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Review Article

Pharmacognostical and physiochemical evaluation of bruhaniya gana siddha ksheerpak for ksheerpan and ksheerbasti in the management of garbhashosh

Sarika Trimbak Shinde*

Department of Kaumarbhritya, Gaud Brahman Ayurvedic College and Hospital, Brahmanwas, Rohtak, Haryana, India

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***Correspondence:**
Dr. Sarika Trimbak Shinde,
E-mail: drsarika843@gmail.com

ABSTRACT

Ayurveda is the most ancient medical science framed on evidence-based principles. So, the standardization of Ayurveda drugs is much needed work in view to asses and maintain the quality of Ayurveda drugs in present era. The WHO appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy. The quality assessment of Ayurveda drug formulations is very important in order to prove their acceptability in modern system of medicine. The present study aims to standardize bruhaniya gana siddha ksheerpaka formulation for ksheerpan and ksheerbasti in the management of garbhashosh (IUGR). The process of standardization is achieved by systematic pharmacognostical and physiochemical evaluative studies. The drug formulation bruhaniya gana siddha ksheer contains Bala, Ashwagandha, Shatawari, Vidari, Kakoli, and Kshirkakoli.[2] Organoleptic characters revelled that formulation was light off white in colour, characteristic fragrant odour, and sweet in taste. The result of pharmacognostical study had shown presence of prismatic crystals of Bala, Scalariform vessels of Shatavari, and trichomes of Ashwagandha, cambial activity of the tubers of Vidari, etc. Physiochemical study showed loss on drying 2.96%, total ash 0.31%, acid insoluble ash 0.01%, pH 6.18%, total solids 34.68. Phyto-chemical analysis showed presence of alkaloids, tannins, steroids, proteins, carbohydrates, reducing sugar, etc. The formulation was prepared and screened for various standardization parameters as per ayurveda drug standard protocols in API.

Keywords: Pharmacognosy, Physiochemical, Phytochemical, HPTLC, Bruhaniya gana siddha ksheerpaka

INTRODUCTION

While going through Ayurveda classics it is revealed that Garbhashosh is one of the disease, which is comparable to intrauterine growth restriction in modern medicine.[4,5] In Ayurveda various drugs and preparations have been described for treatment of garbhashosh.[6]

These drugs are comparatively safe without any unwanted side effects. While describing specific management of garbhashosh, Sushruta suggested use of kshirbasti, specified in brief management of garbhashosh using bruhaniya aushadhi dravya, milk and mansarasa[6]. Selection of bhruhaniya ganashidha kshir having Authentic references, the combination of bhruhaniya gana sidha kshirpan and kshirbasti might give more beneficial effect, these drugs having dominance of snigdha, sheeta, guru guna, sheeta virya, madhura rasa, madhura vipaka, pruthvi- aapa mahabhutadhikya, vata shaman, anuloman, kapha vataghna, bhruhaniya and garbhavrudhikar properties which leads to agnideepan, it also helps for prakruta ahararasanirmiti and prakruta rasadhatunirmiti and rasaprasadana. In this way our aim of garbhavridhi is achieved.[2] Kshirbasti on basis of the dose called as pancha

prasruta basti, on the basis of the chief drug kshira basti, on the basis of the rasa of the drugs madhura rasa skandha dravya basti, on the basis of chief action it is bruhan basti, basti is not given merely to draw out the impacted faeces from the colon but it is one of the routes of the drug administration for the systematic therapy of many diseases.[6]

That is the reason why basti alone has been unanimously claimed as "half treatment" of the diseases.[6] Many Ayurveda formulations lacks standardization which creates difficulty in proving efficacy and quality standards of that drug.

