Comparative pharmacognostical & pharmaceutical evaluation of Vyaghri haritaki avaleha - an ayurvedic formulation

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Abstract: Kasa is well-defined clinical condition in Brihadtrayi, clearly correlate with cough and its pathophysiology exactly correlates with the mechanism of cough reflex in contemporary medical science. Everywhere, Paediatric Outdoor Patients Department (OPD) has more than half of the total patients having respiratory tract complaints. So, to treat the disease Kasa, Vyaghri Haritaki Avaleha (VHA) was taken from Bhaishajya Ratnavali. The present work was carried out to standardize the raw drugs & finished product-VHA with comparing API standards and previous research work done in same institute to conform its identity, quality and purity of the final product. The pharmacognostical work reveals that presence of Epidermis, Cork, Pericycle, Phloem etc. from Kantakari; Lignified fibre, Stone cells etc. of Haritaki; Aluerone grains, Prismatic crystals etc. of Shunthi observed microscopically. Organoleptic features of VHA made out of the crude drugs were within the standard range. The pH value of VHA was 4.5, Water soluble extract was 71.9 %w/w, Loss on drying was 30.41 %w/w, Reducing sugar was 27.92 %w/w and High Performance Thin Layer Chromatography (HPTLC) at 254nm and 366nm resulted into 2 & 5 spots respectively.

Introduction

In year 2011 with 8.27%, respiratory tract disorders stand 3rd in major six categories for death [1]. Also, 2nd major cause for death with 13.58% in age group 1-4 years, 8.45% of total death for age group of 5-14 years. Any type of the disease affects growth and development and also the school performance of the children. Further it has been observed in the Kaumarbhritiya OPD that the incidence of Respiratory Infection presenting with Kasa Roğa is more [2]. In the Samprapti of disease Kasa, vitiated Kapha obstructs the free flow of Pruna Vata in Kantha and Uras. Since Kapha is the main culprit in production of Kasa and Kapha Dosha is dominating in Balyavastha, the incidence is more in this age group. Early intervention is necessary in case of Kasa as it is a potential Nidanarthakara Vyadhi to produce Kshaya [3]. Also it is important to treat any Balaroga at the earliest as it may hamper the proper Vridhi (Growth and development) of child which is clearly described by Acharya Charaka that Avighata as Shareera Vridhikara Bhava (i.e. Vighata hinders Shareera Vridhi) [4]. So the present work was carried out to standardize and evaluate the pharmacognostical as well as to analyze the physico-chemical properties of Vyaghri Haritaki Avaleha (VHA).

Materials and methods

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Drug Material
All the raw drugs except Kantakari (*Solanum surrattense* Berm.), following were obtained from Pharmacy of GAU, Jamnagar. Kantakari Panchanga (whole plant) were collected from local area of Jamnagar city (22° 28’ 0” North & 70° 4’ 0” East). The ingredients of VHA [5] and the part used are given in [Table 1].

**Table 1: Ingredients of VHA**

<table>
<thead>
<tr>
<th>Sanskrit Name</th>
<th>Botanical / English Name</th>
<th>Parts Used</th>
<th>Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kantakari</strong></td>
<td><em>Solanum surrattense</em> Berm.</td>
<td>Whole plant (Fresh)</td>
<td>100</td>
</tr>
<tr>
<td><strong>Haritaki</strong></td>
<td><em>Terminalia chebula</em> Retz.</td>
<td>Fruit (Dry)</td>
<td>50</td>
</tr>
<tr>
<td><strong>Shunthi</strong></td>
<td><em>Zingiber officinale</em> Roxb.</td>
<td>Rhizome (Dry)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Maricha</strong></td>
<td><em>Piper nigram</em> Linn.</td>
<td>Fruit (Dry)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Pippali</strong></td>
<td><em>Piper longum</em> Linn.</td>
<td>Fruit (Dry)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Twak</strong></td>
<td><em>Cinnamomum zylencum</em> Blume.</td>
<td>Bark (Dry)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Patra</strong></td>
<td><em>Cinnamomum tamala</em> Nees. &amp; Eberm.</td>
<td>Leaf (Dry)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Ela</strong></td>
<td><em>Elleteria cardnomum</em> Maton.</td>
<td>Fruit (Dry)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Nagakesara</strong></td>
<td><em>Mesua ferrea</em> Linn.</td>
<td>Stamen (Dry)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Guda</strong></td>
<td>Jaggery</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td><strong>Madhu</strong></td>
<td>Honey</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

