Standardization of Dhanyapanchaka Kvatha Ghana Vati - An Ayurvedic formulation

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Quality assurance is an integral part of all systems of medicine to ensure quality medicament. Thus, there is an urgent need to evaluate such parameters which can be adopted by the pharmaceutical industries. In the communication, attempts have been made to standardize Dhanyapanchaka Kvatha Ghana Vati, an Ayurvedic compound formulation. Standardization and quality control of Ayurvedic formulations is necessary to ensure their quality, strength, purity and authenticity. Present work deals with physico-chemical analysis, high performance thin layer chromatography (HPTLC), microbial limit test and heavy metals analysis of Dhanyapanchaka Kvatha Ghana Vati. The outcomes of the research confirm to the need of ensuring quality and safety of Ayurvedic medicines.

Keywords: Dhanyapanchaka Kvatha Ghana Vati, Drug standardization, HPTLC fingerprint

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In recent years, greater global interest has inclined towards non-synthetic natural drug, derived from plant sources, due to their better tolerance and negligible adverse drug reactions1. The World Health Organization (WHO) has also considered phytotherapy in its health programmes and suggested basic guidelines and procedures for the validation of drug from plant origin both from developed western countries and developing countries like India and China2. Despite various efforts by WHO, there is lack of supporting studies regarding the scientific evaluation of formulation and preparation related parameters. However, India’s ancient system of plant based medicine, Ayurveda is gaining recognition throughout the world and many Ayurvedic drugs are now clinically tested and accepted for manufacture2,3. The Ayurvedic formulations are mainly available in the form of solid dosage (Vati, Ghana and Churna), liquid dosage (Asavas, Aristhas) and semisolid dosage (Ghritas, Avlehas). All of these forms have their specific definition, method of preparation and characteristics. The method of preparation and mode of administration of the Vati is easy and it is retain potency for 3-5 yrs when kept in air tight containers while Curna and Kvatha curna retain potency for 2 yrs4,5.

Standardization is important for ensuring of good quality products as standardized drugs of well defined consistent quality are needed for reliable beneficial therapeutic uses. Thus, there is an urgent need to develop parameters for quality control which are cost effective and can be easily adopted by the manufactures. Efforts are being made in this area that have led to the development of analytical protocols both for single herbal drugs as well as for compound herbal formulations6,8 that can be used as valuable analytical tools in the routine standardization of Ayurvedic drugs and formulations.

Various parameters are considered to standardize these medicinal preparations as safe drugs besides adhering to quality and efficacy as per standards of the Ayurvedic formulations. As most of the tests described in ancient literature appear to be based on observation and seem subjective without valid scientific backing therefore standardization and development of reliable quality protocols for Ayurvedic formulations using modern techniques of analysis is extremely important. The study deals with Dhanyapanchaka Kvatha Ghana Vati (DKGV)9, a common compound preparation used for treatment of Amasula (pain due to indigestion),
Amatisara (Diarrhoea due to indigestion) and Aruci (tastelessness). This compound preparation is composed of five medicinal plants ingredients like fruits of Coriandrum sativum L., rhizome of Zingiber officinale Roscoe., rhizome of Cyperus rotundus L., fruit pulp of Aegle marmelos (L.) Correa. and root of Coleus vettiveroides K.C. Jacob (Table 1). The study was carried out with the aim to develop quality standard for DKGV. The objectives included physico-chemical parameters of plant drug constituents of commercial formulation procured from different Ayurvedic pharmacies; determination of the analytical values for defining the limits of heavy metals, microbial screening and development of high performance thin layer chromatography (HPTLC) fingerprint profile as a rapid analytical tools for authentication of commercial samples.

