the *Triphaladi Kashaya* are given in Table 1.

**Table 1: Ingredients of Triphaladi Kashaya**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>DRUG</th>
<th>BOTANICAL NAME</th>
<th>PART USED</th>
<th>PROPORTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Haritaki</td>
<td>Terminalia chebula</td>
<td>Fruit</td>
<td>1 Part</td>
</tr>
<tr>
<td>2</td>
<td>Bibhitaki</td>
<td>Terminalia bellerica</td>
<td>Fruit</td>
<td>1 Part</td>
</tr>
<tr>
<td>3</td>
<td>Amalaki</td>
<td>Emblica officinalis</td>
<td>Fruit</td>
<td>1 Part</td>
</tr>
<tr>
<td>4</td>
<td>Vishala</td>
<td>Citrullus colocynthis</td>
<td>Root</td>
<td>1 Part</td>
</tr>
<tr>
<td>5</td>
<td>Devdaru</td>
<td>Cedrus deodara</td>
<td>Heart wood</td>
<td>1 Part</td>
</tr>
<tr>
<td>6</td>
<td>Mustaka</td>
<td>Cyprus rotundus</td>
<td>Rhizome</td>
<td>1 Part</td>
</tr>
</tbody>
</table>

**Method of preparation:**

To prepare *Triphaladi Kashaya*, take equal quantity of all the constituents, make *Yavakuta* powder and boil 1 part of the herb with 16 part$^2$ of the water in an open vessel on mild fire till it reduces to one fourth of the original quantity.

**Pharmacognostical study:**

The pharmacognostical study was divided into organoleptic study and microscopic study of the finished product$^3$. 
Organoleptic study:
The genuinity of the polyherbal formulation can be fined with organoleptic characters of the given sample. Organoleptic parameters comprises taste, color, odour and touch of *Triphaladi Kashaya* which was scientifically studies as per standard references.

Microscopic study:
*Triphaladi Kashaya* was dissolved in solvent system Toluence, Ethyl acetate and Formic acid and stationary phase precoated silica gel plates. Microphotographs of *Triphaladi Kashaya* were also taken under Corl-zeissstrinocular microscope.

Physio-chemical analysis:
With the help of various standard physiochemical parameters, *Triphaladi Kashaya* was analysed. The common parameters mentioned for *Kashaya Kalpana* in Ayurvedic Pharmacopoeia of India⁴, and CCRAS⁵ guidelines are loss on drying, total ash, acid insoluble ash, alcohol soluble extract, water soluble extract.

Thin layer chromatography:
TLC is also used for the identification of the completion of any chemical reaction. To determine this, it is observed that at the beginning of a reaction, the entire spot is occupied by the starting chemicals or materials on the plate. As the reaction starts taking place, the spot formed by the initial chemicals starts reducing and eventually replaces the whole spot of starting chemicals with a new product present on the plate. The formation of an entirely new spot determines the completion of a reaction⁶. Furthermore, two-dimensional TLC is frequently used as a method to check if a compound is stable in the stationary phase (such as silica gel, which is usually slightly acidic). For this purpose, the tested compound mixture is eluted twice in a square-shaped TLC plate, first in one direction and then rotated 90°. If the target compound appears on the diagonal of the square, it is stable in silica gel and safe to purify. If it appears below the diagonal, it is decomposing on silica gel⁷. If this is the case, purification can be attempted using neutralized silica gel or an alternative stationary phase such as neutral alumina. TLC is also used as an analytical method for the direct separation of enantiomers and the control of enantiomeric purity, e.g. active pharmaceutical ingredients which are chiral⁸.