Pharmacognostical evaluation
Raw drugs were identified and authenticated by the Pharmacognosy lab, I.P.G.T.&R.A., Jamnagar. The identification was carried out based on the morphological features, organoleptic features and transverse section microscopy of the individual drugs which were personally collected, pharmacognosy of finished product is carried out. Microphotographs were taken using Carl Zeiss Trinocular microscope attached with camera with stain and without stain [6]. The microphotographs were also taken under the microscope.

**Method of Preparation of VHA**
The drug prepared in Pharmacy of GAU, Jamnagar. Method of preparation adopted as standard procedure from *Sharangdhara Samhita Madhyama Khand* [7] [Figure 1].
Method of Physicochemical evaluation

VHA was analysed by using standard qualitative and quantitative parameters. HPTLC was carried out after making appropriate solvent system with Methanolic extract of VHA at the Pharmaceutical Chemistry lab, I.P.G.T. & R.A. Gujarat Ayurved University, Jamnagar. Organoleptical parameters, Physico-chemical analysis, investigations were carried out by following standard procedure. High Performance Thin layer chromatography (HPTLC) studies were carried out with acid hydrolysed methanolic extract on pre-coated silica gel GF 60254 aluminium plate as 5mm bands, 5mm apart and 1cm from the edge of the plates, by means of a Camag Linomat V sample applicator fitted with a 100 µL Hamilton syringe. The mobile phase used was Toluene: Ethyl acetate: Glacial acetic acid: Formic acid (5:5:1:0.5). The plates were developed in Camag twin trough chamber (20 x 10 cm$^2$) and spots were detected in short U.V. (254 nm), Long U.V (366nm). Camag Scanner II (Ver. 3.14) and Cats software (Ver. 3.17) were used for documentation. Presence of more moisture content in a sample may create preservation problem, therefore loss on drying was selected as one of the parameters. For the final product, results were compared with Ayurvedic Pharmacopeia of India (API) and previous published research work from the same institute [8].

Results
Pharmacognostical study

In the transverse section of Kantakari root (Solanum surrattense Berm.) - epidermis, endodermis, phloem, xylem etc. were observed. [Figure 2]

While in transverse section of Kantakari stem (Solanum surrattense Berm.) – hypodermis, cortex, pericycle, central pith etc. were found [Figure 3].
The microscopic characters of prepared VHA are spiral vessels and multi branch trichome with fibers of Kantakari, tannin content of Haritaki, Oil glands with fibres of Twak, stone cells with tannin of Maricha, stone cells of Pippali, simple, oval shaped starch grains of Shunti, Oil content cells with Aleurone grains and epidermal cells with stain of Ela, Epidermal cell with oil content of Patra, pollen grains of Nagakeshara were observed [Figure 4].

**Organoleptic study**

Organoleptic features of VHA [9] like consistency, colour, taste etc were observed as mentioned in [Table-2].

**Figure 4: microscopic characters of prepared VHA**

- Fig. 4.1 - Spiral Vessels Of Kantakari
- Fig. 4.2 - Multi branch Trichome with Fibers of Kantakari
- Fig. 4.3 - Tannin content of Haritaki
- Fig. 4.4 - Epidermal cell with oil content of Patra
- Fig. 4.5 - Oil glands with fibres of Twak
- Fig. 4.6 - Stone cell with tannin of Maricha
- Fig. 4.7 - Stone cell of Pippali
- Fig. 4.8 - Oil content cells with Aleurone grains and epidermal cells with stain of Ela
- Fig. 4.9 - Starch grains of Shunti
- Fig. 4.10 - Pollen grains of Nagakeshara
Table 2: Comparative Organoleptic parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>API standards</th>
<th>Roshy JC et al.</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistency</td>
<td>Semisolid sticky</td>
<td>Semisolid</td>
<td>Semisolid sticky</td>
</tr>
<tr>
<td>Colour</td>
<td>Blackish brown</td>
<td>Brownish Black</td>
<td>Brownish Black</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter and astringent</td>
<td>Sweet – pungent</td>
<td>Sweet - astringent</td>
</tr>
<tr>
<td>Odor</td>
<td>Spicy pleasant odour</td>
<td>Spicy</td>
<td>Spicy odour</td>
</tr>
</tbody>
</table>

