Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 14 (4), 2023; 1082-1086

Physicochemical and preliminary phytochemical analysis of different brands of Hingwashtak churna; a polyherbal medicine in Sri Lanka

Research Article

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Abstract

Hingwashtak churna is a polyherbal medicine, used to treat gastrointestinal diseases like gastric ulcers, bloating, acidity, and joint disease. The quality evaluation of hingwashtak churna is needed for its acceptability and safety for stakeholders. Therefore, this study focused on the evaluation of physiochemical and preliminary phytochemicals of different selected marketed brands of polyherbal medicine "*hingwashtak churna*" in Kurunegala, Sri Lanka. Three brands of powder form (H_1, H_2 & H_3) of *Hingwashtak churna* samples were purchased in Kurunegala, Sri Lanka. Preliminary phytochemical screening and physio-chemical tests were carried out for each brand. The results were analyzed with one-way ANOVAs using SPSS 22. The results of physico-chemical parameters obtained from the study showed that there was a significant difference ($P > 0.05$) among all three marketed brands as well as between each and other brands. Phytochemical screening of aqueous extract revealed the presence of carbohydrates, reducing sugar, flavonoids, saponins, tannins, steroids, phenols and ascorbic acid in all the brands. There was a significant difference in the flow properties of the powders from all three brands. Most of the parameters of the physiochemical tested brands of Hingwashtak Churna had roughly similar values to previous studies. As no published data are available in Sri Lanka, this preliminary profile could be a reference in future studies. However, further analysis has to be done in order to standardize the product according to the WHO guideline on Quality control methods for medicinal plant materials.

Keywords: Polyherbal medicine, *Hingwashtak churna*, Sri Lanka, Phytochemical, Physio-chemical.

Introduction

Herbal has been used as medicine in all cultures and it is an ancient method to treat diseases. Different parts of the plant are used for the preparation of herbal medicines. Those are leaves, roots, seeds, bark, and flowers. They are consumed, ingested, drank, inhaled, or topically applied to the skin. There are different types of plant biochemicals compounds present in the herbal products, many of which contribute to the therapeutic benefits of the plant (1).

Herbal medicines have lots of advantages. They have greater empathy for patients as well as acceptance. The prolonged and seemingly ordinary use of herbal medicines may provide evidence of their protection and effectiveness. Herbal medicines have supplied many of the most effective medicines worldwide to the large arsenal of medicines available to modern medical science, both in crude and pure form, on which modern medicines are founded (2).

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Hingwashtak churna is a polyherbal medicine which consists of eight ingredients viz., Piper nigrum L. (Black pepper powder), Piper longum (Long pepper powder), *Zingiber officinale* Roscoe (Dry ginger powder), *Nigella sativa* (Black Cumin Seed), *Trachyspermum ammi* L. Sprague (Carom seeds), *Cuminum cyminum* L. (Cummin), *Ferula assa-foetida* L. (Asafetida powder), and *Saindhava Lavana* (Rock salt) (3). It is used to treat digestive disorders by serving as a digestive aid, antispasmodic and anticarminative and it is a potent anthelminthic and helps to treat all painful conditions, such as sciatica, rigidness in the back (4). In addition, *Hingwashtak churna* ensure the proper function of the gastrointestinal track. It is also used in the treatment of the imbalance of vata and vata related diseases, joint bloating diseases (1).

The World Health Organization (WHO) has recognized the value of medicinal plants in developing countries for public health care and has established guidance to help Member States in their efforts to formulate national policies on conventional medicine and to research their potential usefulness, including evaluation, protection, and efficacy (5). Quality of herbal medicines is assessed by organoleptic evaluation, pharmaceutical evaluation, macroscopical and microscopical studies, physio-chemical evaluation, qualitative and quantitative phytochemical evaluation, chromatographic techniques (Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography

(HPTLC), fingerprinting, determination of heavy metals (elemental determination), determination of microbial contamination, toxicological studies and therapeutic (pharmacological) evaluation.

This study was carried out only to put view on general scenario of quality by using physio-chemical, and phytochemical variabilities in available different marketed brands of hingwashtak churna in Sri Lanka without fortifying or criticizing any brand. Phytochemical constituents are bioactive constituents which possess some therapeutic effect. Thus, preliminary phytochemical screening is important to evaluate the quality of *Hingwashtak churna* in order to ensure its therapeutic activity by assessing the presence of relevant phytoconstituents. In this study, qualitative phytochemical evaluation was done. Physio-chemical properties are also very important for herbal medicines in order to ensure the quality of the product by complying with standard values of those physio-chemical parameters such as pH value, moisture content, ash values and extractive values. Thus, evaluation of these physio-chemical parameters plays a major role in this study.