Aim

The aim of the study was to perform quality evaluation of bruhaniya gana siddha ksheerpak formulations is achieved through organoleptic, botanical, physical, chemical and biological methods, which are useful to check adulteration and purity of bruhaniya gana siddha ksheerpaka.[1,7]

METHODS

Pharmacognostical study

Preliminary identification was carried out based on organoleptic and botanical characteristics of individual drugs. Organoleptic examination was done with the help of five senses by assessing colour, odour, taste, texture of individual drug. It is simplest and quickest method of drug standardization for its identity and purity. Botanical identification which was very important in the sense of authentication of raw drugs in two types macroscopic and microscopic. Macroscopic examinations which can be seen through normal eyes like shape, external features, any markings.[9] In microscopical study internal structures of cell contents like parenchyma, collenchyma, description of root, bark etc. studied with help of various microscopes.[9]

Collection, identification, authentication of raw drugs

The raw drugs Shatavari, Ashwagandha, Bala, Vidari, Kakoli, Kshirkakoli were collected from the authentic Ayurveda pharmacy. Sita (sugar), cow ghee, honey, til taila (sesame oil) cow milk used to prepare ksheerpaka for kshirpan and kshirbasti formulation were purchased from local market. All the ingredients were authenticated in a laboratory. This includes organoleptic and botanical (macroscopic and microscopic) study.

Preparation of bruhaniya gana siddha ksheerpaka according to guidelines of Shargdhar Samhita.[8] (a) Kshirpan formulation: Bharad churna (course powder) of Shatavari, Ashwagandha, Bala, Vidari, Kakoli, Kshirkakoli, Sarkara in equal quantity of 1.5 g each total of 10.5 g, eight parts of milk (80 ml), four parts of water (320 ml) are boiled until only milk part remains; and (b) Kshirbasti formulation: Bharad churna (course powder) of Shatavari, Ashwagandha,Bala, Vidari, Kakoli, Kshirkakoli, Sarkara in equal quantity of 3 g each total of 21 g, eight parts of milk (160 ml), four parts of water (640 ml) are boiled until only milk part remains. Til tail, cowghee, honey 80 ml each added to sukhoshna (lukewarm) kshirpak, and it was well churned using churning staff.

Figure 1: Bruhaniya gana siddha ksheerpaka raw drugs.

Table 1: Drugs used for preparation of bruhaniyagana siddha kshirpak.

Sanskrit name of the drug	Latin name	Parts used	Drug form	Quantity used for kshirpan formulation	Quantity used for kshirbasti formulation
Shatavari	*Asparagus racemosus*	Root	Bharad churna	3 g	1.5 g
Ashwagandha	*Withania somnifera*	Root	Bharad churna	3 g	1.5 g
Bala	*Sida cordifolia*	Whole plant	Bharad churna	3 g	1.5 g
Vidari	*Pueraria tuberosa*	Tuberous root	Bharad churna	3 g	1.5 g
Kakoli	*Lillium polyphyllum*	Root, bulb	Bharad churna	3 g	1.5 g
Kshirkakoli	*Fritilloria roylei*	Root, bulb	Bharad churna	3 g	1.5 g
Jal (water)				640 ml	320 ml
Godugdha (cow milk)				160 ml	80 ml
Sita: sugar	*Saccharum purificum*		Akhand	3 g	1.5 g
Til tail	*Sesamum indicum*			80 ml	-
Goghrita (ghee)	*Butyrum departum*			80 ml	-
Madhu (honey)	*Mal depuratum*			80 ml	-
Final product: decoction (medicated milk): ksheerpak				400 ml	80 ml

Physiochemical examination

It includes study of physical standards like moisture content, specific gravity, viscosity, density, solubility, ash values, extractives, foreign organic matter, fluorescence analysis etc. The plant extracts in ethanolic solutions were assessed for the existence of the phytochemical analysis by using the following standard methods. Tests for different active principles in bhruhaniya gana siddha kshirpak was subjected to qualitative tests to detect the various phytoconstituents as- carbohydrates, lipids, alkaloids, terpenoids, tannins, proteins etc. Materials were dissolved separately in 5 ml of alcohol and filtered; the filtrates were used to test the presence of carbohydrates. The methanol extract was subjected to preliminary phytochemical analysis.[1]

Ethanol extract was prepared in the same manner and phytochemical analysis was carried out. Dragendorff's test for alkaloids, alkaline reagent test for flavonoids, ferric chloride test for tannins, froth formation test for saponins, Keller-Killiani test for glycosides, Libermann Burchard test for steroids and Salkowski test for triterpenoids were conducted.[10]

Chromatography (HPTLC)

HPTLC profile

Instrument used was CAMAG make HPTLC with WINCATS 1.4.3 software and Linomat 5 sample applicator. The stationary phase used was HPTLC plate's silica gel 60 F254 and mobile phase was toluene: ethyl acetate: formic acid (6:3:1) v/v. The sample was prepared in methanol, and 2 ml sample was applied as 8 mm band for each spot. The plate was visualized under short and long ultraviolet (UV) radiations and density of the separated spots was recorded using scanner III.