Physico-chemical Parameters
Physico-chemical Parameters [10] of the VHA like loss on drying, water soluble extract, methanol soluble extract etc were found as in [Table-3].

HPTLC study

Table 3: Comparative results of Physico-chemical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>API standards</th>
<th>Roshy JC et al.</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on Drying (110°C) (% w/w)</td>
<td>Not more than 23%</td>
<td>9.07</td>
<td>30.41</td>
</tr>
<tr>
<td>Water soluble extract (% w/w)</td>
<td>Not less than 68%</td>
<td>75.18</td>
<td>71.9</td>
</tr>
<tr>
<td>Methanol soluble extract (% w/w)</td>
<td>Not less than 20%</td>
<td>51.07</td>
<td>81.9</td>
</tr>
<tr>
<td>pH</td>
<td>5.5 – 5.6</td>
<td>4.34</td>
<td>4.5</td>
</tr>
<tr>
<td>Reducing sugar (% w/w)</td>
<td>-</td>
<td>31.92</td>
<td>27.92</td>
</tr>
<tr>
<td>Non-reducing sugar (% w/w)</td>
<td>-</td>
<td>27.32</td>
<td>17.03</td>
</tr>
<tr>
<td>Total sugar (% w/w)</td>
<td>-</td>
<td>59.24</td>
<td>44.95</td>
</tr>
</tbody>
</table>

Table 4: Comparative results of HPTLC

On performing HPTLC, visual observation under UV light showed few spots but on analysing under densitometer much more was observed and at 254nm the chromatogram showed 2 peaks, at 366nm the chromatogram showed 5 peaks [Table-4 and Figure-5].

Figure-5: Results of HPTLC

![Figure-5: Results of HPTLC](image-url)
Discussion

Standardization of herbal products is the need of time because of several reasons. Microscopically evaluation is very important in the initial identification of ingredients as well as in the detection of adulterations. Identification of original drug is the first step to maintain the quality of the final product. All the ingredients were authenticated with help of characters mentioned in the API. Physico-chemical parameters were compared with API and previous published research work from the same institute for the validation of the data. pH shows that the aqueous solution of Avaleha is acidic in nature (Table-3). Loss on drying parameter was 30.41% as the humid contains found more. Total sugar was 44.95%, could not assessed according to standards as the parameter not mentioned in API. HPTLC fingerprinting for VHA reveals eight spots of Rf values 0.01, 0.14, 0.30, 0.41, 0.50, 0.72, 0.82, 0.92 in short wave UV 254 nm. In long wave UV 366 nm five spots at 0.01, 0.12, 0.40, 0.62, 0.69 Rf values were observed [Table 4 and Figure 5].

Conclusion

The data evolved Pharmacognosy and physico-chemical evaluation of VHA in the present study will be very useful for routine quality control and also to control the batch to batch variation. Preliminary morphological features, transverse section microscopy, powder microscopy of prepared drug results show the ingredients used confirm their genuinity as raw drugs. All ingredients were proved authentic comparing to the parameters mentioned in API. Organoleptic features and physico-chemical parameters assessed which were within the limits according to API except loss on drying. Reducing sugar, Non-reducing sugar, Total sugar and HPTLC of VHA were not mentioned in API standards so it needs repeated study for producing data for the validation of VHA and identification of all the active chemical constituents of the test drug to substantiate the clinical efficacy.

Acknowledgement

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Conflict of interest: We declare that we have no conflict of interest.

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1. Anonymous; Medical certification of cause of death, Gandhinagar: Vital statistic division, Office of the Chief Registrar (Births and Deaths) and Commissionerate of Health, Medical Services And Medical Education (Health), Govt. of Gujarat, 2011, p. 4