Thus, this study will provide the platform for stakeholders which wish and require knowledge about quality of marketed brands of *Hingwashtak churna*.

Materials and Methods

Sample collection

Three different market brands of powder form of *Hingwashtak churna* samples were purchased from the local market from the registered Ayurvedic Pharmacy in Kurunegala, Sri Lanka, coded as, H_1, H_2 and H_3 (Table 1).

Table I: Procurement of Sample materials

Code	Manufacturing date	Expiry date	Batch No
H_1	27/07/2020	26/07/2022	250
H_2	30/01/2021	30/01/2023	6697
H_3	08/11/2019	08/11/2023	124 10

Evaluation of physio-chemical parameters

Physicochemical parameters such as pH, extractive values, ash values and moisture content were evaluated according to method as described in World Health Organization (WHO) guidelines of quality control methods for herbal materials and the Ayurvedic Pharmacopoeia in India (6)(7). Each test was done triplicate.

Determination of pH value

The powder sample of 5 g of *hingwashtak churna* was weighed and dispersed in 100 mL of distilled water in a beaker. It was kept at room temperature for 24 hours. The supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated digital pH meter.

Determination of moisture content

The sample of 10 g was place in the evaporating dish, and it was heated at 105°C until difference between two successive weighing corresponded do not differ by more than 5 mg.

Determination of Ash values

The sample of 2 g was taken accurately in a previously ignited and tarred silica crucible, and it was ignited in a muffle furnace at 500-600 ^{o}C until it was white, indicating the absence of carbon. Total ash of air-dried material was calculated.

Acid-insoluble ash value

The available amount of ash, which was obtained from total ash experiment procedure, was boiled after covering with watch glass for 5 minutes with 25 mL of 1M HCl and the watch glass was rinsed with 5 mL of hot water. The Insoluble matter was transferred to the original crucible and dried on a hot plate and ignite to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air-dried powdered hingwashtak churna sample.

Water soluble ash value

The available amount of ash, which was obtained from total ash experiment procedure, was boiled for 5 minutes with 25 mL of water and insoluble matter which had been collected and it was ignited for 15 minutes at a temperature not exceeding 450 °C in the muffle furnace. Difference in weight of total ash and weight of insoluble matter was determined and that value was the weight of water soluble ash residue. The percentage of water soluble ash was calculated with reference to the air-dried powdered hingwashtak churna sample.

Ethanol soluble extractive value

Hingwashtak churna powder (5 g) was accurately weighed out and placed inside a glass stoppered conical flask. It was then macerated with 100 mL of ethanol. Flask was shaken frequently during the first 6 hours and was kept aside without disturbing for 18 hours. Then, it was filtered rapidly with taking care not to lose any solvent and about 25 mL of filtrate was transferred into a tared flat-bottomed shallow dish and evaporated to dryness on a water bath. Then, it was dried to 105 °C for 6 hours and then cooled in a desiccator for 30 minutes and finally weighed out without delay. The percentage of ethanol soluble extractive were calculated with reference to the air-dried powdered hingwashtak churna sample.

Water soluble extractive value

Hingwashtak churna powder (5 g) was accurately weighed out and placed inside a glass stoppered conical flask. It was then macerated with 100 mL of 0.25% chloroform. Flask was shaken frequently during the first 6 hours and was kept aside without disturbing for 18 hours. Then, it was filtered rapidly with taking care not to lose any solvent and about 25 mL of filtrate was transferred into a tared flat-bottomed shallow dish and evaporated to dryness on a water bath. Then, it was dried to 105 °C for 6 hours and then cooled in a desiccator for 30 minutes and finally weighed out

without delay. The percentage of ethanol soluble extractive were calculated with reference to the air-dried powdered hingwashtak churna sample.

Preliminary phytochemical screening

The aqueous extracts of each brand of hingwashtak churna powder were prepared and subjected to preliminary phytochemical screening using standard method describe in the Gupta et.al (8).

Tests for alkaloids

Mayer's test for alkaloids

A few drops of HCl were added to 2 mL of extract. Then, a few drops of Mayer's reagent were added. The presence of cream precipitation was evidence of alkaloids.

Wagner's test for alkaloids

A few drops of HCl were added into 2 mL of extract. Then, a few drops of Wagner's reagent were added. The presence of reddish brown colour was evidence of alkaloids.

Tests for carbohydrates

Molish test for carbohydrates

A few drops of Molish reagent were added into 2 mL of extract. Then, a few drops of conc.H_2SO_4 were added. The presence of violet or reddish colour was evidence of carbohydrates.