The plate was sprayed with vanillin-sulphuric acid reagent and observed in daylight. The Rf values were recorded. HPTLC and peak display densitogram were noted at 254 and 366 nm (E Stahl, 1969).[15]

Heavy metals analysis

The Bruhaniya gana drugs course powder sample was digested in HNO_3 and $HCLO_4$ (5:2). The volume of the digested samples was then made up to 100 ml with deionized water and analysed using standard less Omnian software[12].

Pesticide residue

Analysis of pesticide residue in bruhaniya gana drug course powder sample is done on GC-MS Auto system XL turbo mass (column use- PE-5MS-30 m×0.250 mm×0.250 µm). The sample was extracted in hexane. Extracted solvent is concentrated in nitrogen evaporator and injected in GC-MS bruhaniya gana drug course powder.[13]

OBSERVATIONS

Organoleptic properties ksheerpaka formulation and its ingredients

Organoleptic evaluation of the ksheerpaka *r*evealed that it was sweet in taste, light off white in colour, has characteristic odour and was a liquid suspension.

Table 2: Organoleptic properties of bhruhaniya gana siddha ksheerpaka.

Parameters	Ksheerpaka formulation
Colour	light off white
Odour	characteristic odour
Taste	Sweet
Appearance	Liquid suspension

Table 3: Organoleptic properties of bhruhaniya gana course powder and its ingredients.

Properties	Shatavari	Ashwagandha	Bala	Vidari	Kakoli	Kshirkakoli
Colour	Off white	Off white	Cream	Light cream	Greyish yellow	Creamish
Odour	No specific odour	Characteristic odour	Pleasant	No specific odour	Slightly aromatic	Pleasant
Taste	Sweetish	Bitter and astringent	Little bitter	Sweet	Acrid	Bitter
Appearance	Course powder	Course powder	Course powder	Course powder	Course powder	Course powder

Microscopic observation

Shatavari, Bala, Ashwagandha, Vidari, Kakoli, Kshirkakoli were studied under the microscope revealed annular vessels, acicular crystals of Shatavari, simple and compound starch grains, stellate trichomes, simple trichomes, pitted vessels, septate fibers, prismatic crystals of Bala, scaliform vessels, simple hair pitted vessels, simple and compound starch grains and trichomes were found in Ashwagandha, authenticating features such as starch grains, cluster crystals, latex cells (laticifers), vessel element types and cambial activity of the tubers of Vidari. Kakoli- showed cork cells in surface view and section view, in sectional view cork cells with underlying cortex cells, cortex cells filled with cristelline material, simple ovoid to ellipsoidal starch grains measuring 5-11 µm in diameter, simple pitted vessels and tracheids.

Kshirkakoli- transverse section of root shows single layered epidermis the cells of epidermis were pentagonal in shape cortex is made up of parenchymatous cells which are hexagonal in shape without intercellular spaces. Below the cortex is a thick walled single layered endodermis. Pericycle was present which was composed of thin and single layered cells. Primary xylem was distributed towards the pith zone and surrounded by phloem. The main part of pith area was occupied by metaxylem. Pith was parenchymatous.

Physico-chemical parameters of ksheerpaka formulations

The specific gravity of the ksheerpaka *wa*s found to be 1.03, the pH was found to be 6.18 and total solids found in ksheerpak formulation was 34.68. Water soluble extractive value of bruhaniya gana siddha Ksheerpak is 61.68. The alcohol soluble extractive value was 41.74. Total ash content was 0.31, loss on drying was 2.96. Thus, it was found that the extractive value was highest for water followed by alcohol.

Physico-chemical parameters of powder like loss on drying, ash value, water soluble extract, methanol soluble extract and pH were all found to be within the normal range as per API guidelines.[14]

Table 4: Physico-chemical parameters of ksheerpaka formulations.