From ancient time, all Ayurvedic preparations have their traditional methods of preparation, mode of administration, dose and time duration on the basis of traditional knowledge on medicine by local peoples or Baidyas. Due to the advent of commercialization longer shelf life has become the need of hour, especially for the preparation of Kvatha (decoction) which are highly perishable. Even though preservatives and additives are considered to be inert, one cannot expect the same result as that of freshly prepared Kvatha. Kvatha (decoction) Kalpana is one amongst the basic preparations in herbal pharmaceutics. Marketing these formulations is not possible because of its shorter shelf life and hence Dhanyapanchaka Kvatha is converted to Dhanyapanchaka Kvatha Ghana Vati (solidified aqueous extract) form by using the method of Anukta paribhasha explained in the classical texts of Ayurveda. Converting Kvatha into different dosage forms like Ghana vati, (solidified aqueous extract) Arishta (self generated alcoholic liquid) may help to increase the shelf life without much change in the property of the particular formulation. This form of drug is easy to take, storage, transport, easily absorbed, and have longer shelf life (2-5 yrs) with enhance therapeutic action and fewer side effects. These types of drugs are very pure, safe, good quality and traditionally have strong faith and acceptability. Dhanyapanchaka Kvatha Ghana Vati is widely used in traditional practices to maintain Amasula (pain due to indigestion), Amatisara (diarrhoea due to indigestion) and Aruci (tastelessness).

Materials and methods

Collection and authentication of raw materials

All the ingredients used in the preparation of Vati were collected from different places, viz. fruits of Dhanyaka (Coriandrum sativum L., specimen voucher No. 102) and rhizome of Nagara (Zingiber officinale Roscoe., specimen voucher No. 130) from Karwi, Chitrakoot (UP) during 2013, rhizome of Musta (Cyperus rotundus L., specimen voucher No. 435) and fruit pulp of Bilva (Aegle marmelos (L.) Correa., specimen voucher No. 52) were collected from Chitrakoot forest during year 2013 and the specimens were deposited in herbarium section Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (MP). Root of Balaka (Coleus vettiveroides K.C. Jacob, specimen voucher No. J/165-Rt 8) was procured from Khari Bawli market, New Delhi during 2013, and the dried specimen was deposited in the crude drug museum of CSMDRIA, Chennai. All the ingredients were authenticated with the help of taxonomist Dr RLS Sikarwar and available literature.

Preparation of the Dhanyapanchaka Kvatha Ghana Vati

The authenticated crude drugs were crushed to a coarse powder separately (180 μm IS sieve or old sieve number 85 and then mixed thoroughly with equal proportion of each ingredients (1:1:1:1:1) and 8 parts of water in a stainless steel container and then continuous mild heat was supplied until it was reduced to one-fourth of its initial quantity. During the heating process, continuous stirring was done to facilitate the evaporation and avoid any deterioration due to burning of materials. After a desirable reduction in volume was achieved, the Kvatha was filtered through single folded cotton cloth and collected in a separate vessel.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of ingredients</th>
<th>Botanical name</th>
<th>Parts used</th>
<th>Proportion of ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dhanyaka</td>
<td>Coriandrum sativum L.</td>
<td>Fruit</td>
<td>1 part</td>
</tr>
<tr>
<td>2.</td>
<td>Nagara (Sunthi)</td>
<td>Zingiber officinale Roscoe.</td>
<td>Rhizome</td>
<td>1 part</td>
</tr>
<tr>
<td>3.</td>
<td>Musta (Musta)</td>
<td>Cyperus rotundus L.</td>
<td>Rhizome</td>
<td>1 part</td>
</tr>
<tr>
<td>4.</td>
<td>Balaka (hrivera)</td>
<td>Coleus vettiveroides K.C. Jacob</td>
<td>Root</td>
<td>1 part</td>
</tr>
<tr>
<td>5.</td>
<td>Bilva</td>
<td>Aegle marmelos (L.) Correa.</td>
<td>Fruit pulp</td>
<td>1 part</td>
</tr>
</tbody>
</table>
**Kwatha** was boiled again over slow fire on a gas stove, maintaining the temperature between 90 °C and 95 °C till a semisolid consistency is obtained. As the water evaporates, the viscosity of the extract increases, resulting in *Ghana*\(^6\) form. Then, the *Ghana* was mixed with the *Curnas of Dhanyapanchaka* (up to 10% of extract) further forming a solid mass.