Fehling's test for reducing sugar

Around 2 mL of mixture of Fehling's solution A and B were added into 1 mL of extract. Then, the test tube was placed in the water bath at 60 °C. First yellow and then brick red precipitation with a green suspension were indicated the presence of reducing sugar.

Tests for flavonoids

Alkaline reagent test for flavonoids

A few drops of 40% NaOH solution was added into 2 mL of extract. Intense yellow colour was formed which becomes colourless on addition of diluted acid was indicated the presence of flavonoids.

Lead acetate test for flavonoids

Around 2 mL of extract was mixed with a few drops of Lead acetate solution. The presence of yellow precipitation was evidence of flavonoids.

Tests for saponins

Foam test for saponins

Around 4 mL of distilled water was added into 2 mL of extract. Then, it was mixed well and shaken vigorously. The foam formation was indicated by the presence of saponins.

Tests for tannins

Braymer's test for tannins

Around 2 mL of extract was mixed with 2 mL of water. Then 2-3 drops of 5% $FeCl_3$ was added. The presence of black, green or bluish colour was evidence of tannins.

Tests for steroids

Salkowski's test for steroids

Around 2 mL of extract was mixed with 2 mL of chloroform. Then, 2 mL of conc.H_2SO_4 was added to it. The red colour of chloroform layer and greenish yellow fluorescence of acid layer were indicated the presence of steroids.

Tests for proteins

Millon's test for proteins

Around 5 mL of millon's reagent was added into 3 mL of extract. Then the mixture was heated gently on a water bath. The presence of white precipitate which turns brick red on warming was evidence of proteins.

Tests for amino acids

Ninhydrin test for amino acids

A few drops of 5% Ninhydrin solution was added to 3 mL of extract. Then the mixture was kept in boiling water bath for 10 minutes. The presence of purple or bluish colour was evidence of amino acids.

Tests for glycosides

Keller Killiani's test for glycosides

Around 2 mL extract was mixed with a few milliliters of glacial acetic acid and one drop of 5% $FeCl_3$. Then conc.H_2SO_4 was added to it. The presence of reddish brown colour at the junction of two layers and bluish green in upper layer was evidence of glycosides.

Tests for phenols

$FeCl_3$ test for phenols

A few drops of 5% $FeCl_3$ solution was added into 2 mL of extract. The presence of deep blue - black colour was evidence of phenols.

Lead acetate test for phenols

A few drops of Lead acetate were added to 2 mL of extract. The presence of white precipitate was evidence of phenols.

Tests for terpenoids

Copper Acetate test for terpenoids

Around 2 mL of extract was dissolved in water and 3-4 drops of copper acetate were added to it. The presence of emerald, green colour was evidence of terpenoids.

Tests for vitamins

Test for ascorbic acid (Vitamin C)

Around 1 mL of extract was diluted with 5 mL of distilled water and a drop of 5% sodium nitroprusside and 2 mL of NaOH were added to it. Then a few drops of HCl were added dropwise. The yellow colour which turns blue was indicated the presence of vitamin C.

Data analysis

All analysis were undertaken in triplicate and quantitative values were presented as means ± standard deviation. The statistical significance was evaluated by the analysis of variance (ANOVA) followed by Tukey's

test. Differences between means were considered significant if P-values lower than 0.05 ($p<0.05$)(9)(10).

Results and Discussion

Physiochemical and phytochemical parameters were evaluated for three different brands of hingwashtak churna. The analysed data of physiochemical and phytochemical showed in the table 2 and table 3 respectively.

Table 2: Summary results of physiochemical parameters of different brands of *Hingwashtak churna*

Properties	Brand H_1	Brand H_2	Brand H_3
pH	5.09 ± 0.005^a	5.05 ± 0.000^b	4.92 ± 0.005^c
Loss Of Dryness (%)	9.47 ± 0.045^c	9.73 ± 0.092^b	11.14 ± 0.105^a
Ethanol soluble extractive value (%)	29.58 ± 0.351^a	23.21 ± 0.277^b	19.86 ± 0.583^c
Water soluble extractive value (%)	36.63 ± 1.100^a	35.08 ± 1.056^a	31.39 ± 0.600^b
Total ash value (%)	19.07 ± 0.857^a	17.56 ± 0.965^a	15.33 ± 0.309^b
Acid-insoluble ash value (%)	0.97 ± 0.066^b	1.05 ± 0.025^b	1.52 ± 0.099^a
Water soluble ash value (%)	15.70 ± 0.095^a	13.67 ± 0.286^b	15.33 ± 0.536^a

Values are represented as mean±SD; Values with different superscripts in the same row differ significantly ($P<0.05$).