Parameters	Ksheerpaka formulations
Loss on drying (%)	2.96
Total solids (%)	34.68
Total ash content (%)	0.31
Water soluble extractive (%)	61.68
Alcohol soluble extractive (%)	41.74
Acid insoluble ash content (%)	0.14
Water soluble ash content (%)	0.01
pH	6.18
Specific gravity (kg/m^3)	1.03

Heavy metal analysis

Bruhaniya gana siddha ksheer formulation revealed that the formulation concentration was below the permissible limits. Sample was analysed at SICART, Anand, Gujarat, by using standard less Omnian software and WD-XRF analysis.

Phytochemical analysis

The detail composition of bruhaniya gana drugs course powder and its activity was depending upon the major types of phytoconstituents present in the formulation which was expressed in Table 6, preliminary phytochemical analysis of methanol and ethanol extracts

of bruhaniya gana drugs course powder revealed the presence of alkaloids, flavonoids, tannins, glycosides, sterols, triterpenoids and saponins in both the extracts.

Table 5: Heavy metal analysis ksheerpaka formulations.

Metal	Concentration
Na	0.499
Mg	7.207
Si	14.207
P	1.751
K	9.878
Ca	9.142
Fe	9.267

HPTLC study

Polyherbal formulation is a complex mixture of more than one herb in a particular proportion. The absence of any ingredient or the presence of an undesirable ingredient knowingly or unknowingly can lead to reduction of therapeutic value or even serious health problems to the consumers. Hence it was essential to ensure that only desirable drugs in precise proportion are included in the preparation of polyherbal drugs. This can be assured by quality checks and standardized manufacturing practices. For finished products quality can be ensured through fingerprinting by HPLC and HPTLC with suitable markers.[15]

Table 6: Phytochemical screening results of the ksheerpaka formulation.

Phytoconstituent	Name of the test	Result of kshirpak
Alkaloids	Dragendorff's	+
Flavonoids	Alkaline reagent	+
Tannins	Ferric chloride	+
Glycosides	Keller-Killiani	+
Sterols	Libermann Burchard	+
Triterpenoids	Salkowski	+
Saponins	Froth formation	+

