PHARMACOGNOSTICAL AND PHYTOCHEMICAL STANDARDIZATION OF
VASAGDUCHYADI KWATHA A POLY HERBAL AYURVEDIC FORMULATION

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ABSTRACT
Ayurveda has a large number of effective formulations for many diseases. Absence of reference standards for compound formulations is one of the hindrances on the way towards standardisation. The present study was aimed at setting up a standard profile of Vasaguduchyadi kwatha which was prepared using pharmacognostically authenticated raw drugs followed by subjecting it to detailed pharmacognostical, physicochemical and phytochemical (including High Performance Thin Layer Chromatography) analysis as per standard protocol. The observations were systematically recorded. Pharmacognostical findings (crystals, fibers, stone cells etc.) confirm the ingredients present in the finished product. Identified phytochemical components (Gallic acids, Flavonoids, Alkaloids, Phenolic compounds etc.) support the intended action of the formulation in liver diseases. The results of this study may be used as the reference standard in further research undertakings of its kind.

Keywords: Vasaguduchyadi kwatha, Pharmacognosy, phytochemistry, HPTLC, Standardization.

INTRODUCTION
Vasaguduchyadi kwatha, a compound ayurvedic formulation explained in Astangahridaya [1], for the treatment of liver diseases especially for kamala (Jaundice) and panduroga (Anaemia). Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [2]. In contrast to the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders [3]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to provide scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.
The first step for scientific basis research is to provide quality standardisation of compound formulations by establishing the presence of each ingredient in the finished product followed by physico-chemical and phytochemical analysis.

**MATERIALS AND METHODS**

**Plant Material:**
Raw drug materials were collected from the pharmacy department of Gujarat Ayurved University and local market of Jamnagar, the ingredients and the part used are given in Table 1.

**Pharmacognostical Evaluation**
As per API [4] raw drugs were identified and authenticated by the Pharmacognosy department. The identification was carried out based on the morphological, organoleptic features and powder microscopy of the individual drugs. Later, pharmacognostical evaluation of the Vasaguduchyadi kwatha churna was carried out. Churna dissolved in small quantity of distilled water, studied under the Carl zeiss binocular microscope attached with camera, with stain and without stain. The microphotographs were also taken under the microscope.

**Methods of Preparation of the Vasaguduchyadi kwatha**
Coarse powder (sieve No. 10) of the ingredients was taken and soaked in 16 times of portable water for overnight (12 hrs). Next day, it was subjected to mild heat with continuous stirring without covering with lid. Reduction was done until the quantity was reduced to 1/4th of the initial volume. The kwatha was filtered through four folded clean cotton cloth and stored in cleaned vessel.

This kwatha was analyzed using various standard physicochemical parameters such as pH, Specific gravity, Refractive Index and total solid content as per API [4] at the Pharmaceutical chemistry lab, IPGT & RA.

**Qualitative tests** [5]
Methanol extract of the sample was analyzed for different functional groups. The presence of Alkaloids, Steroids, Glycosides, Flavonoids and Tannins were confirmed through suitable tests.
HPTLC [6]

HPTLC study was carried out with Standard Gallic acid. Methanol extract of sample & standard Gallic acid were spotted on pre coated silica gel GF 60\textsubscript{254} aluminum plates by means of Camang Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene (5ml), Ethyl acetate (3.5ml) and Formic acid (0.5ml) was used as the mobile phase. After development densitometric scan was performed with a Camag T. L. C. scanner III in reflectance absorbance mode at 254 and 366nm under control of Win CATS Software (V 1.2.1. Camag). Then the plate was sprayed with 10 % FeCl\textsubscript{3} followed by heating and then visualized in day light.

Preparation of methanol extract

The 25 ml Vasaguduchyadi Kwatha was evaporated on water bath. 5 ml ether was added to it and decant ether layer after ether was evaporated 5ml methanol was added. This solution was used for qualitative tests & spotting.

RESULTS AND DISCUSSION

Pharmacognostical study

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of Vasaguduchyadi kwatha. For that coarse powder of all the ingredients were subjected to organoleptic and microscopic evaluation separately. Results matched with the API and thus confirmed the genuineness of all the raw drugs. Later, after the preparation of the Vasaguduchyadi kwatha churna’s pharmacognostical evaluation was carried out.

Microscopic Characters of Vasaguduchyadi kwatha churna

Microscopic evaluation was conducted by dissolving the Vasaguduchyadi kwatha churna in distilled water and studied under microscope with and without stain for the presence of the characteristics of the ingredient drugs and for the probable changes in features if any. The microphotographs were taken by using Carl Zeiss Trinocular microscope. Characteristics of all the ingredient drugs were identified in Vasaguduchyadi kwatha churna also. Details are placed in Table 2.