Physiochemical analysis

According to table 2, Brand H_1 showed higher pH, ethanol soluble extractive value, water soluble extractive value, total ash value and water-soluble ash value while Brand H_3 showed higher loss of dryness and acid-insoluble ash value. The results of physico-chemical parameters obtained from the study showed that there was a significant difference ($P > 0.05$) among all three marketed brands as well as between each and other brands.

The amount of moisture in herbal remedies and their pH are closely related to the level of microbial contamination and is useful for determining constituent stability and crude drug sensitivity to microbial attack. In the current study, the pH value of all the Brands showed in the range from 4.92 to 5.09 indicating suitability for human use. It revealed that the formulations were acidic. It might be due to the presence of ascorbic acid. A similar study conducted by the Vakhariya (1) showed similar pH range 5.5 and 5.3. Loss on drying was used to determine the moister content of the powdered drugs. In this study, loss on drying value showed less than 12%.

The extract value indicated the nature of the chemical ingredients contained in crude medicine. The lower extract value signifies addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating (11). In this study all the bands exhibited the ethanol extract value in the range of 19.86% and 29.58% and water-soluble extract value in the range of 31.39%and 36.63%. These results were almost similar to the previous studies(12). Even though some of the values are deviating from the previous studies (13). Present study indicated that the value of water soluble extract showed higher value than alcohol extract value which indicates the presence of more water soluble constituents in formulation.

Total ash value gives an estimation about purity and quality of marketed polyherbal medicine. The sample might contain physiological ash which comes from the plant tissue itself and non-physiological ash which is the remnant of foreign substances clinging to the plant surface (such as sand and soil) (6). Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth and water-soluble ash value gives an estimation of inorganic contents (6). A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the herbal drug or drug combinations for marketing. In this study, total ash value of H_1 was 19.07% ± 0.857, H_2 was 17.56% ± 0.965 and H_3 was 15.33% ± 0.536. These values were found to be reasonably low indicating the low contamination. Acid insoluble ash value found in the range from 0.97 to 1.52 % w/w. which was comparable with previous study. all three brands were within the standard limit in accordance with previous studies. The water soluble ash values of the all brands were in the range from 13.67 to 15.70 % w/w. These results were higher than the marketed brand and approximately similar with house formulation (3).

Preliminary phytochemical screening

According to Table 3, aqueous extract of all three brands showed the presence of carbohydrates, reducing sugar, flavonoids, saponins, tannins, steroids, phenols and ascorbic acid. Alkaloids, proteins, amino acids, glycosides and terpenoids were absent in all the brands. In the previous study the phytochemical screening revealed the presence of alkaloids, terpenoids, glycosides, flavonoids, saponins and steroids (14). Another study done on prepared formulation showed the presence of tannins, alkaloid, glycoside, flavonoids, saponin and terpenoid, with absence of steroids, phenolic compound, sterols, anthocyanin, starch and carbohydrate (12). There are some variations in the result of phytochemical analysis. Significant phytochemical difference may be due to type and the quality of the raw material and the mode of processing which may influence the amount of active constituents in formulations. As well as the raw materials may be varied due to the different climates and geographical conditions. Therefore, the researchers cannot have standard amount of active ingredients or phytoconstituents from raw materials and there are no standard parameters for *Hingwashtak churna* to maintain standard minimum levels of phytoconstituents. But, quality evaluation of *Hingwashtak churna* is important for its acceptability as well as safety. Thus

standardization of *Hingwashtak churna* is very important.

Table 3: Phytochemical screening of different brands of *Hingwashtak churna*

Phytoconstituent	Test	H_1	H_2	H_3
Alkaloids	Mayer's test	-	-	-
	Wagner's test	-	-	-
Carbohydrates	Molisch's test	+	+	+
Reducing sugar	Fehling's test	+	+	+
Flavonoids	Alkaline reagent test	+	+	+
	Lead acetate test	+	+	+
Saponins	Foam test	+	+	+
Tannins	Braymer's test	+	+	+
Steroids	Salkowski's test	+	+	+
Proteins	Millon's test	-	-	-
Amino acids	Ninhydrin test	-	-	-
Glycosides	Keller Killiani's test	-	-	-
Phenols	$FeCl_3$ test	+	+	+
	Lead acetate test	+	+	+
Terpenoids	Copper acetate test	-	-	-
Ascorbic acid	Vitamin C test	+	+	+

Conclusion

Here was a significant difference in the flow properties of the powders from all three brands. Most of the parameters of the physiochemical tested brands of Hingwashtak Churna had roughly similar values with previous studies. As no published data are available in Sri Lanka, this preliminary profile could serve as a reference in future studies.

However, further analysis has to be done in order to standardize the product according to the WHO guideline on Quality control methods for medicinal plant materials.

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