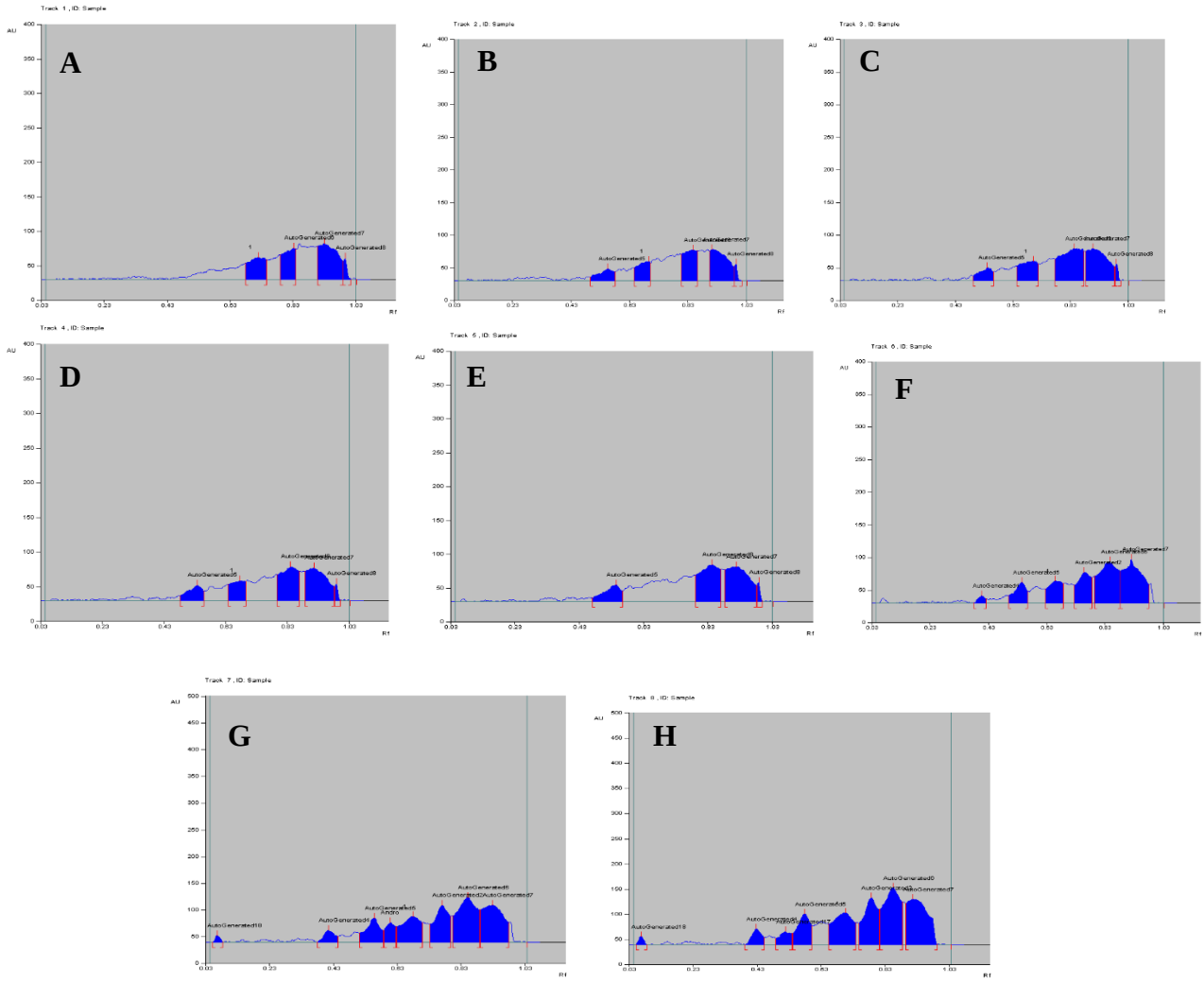

Figure 2: HPTLC fingerprints of bruhaniya gana course powder at 254 nm and 366 nm after derivatisation.

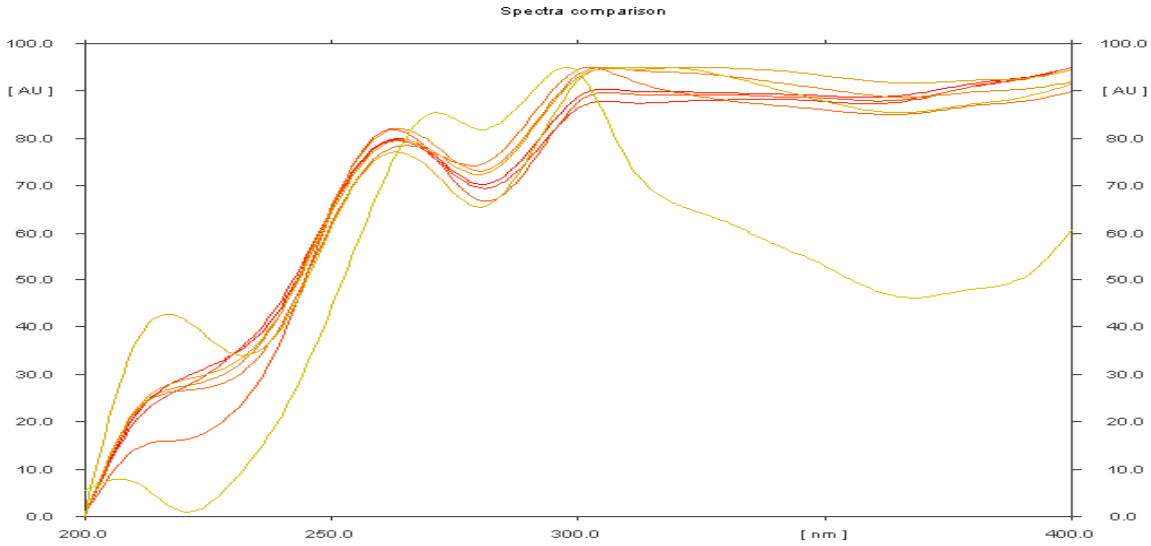

Figure 3: Densitogram of bruhaniya gana course powder.