The solid mass (*Ghana*) was forced through a number of 16 sieve and granules were prepared and then dry the rounded *Vatis* in a tray-dryer at a temperature not exceeding 50 °C to 60 °C for 10 to 12 h. The formulation was then compressed in a single-punch press with a target weight of 250 mg. Stored *Vatis* in containers and packed them in air-tight condition to protect them from light and moisture\(^17,18\).

Two samples were prepared at research laboratory *Ayurveda* Sadan, Chitrakoot Batch-A and Batch-B; whereas, Batch-C was prepared by Chitrakoot Rasshala Pharmacy, Chitrakoot.

**Physicochemical and quantitative parameters**

Organoleptic characters, average weight loss on drying at 105 °C and physicochemical analysis of all the samples were carried out. Quantitative analysis for friability test (Digital friability test apparatus, Model 902, Electronic India, New Delhi) and disintegration time (Disintegration test apparatus, Model 1901, Electronic India, New Delhi) were checked in triplicate according to the prescribed Standard methods in Indian Pharmacopoeia\(^18\).

**High performance Thin Layer Chromatography (HPTLC) profile**

For HPTLC\(^{19,20}\), 0.25 g of each dried samples and powdered *Ghana Vati* macerated with 10 mL ethanol were taken. Filtered each of the extracts and combines together. Added 5 g of anhydrous sodium sulphate, kept it for 10 min, filtered and concentrated. HPTLC of extracts of all the samples were carried out on silica gel 60 F\(_{254}\) pre-coated plates. Toluene: Ethyl acetate: Methanol: Water (5: 3.5: 1: 0.5) were used as mobile phase. The plate was developed and visualized under ultraviolet at 366 nm, visible light and after spraying with 5% methanolic-sulphuric acid reagent followed by heating at 105 °C for 5 min.

**Heavy metal**

Heavy metals analysis\(^{17,18}\) (lead, cadmium, arsenic and mercury) were carried out using Atomic absorption spectrophotometry (Shimadzu-Model-AA-7000). All the samples were digested with concentrated HNO\(_3\); HClO\(_3\) (4:1). Standard solutions were made of different dilution to get linear calibration (Merck). Pb and Cd were performed using graphite oven method, while As (Arsenic) was determined as hydride method and Hg was determined using cold absorption method.

**Test for microbial limits**

Following tests were carried out as per standard methods\(^{17,18,21}\) to determine the microbial load in three batches of *Dhanyapanchaka Kvatha Ghana Vati*.

1. Enumeration of *Staphylococcus aureus* /g
2. Enumeration of *Salmonella* sp. /g
3. Enumeration of *Pseudomonas aeruginosa* /g
4. Determination of *E. coli*
5. Determination of total bacterial plate count (TBC)
6. Determination of yeast and mould.

The microbiological tests were determined using specified agar and enrichment media from Himedia and Privet Limited Mumbai.

**Results and discussion**

Standardization and quality control of *Ayurvedic* formulations is necessary to ensure their quality, strength, purity and authenticity. Present work deals with organoleptic, physico-chemical analysis, high performance thin layer chromatography (HPTLC), microbial profile and heavy metals analysis of *Dhanyapanchaka Kvatha Ghana Vati*.

The organoleptic parameters form the basic criteria for selecting a raw drug and also to confirm the finished product. Texture of *Ghana Vati* was smooth indicating the surface uniformity without cracks. This is the primary character to assess the quality of tablets. Color was greenish brown, taste was bitter and odor was characteristic due to the specific properties of the various ingredients.