**RESULTS:**
The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Triphaladi Kashaya*. For this, all the ingredients of *Triphaladi Kashaya* was subjected to organoleptic and microscopic evaluations to confirm the genuineness of all the raw drugs. Later after the preparation of formulation, pharmacognostical evaluation was carried out. Organoleptic features like color, odour and taste of the *Triphaladi Kashaya* were recorded and are placed in Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Odour</td>
<td>Unpleasant</td>
</tr>
</tbody>
</table>
Taste | Astringent and bitter

Microscopic evaluation: Microscopic evaluation was conducted by dissolving *Triphaladi Kashaya* in solvent system and studied under microscopic for the presence of characteristics of ingredient drug the diagnostic characters are starch grains of *Haritaki* (Figure -1 A,B,C), endosperm cells of *Bibhitaki* (Figure-1 D), stone cells of *Amlaki* (Figure-1 E,F,), cork cell view of *Vishala* (Figure-1 G,H,I), prismatic crystal of calcium oxalate in *Devdaru* (Figure-1 J) and tracheid cells of *Devdaru* (Figure-1K,L,M,N,O), Parenchyma contain starch grains of *Musta* (Figure-1 P).

Physico-chemical parameters:
Physico-chemical parameters like loss on drying found within normal limit. Alcohol- soluble extract and water-soluble extract value of *Triphaladi Kashaya* were found to be 2.49% and 2.00% respectively. Details are shown in Table 3.

### Table 3: Physico-chemical parameters of *Triphaladi Kashaya*

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>VALUES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105°c (w/w)</td>
<td>3.80 %</td>
</tr>
<tr>
<td>Total ash (w/w)</td>
<td>2.22 %</td>
</tr>
<tr>
<td>Acid insoluble ash (w/w)</td>
<td>0.18 %</td>
</tr>
<tr>
<td>Alcohol- soluble extract (w/w)</td>
<td>2.49 %</td>
</tr>
<tr>
<td>Water-soluble extract (w/w)</td>
<td>2.00 %</td>
</tr>
</tbody>
</table>

Thin layer chromatography:
Destinometry scanning of the TLC pattern showed 2 spots at corresponding Rf values in visible light- 0.325 and 0.412; 06 spots at corresponding Rf values- 0.325,0.412,0.5,0.65,0.837 and 0.887 in UV 254 nm and 04 spots at corresponding Rf values- 0.075,0.325,0.45 and 0.662 obtained in UV 366 nm (Table 4). Though it is not possible to identify particular chemical constituent from the spot obtained, the pattern may be used as a reference standard for further quality control research.

### Table 4: Rf values of *Triphaladi Kashaya*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rf value in visible light</th>
<th>Rf value at 254 nm</th>
<th>Rf value at 366 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>0.325 and 0.412</td>
<td>0.325,0.412,0.5,0.65,0.837 and 0.887</td>
<td>0.075,0.325,0.45 and 0.662</td>
</tr>
</tbody>
</table>
Figure 1
DISCUSSION:

Study on *Triphaladi Kashaya* was a step towards pharmacognostical and pharmaceutical standardization of the drug. The pharmacognostical study revealed the presence of the diagnostic characters starch grains of *Haritaki*, endosperm cells of *Bibhitaki*, stone cells of *Amlaki*, thick walled tangentially elongated cork cell view of *Vishala*, prismatic crystal of calcium oxalate in *Devdaru*, tracheid cells of *Devdaru*, Parenchyma contain starch. This confirms the presence of all ingredients of raw drugs in the...
final product and there is no major change in the microscopic structure of raw drug during the pharmaceutical process of preparation of Kashaya, this showed the genuinity of the final product. The Physicochemical parameters showed that the ash values are the criteria to identify the impurity of drugs. Triphaladi Kashaya contained 2.22% w/w total ash. The results revealed that Triphaladi Kashaya is free from unwanted organic compounds and production site was good enough keeping sample free from dust and other solid matters. The 2.00% w/w of water-soluble extractives and 2.49% w/w methanol soluble extractives were present in Triphaladi Kashaya indicates that drug is having good solubility in water and methanol. In TLC study, 6 spots at 254 nm and 4 spots at 366 nm were obtained, indicating its possible components of matrix which may possess its therapeutic effect.

CONCLUSION:
The pharmacognostical and Physio-chemical study of Triphalad Kashaya confirmed the purity of the drug. As no standard fingerprint is available for this formulation, an attempt has been made to evolve pharmacognostical and physio-chemical profiles of Triphaladi Kashaya. Information acquired from this study may be beneficial for further research work and can be used as a reference standard for quality control researches.

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Conflict of interest: None

Declares Ethical approval: The study was approved by the Institutional Ethics Committee

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