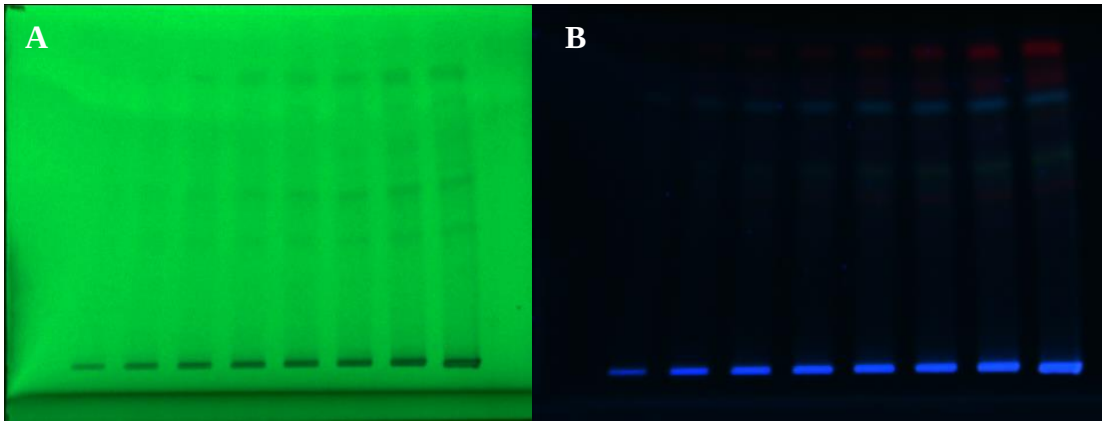

Figure 4: Photographs of HPTLC plates of bruhaniya gana course powder and its ingredients at (A) 254 nm before derivatisation; and (B) at 366 nm after derivatization.

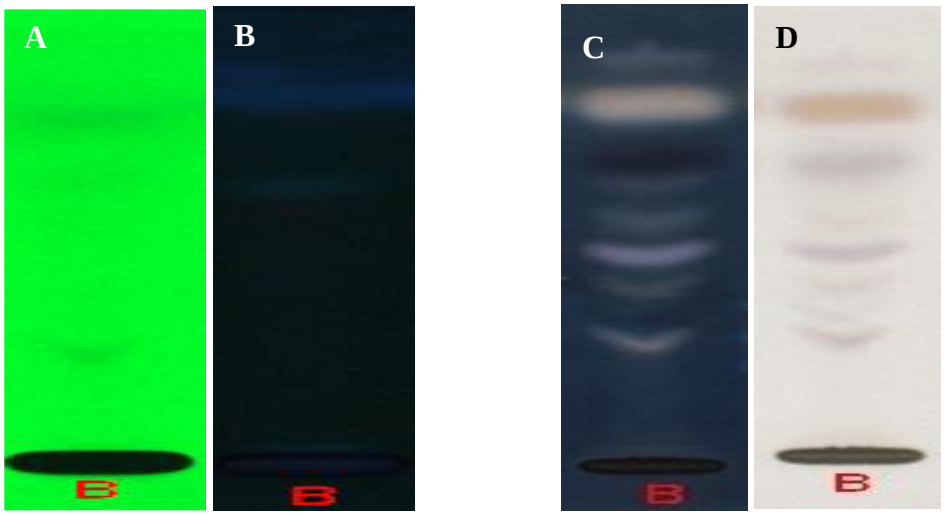

Figure 5: Photographs of HPTLC plates of bruhaniya gana siddha ksheerpaka (A) at 254 nm before derivatisation; (B) at 366 nm before derivatization; (C) at 366 nm after derivatization; and (D) under visible after derivatization.

Table 7: HPTLC Rf values of bruhaniya gana course powder and its ingredients.

Peak	Start position (Rf)	Start height (AU)	Max position (Rf)	Max height (AU)	Max percentage (%)	End position (Rf)	End height (AU)	Area (AU)	Area (%)	Assigned substance (auto-generated)
1	0.05	0.0	0.07	16.5	3.35	0.09	0.2	182.2	1.04	18
2	0.39	0.2	0.43	31.4	6.35	0.45	14.6	683.1	3.91	4
3	0.49	12.7	0.52	26.2	5.30	0.54	22.1	682.1	3.91	17
4	0.54	22.4	0.58	61.3	12.39	0.60	37.6	1678.2	9.61	5
5	0.65	40.7	0.71	63.3	12.80	0.74	43.9	2851.9	16.33	1
6	0.75	42.5	0.79	93.0	18.81	0.81	69.8	2902.2	16.62	2
7	0.82	70.0	0.86	112.6	22.78	0.89	35.2	4218.3	24.16	6
8	0.90	82.0	0.92	90.1	18.22	1.00	0.6	4264.9	24.42	7

Table 8: HPTLC Rf values of bruhaniya gana siddha ksheerpaka.