Phytochemical Analysis

Physico-Chemical Parameters
Physico-Chemical parameters of the vasaguduchyadi kwatha like pH, Specific gravity, Refractive Index and total solid content were all found to be within the normal range. Primary qualitative study carried out with the methanol extract of Vasaguduchyadi kwatha and tests shows alkaloids, steroids & triterpenoids, cardiac & coumarin glycosides, tannins, phenolic compounds and flavonoids are present in the sample. Details are shown at Table 3.

High-Performance Thin Layer Chromatography Study (Methanolic extract)

HPTLC study shows presence of gallic acid in the test formulation(Table 4). Densitometry scanning of the HPTLC pattern showed 4 spots other than gallic acid corresponding to hRf values 0.29, 0.59, 0.75, 0.90 in short wave UV 254 nm and 4 spots corresponding to hRf values 0.29, 0.65, 0.76 and 0.88 in long wave UV 366 nm (Table 5, plate 2). Though it may not be able to identify particular chemical constituent from other spots obtained, the pattern may be used as a reference standard for further quality control researches.

DISCUSSIONS

Study on Vasaguduchyadi kwatha is a beginning towards pharmacognostical and Physico-chemical standardization of herbal drugs in kwatha and kwatha churna form. Powder microscopy of kwatha churna showed the striking characters of all individual drugs of Vasaguduchyadi kwatha. This confirms the ingredients present in the finished product and there is no major change in the microscopic structure of the raw drugs during the pharmaceutical processes of preparation of kwatha churna. All the pharmaceutical parameters analyzed were within the normal reference range. Presence of phytochemicals such as tannin, alkaloids, coumarin glycosides, flavonoids, phenolic compound and triterpenoids which are present in sample are proven for their hepato protective activity \[7-16\] and it hypothesized they are responsible for hepatoprotective activity of Vasaguduchyadi kwatha. The present work can be used as a reference for further standardization works \[17\].

CONCLUSIONS:

Pharmacognostical findings confirm the ingredients present in the finished product and there is no major change in the microscopic structure of the raw drugs during the pharmaceutical processes of preparation of kwatha churna. Identified phytochemical
components support the hepatoprotective potential of the formulation in liver diseases. It is inferred that the formulation meets minimum qualitative standards as prescribed by API at preliminary level. The results of this study may be used as the reference standard in further research undertakings of its kind.

**TABLE 1: INGREDIENTS OF VASAGUDUCHYADI KWATHA FOR 100 ML**

<table>
<thead>
<tr>
<th>Drugs API</th>
<th>Quantity used (Coarse powder)</th>
<th>Botanical name</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasa</td>
<td>3.125 g</td>
<td>Adhatoda vasica Nees</td>
<td>Root</td>
</tr>
<tr>
<td>Guduchi</td>
<td>3.125 g</td>
<td>Tinospora cordifolia (willd.)Miers.ex.HK f and Th</td>
<td>Stem</td>
</tr>
<tr>
<td>Amalaki</td>
<td>3.125 g</td>
<td>Emblica officinalis Gaertn</td>
<td>Pericarp</td>
</tr>
<tr>
<td>Haritaki</td>
<td>3.125 g</td>
<td>Terminalia chebula Retz.</td>
<td>Pericarp</td>
</tr>
<tr>
<td>Bibhitaka</td>
<td>3.125 g</td>
<td>Terminalia bellerica Roxb.</td>
<td>Pericarp</td>
</tr>
<tr>
<td>Chirayita</td>
<td>3.125 g</td>
<td>Swertia chirayita (Roxb.ex Flem) Karsten</td>
<td>Whole Plant</td>
</tr>
<tr>
<td>Kutuki</td>
<td>3.125 g</td>
<td>Picrochiza kurroa Royle ex Benth</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Nimb</td>
<td>3.125 g</td>
<td>Azadirachta indica A.Juss.</td>
<td>Stem Bark</td>
</tr>
</tbody>
</table>