The results of physicochemical parameters, viz. loss on drying (1.70-1.80 %), Average weight (4.97-5.03 %), Friability (0.47-0.51 %) and disintegration time as minute (76-80) were found (Table 2).

HPTLC fingerprint profiles of the formulations are depicted in Fig. 1a-c. The TLC plate was examined under 366 nm, after derivatization 366 nm and visible light. The R\(_f\) values and colour of the bands obtained were recorded. It shows major spots at 366 nm R\(_f\) 0.05

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss on drying at 105 °C as %</td>
<td>1.70-1.80</td>
</tr>
<tr>
<td>2.</td>
<td>Average weight : as %</td>
<td>4.97-5.03</td>
</tr>
<tr>
<td>3.</td>
<td>Friability as %</td>
<td>0.47-0.51</td>
</tr>
<tr>
<td>4.</td>
<td>Disintegration time as minute</td>
<td>76-80</td>
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Table 3 — Determination of heavy metals in Dhanyapanchaka Kvatha Ghana Vati

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Dhanyapanchaka Kvatha Ghana Vati</th>
<th>API Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Batch A</td>
<td>Batch B</td>
</tr>
<tr>
<td>1.</td>
<td>Lead (Pb)</td>
<td>0.1111</td>
<td>0.1103</td>
</tr>
<tr>
<td>2.</td>
<td>Cadmium (Cd)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3.</td>
<td>Arsenic (As)</td>
<td>0.0068</td>
<td>0.0064</td>
</tr>
<tr>
<td>4.</td>
<td>Mercury (Hg)</td>
<td>0.0160</td>
<td>0.0164</td>
</tr>
</tbody>
</table>

Note: ND - Not detected

These recorded levels are less than the limit set by Ayurvedic Pharmacopoeia of India (API). Pathogenic bacteria, i.e., Salmonella, Pseudomonas, Staphylococcus and E. coli were found to be absent in the formulation. These results thus revealed that the formulation developed is safe for consumption. Medicinal plant matters normally carry bacteria and moulds often originating in soil in high numbers. In the present formulation, the microbial count was within permissible limits,

Fig. 1 — (a) HPTLC fingerprints profile at 366 nm (before derivatization), (b) HPTLC fingerprints profile at 366 nm (after derivatization) and (c) HPTLC fingerprints profile at visible light (after derivatization), Track T1: Batch A- Dhanyapanchaka Kvatha Ghana Vati; Track T2: Batch B- Dhanyapanchaka Kvatha Ghana Vati (Two samples were prepared at research laboratory Ayurveda Sadan, Chitrakoot Batch-A and Batch-B); Track T3: Batch C- Dhanyapanchaka Kvatha Ghana Vati (Batch-C was prepared by Chitrakoot Rasshala Pharmacy, Chitrakoot).

Figures and tables have been placed after the main text.

Conclusion

The Ayurvedic system of medicines is prevalent in India since the Vedic period and as early as the dawn of human civilization. Though, Ayurveda has undergone many changes in the course of its long history, it still remains the mainstay of medical relief to a large section of population of the nation. Due to urbanization and dwindling of forests, the Vaidya by and large is no longer a self contained unit collecting and preparing his own medicines as before. He has now to depend on the newly developed agencies like one collecting and supplying the crude drugs and the other undertaking mass production of medicines in the Ayurvedic pharmaceutical units run on the commercial scale.

The standardization of such formulation is need of the hour for wider acceptability. Hence, the organoleptic, physicochemical parameters, quantitative analysis, HPTLC fingerprint profiles, heavy metals and microbial profiles together may be used for quality evaluation and the standardization of compound formulations. From ongoing observations it can be concluded that the distinguishing band in the HPTLC profiles may be utilized as marker parameters for monitoring the quality of the formulation. The data generated indicates genuineness, purity and safety of the finished product and can be useful as diagnostic tool for standardization of same drugs.
Acknowledgement

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References