Peak	Max Rf	Area	Area (%)
1	0.04	83.2	0.20
2	0.28	3321.8	8.15
3	0.33	732	1.80
4	0.39	2226.2	5.46
5	0.45	4685.4	11.50
6	0.52	2114.0	5.19
7	0.63	9692.0	23.79
8	0.76	16219.3	39.80
9	0.85	1672.7	4.10

HPTLC analysis of bruhaniya gana row drugs course powder used for ksheerpak formulation, gave finger print patterns and bands of Rf value were observed within normal limits. Chromatographic study (HPTLC) was carried out at SICART (sophisticated instrumentation centre for applied research and testing) Vallabh-Vidhyanagar, Anand, Gujarat.

DISCUSSION

Organoleptic analysis is the appraisal of a product as it is perceived by sense organs and helps in the preliminary quality evaluation. Hence the organoleptic parameters of bruhaniya gana drugs used for ksheerpaka were analysed as preliminary quality check (Table 2). All the microscopic characteristics identified in powder were equivalent to standard profile.

Physicochemical analysis can be employed for routine evaluation at the sites of manufacture and established physicochemical standards can furnish information for further investigation and facilitate identification of formulations. Hence the specific gravity, pH, total solids and extractive values of the bruhaniya gana siddha ksheerpaka were determined, which will serve as reference for future analysis (Table 4). Plants are nature's drug stores. The enormous chemical compounds present in plants are to be explored for solving present day health problems. Phytochemical analysis revealed that the bruhaniya gana siddha ksheerpaka contained secondary metabolites like alkaloids, flavonoids, tannins, sterols, triterpenoids, amino acids and glycosides conferring cumulative pharmacological properties to the formulation (Table 6).

The fear of presence of detrimental elements including heavy metals is a major apprehension causing scepticism in minds of common man regarding the safety and quality of Ayurveda formulations. Hence, it is indispensable to rule out the occurrence of undesirable elements to augment the acceptance of formulations. Cadmium and lead are reported to be of great concern since they cause adverse health issues in humans. Elemental analysis of bruhaniya gana drugs done by WD-XRF analysis revealed that toxic heavy metals like lead and cadmium were below detectable limits and mercury was below detectable and permissible limit (Table 5). So, it was found that bruhaniya gana drugs siddha ksheerpaka was safe to consume as the concentration of heavy metals was very much below permissible limits (Table 5).

Herbal drugs may be contaminated with pesticides from soil, air or water. Some of these can cause health problems to their consumers. The therapeutic activity of herbal products may be reduced or even nullified by the presence of pesticide residues contaminants causing ill health to patients taking the drug. Hence it is vital to ensure that the finished product is free from pesticide Residues

contamination. Analysis of pesticide residues in bruhaniya gana drugs was done on GC-MS. The absence of pesticide residues in bruhaniya gana drugs assures safety of its consumption.

HPLC/HPTLC method has been used for the estimation of markers and standardization of bruhaniya gana row drugs and bruhaniya gana row drugs siddha ksheerpaka formulation. bruhaniya gana row drugs and bruhaniya gana row drugs siddha ksheerpaka formulation were subjected to HPTLC analysis which generated a fingerprint, wherein Rf, intensity and colour of bands were obtained (Table 7). The findings of HPTLC, which is mainly performed for the confirmation of the phytoconstituents present sounds similar as mentioned in the Ayurvedic Pharmacopoeia (Figure 4-5).

CONCLUSSION

Present study reveals that quality of bruhaniya gana row drugs used for ksheerpaka formulation was characterised as per pharmacognostical, physicochemical, chromatographic and toxicological parameters, which helps in justifying the quality of formulation and meet the desired quality. The analytical specifications were established for the product with respect to quality based raw materials. The chromatographic data showed presence of ingredients in the ksheerpaka formulation with their unique Rf values. This study may be adopted as standard reference for further quality control research works and clinical studies.

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