**TABLE 2: POWDER MICROSCOPIC RESULTS OF VASAGUDUCHYADI KWATHA (PLATE 1).**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Botanical Name</th>
<th>Microscopical Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vasa</td>
<td>Adhatoda vasica Nees</td>
<td>Pitted vessels (Fig-1) Prismatic crystals (Fig-2) Simple starch grains (Fig-3) Compound starch grains (Fig-4)</td>
</tr>
<tr>
<td>2</td>
<td>Guduchi</td>
<td>Tinospora cordifolia (willd.)Miers.ex.HK f and Th</td>
<td>Compound starch grains (Fig-5) Fragment border of pitted vessels (Fig-6) Collenchymas (Fig-7) Cork cells (Fig-8)</td>
</tr>
<tr>
<td>3</td>
<td>Amalaki</td>
<td>Emblica officinalis Gaertn</td>
<td>Pitted scleroides (Fig-9) Lignified fibers (Fig-10) Mesocarp (Fig-11) Stone cells (Fig-12)</td>
</tr>
<tr>
<td>4</td>
<td>Haritaki</td>
<td>Terminalia chebula Retz.</td>
<td>Mesocarp (Fig-13) Pitted scleroides (Fig-14)</td>
</tr>
<tr>
<td>No.</td>
<td>Plant</td>
<td>Scientific Name</td>
<td>Physico-Chemical Parameters</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>-----------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>5</td>
<td>Bibhitaki</td>
<td>Terminalia bellerica Roxb.</td>
<td>Stone cells (Fig -15), Scleroides (Fig -16), Tannins containing cells (Fig -17)</td>
</tr>
<tr>
<td>6</td>
<td>Chirayita</td>
<td>Swertia chirayita (Roxb.ex flem)Karsten</td>
<td>Rosette crystals (Fig -18), Pitted vessels (Fig -19), Tannins containing cells (Fig -20), Pitted scleroides (Fig -21), Trichomes (Fig -22)</td>
</tr>
<tr>
<td>7</td>
<td>Kutuki</td>
<td>Picrochiza kurroa Royle ex Benth</td>
<td>Rosette crystals (Fig -18), Pitted vessels (Fig -19), Tannins containing cells (Fig -20), Pitted scleroides (Fig -21), Trichomes (Fig -22)</td>
</tr>
<tr>
<td>8</td>
<td>Nimb</td>
<td>Azadirachta indica A.Juss.</td>
<td>Spiral vessels (Fig -23), Trichomes (Fig -24), Pollen grains (Fig -25), Lignified parenchyma (Fig -26), Stomata (Fig -27)</td>
</tr>
</tbody>
</table>

**TABLE 3: PHYSICO-CHEMICAL PARAMETERS OF VASAGUDUCHYADI KWATHA**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Vasaguduchyadi Kwatha</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>Specific gravity</td>
<td>1.0177</td>
</tr>
<tr>
<td>3</td>
<td>Refractive Index</td>
<td>1.348</td>
</tr>
<tr>
<td>4</td>
<td>Total Solid Contents (%v/w)</td>
<td>5.13</td>
</tr>
</tbody>
</table>

**Qualitative Parameters – Methanol Extract**

- a Test for alkaloids: +ve
- b Test for proteins: -ve
- c Test for Fats & Oils: -ve
- d Test for Steroids & Triterpenoids: +ve
- e Test for Volatile Oils: -ve
- f Test for Cardiac Glycosides: +ve
- g Test for Coumarin Glycosides: +ve
- h Test for Anthraquinone Glycosides: -ve
- i Test for Saponin Glycosides: -ve
- j Test for Tannins & Phenolic Compounds: +ve
- k Test for Flavonoids: +ve
TABLE 4: HPTLC PROFILE OF VASAGUDUCHYADI KWATHA WITH STANDARD GALLIC ACIDS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Short U.V.(254 nm) Rf value</th>
<th>Long U.V.(366nm) Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>vasaguduchiyadi kwatha</td>
<td>0.52</td>
<td>0.52</td>
</tr>
</tbody>
</table>

TABLE 5: HPTLC STUDY OF VASAGUDUCHYADI KWATHA

<table>
<thead>
<tr>
<th>Spots</th>
<th>Short U.V.(254 nm) Rf value</th>
<th>Long U.V.(366nm) Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>0.59</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>0.76</td>
</tr>
<tr>
<td>4</td>
<td>0.90</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Plate: 1

Fig. 1 Pitted vessels
Fig. 2 Prismatic crystals
Fig. 3 Simple starch grains
Fig. 4 Compound starch grains
Fig. 13  Mesocarp cells
Fig. 14  Pitted scleroides

Fig. 15  Stone cells
Fig. 16  Scleroides

Fig. 17  Tannins containing cells
Fig. 18  Rosette crystals

Fig. 19  Pitted vessels
Fig. 20  Tannins containing cells
Fig. 21 Pitted scleroides

Fig. 22 Trichomes

Fig. 23 Spiral vessels

Fig. 24 Trichomes

Fig. 25 Pollen grains

Fig. 26 Lignified parenchyma

Fig. 27 Stomata

Fig. 28 Lignified parenchyma
Fig. 29 Oleo resin cells

Fig. 30 Pitted vessels

Fig. 31 Lignified fibers

Fig. 32 Prismatic crystals

Plate: 2 HPTLC densitogram
REFERENCES

8. Vasanth P. Raj, Raghu H. In vitro and in vivo hepatoprotective effects of the total alkaloid fraction Hygrophila auriculata leave Indian J Pharmacol. 2010 April; 42(2): 99–104

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