Research Article

Pharmocognostic and Phytochemical Evaluation of Polyherbal Formulation Kathakakhadiradi Kashyam

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ABSTRACT

"kathakakhadiradi kashyam" is an ayurvedic medicinal preparation which is used in effective management of diabetes and reducing associated symptoms. The present study is an approach towards standardising the formulation. The study includes organoleptic charecterisation, physic chemical analysis such as ash values, extractive values loss on drying, determination of P^H and Photochemical screening. The generated data in the present study is expected to be useful in the correct identification and authentification of the polyherbal formulation in future.

Keywords: kathakakhadiradi kashyam, Phytochemical Evaluation, Polyherbal Formulation.

INTRODUCTION

As per the estimates of World Health Organization (WHO), more than 80% of global population uses plants or their products as the primary source of medicinal agents[1]. Among the 21,000 plants with medicinal property listed worldwide by WHO, 2500 species are from India, out of which 150 species are used commercially on a fairly large scale[2]. India is the largest producer of medicinal herbs and is called as botanical garden of the world[3]. India is sitting on a gold mine of well recorded and traditionally practiced knowledge of herbal medicine especially plants grown in higher altitudes (Himalayan regions) are world renowned and are a major contributor to the herbal pharmaceutical industry, both of India and other countries[4].

Plants have been a source of medicine for thousands of years and it has become a part of Indian culture and Indian health care. India can emerge as the major country and play the lead role in production of standardized, therapeutically effective herbal drugs and formulations, this can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. Use of herbs and herbal products in both developing and developed countries for treatment of various diseases has increased dramatically in recent years. Common trouble in their wide use and establishment are problems with standardization, confusions in identification, adulteration and availability.

"kathakakhadiradi kashyam" is an ayurvedic medicinal preparation which is used in effective management of diabetes and reducing associated symptoms such as polyuria, fatigue, constipation, dryness of mouth, polydipsia and excessive swetting. It can also be used against diabetic carbuncles and infections. Normal human doses ranges from 10-20 ml twice a day before food. The formulation also claims to have effects on urinary problems and immunity. Scientific data regarding the approaches towards standardisation of kathakakhadiradi kashyam is lacking hence the present study aims in evaluating the primary level parameters of standardisation.

Plant materials

All the plant materials like *Strychnos potatorum, Acacia catechu, Woodfordia fruticose, Salacia reticulate, Curcuma longa, Biophytum Senstivium, Ziziphus jujube, Cyclea Peltata, Mangifera indica, Terminalia chebula and Cyperus rotundus* were collected from the herbal garden and drug store of Ayurveda college, Coimbatore. All the individual drugs were authenticated at Agricultural university, Coimbatore.

Formulation of KKS

As per the references from sahashrayoga the ayurvedic formulation "kathakakhadiradi kashyam" was formulated using following ingredients listed in table 1

Botanical Name	Quantity (g)
Strychnos potatorum	50
Acacia catechu	50
Woodfordia fruticose	50
Salacia reticulate	50
Curcuma longa	50
Biophytum Senstivium	50
Ziziphus jujube	50
Cyclea Peltata	50
Mangifera indica	50
Terminalia chebula	50
Cyperus rotundus	50

50 g of all the listed herbs in table 1 in dried form are collected and are size reduced to form coarse powder by cutter mill. Water (8 times of the powdered drug) is added to the coarse powder. This mixture is stirred for one to two hours. After that this mixture is boiled in a wide mouth beaker with the help of water bath for two to three hours. Keep on stirring the contents with a spatula. Boil the Kashayam till it reduces to one fourth quantity. Filter the Kashayam across a clean cloth immediately. Thus prepared Kashayam is stored in an air tight container and is used for further studies.

The standard marketed kathakakhadiradi kashyam sample was collected from the pharmacy of Ayurveda college, Coimbatore (AVP pharmaceuticals).

Chemicals and Drugs

Major chemicals used in the study like hydrochloric acid, ∞- napthol, Sulphuric acid, Fehling A&B, Benedict reagent, sodium hydroxide, nitric acid, ammonia, lead acetate, ninhydrin, sudan red III reagent, glycerin, picric acid, chloroform, acetic anhydride, ferric chloride, zinc, dragendroff's reagent, Wagner's reagent, Mayer's reagent, sodium chloride and bromin water were collected from the store of RVS College of Pharmaceutical Sciences. All the chemicals used in the study are of analytical grade.

Standardisation of KKS

***I. Organoleptic evaluation*[5]**

Organoleptic evaluation were done by means of sense organs. Here sensory characteristics like colour, odour, taste, texture etc. are analysed and recorded. The evaluations were based on methods described in siddiqui.

II. Physico-chemical analysis

Determination of p^H

Digital p^H meter were standardized with standard p^H solutions. Then the electrodes are cleaned with fine tissue paper and it is then dipped into a beaker containing 10 ml test substance suspended in 100 ml of water. The readings were noted.

***Determination of Total dissolved solids [TDS]*[6]**

10 ml of the sample was evaporated in calibrated Petri dish. The residue is then dried for1 hr in an oven at 180±2°C, Cooled and the dish is weighed.

***Determination of moisture content [Loss on drying]*[7]**

Two ml of the drug were taken in a tarred china dish. Dried in the oven at 100°C or 105°C, and then cooled in a desiccator and watch. After that the loss was recorded as moisture. Continue the drying and weighing at one hour interval until difference between two successive weighing to not more than 0.25 percentage. The percentage difference between the china dish containing the test sample before and after the experiment is calculated as the percentage of moisture content.

***Determination of Total ash*[8]**

Incinerate about 2-4ml of the sample in a tarred crucible (usually of platinum or silica) heated at 450°C for removal of carbon atom (it becomes white), cool and determine the weight of the product. Estimate the percentage of ash with blank air dried crucible.

Determination of acid insoluble ash

It is the amount of ash which is insoluble in dilute hydrochloric acid. The ash obtained in the total ash was boiled with 25 ml of 2N hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash along with the filter paper was transferred into silica crucible. It was ignited and weighed. The percentage of acid insoluble ash value was calculated with reference to air-dried drug.

Determination of water soluble ash

The water soluble ash value is used to detect the presence of material exhausted by water. The ash obtained in the total ash was boiled with 25 ml of water for 5 minutes. The insoluble ash was collected and transferred into silica crucible, ignited for 15 minutes and weighed. The weight of the soluble matter was subtracted from the weight of total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash value is calculated with reference to air-dried drug.

Determination of sulphated ash

Heat the crucible 10 minutes to remove the contamination and allowed to retain the room temperature and weigh this crucible. Add 1 to 2 ml of the formulation, into the crucible, ignite gently at initial one hour and Cool, add the residue with 1 ml of sulphuric acid, and ignite at 800°C for three hours after allowing the crucible to come room temperature, add 1 ml of sulphuric acid and heat. Repeat these above procedure until two successive weighing should not have a difference of 0.5 mg difference.

Extractive values[9]

The percentage of extracts obtained from the drug(s) with the help of various solvents are called extractive values.

Determination of alcohol soluble extractive value

This is the amount of extract obtained from formulation with the help of alcohol as solvent. 5 ml of test sample is macerated with 100 ml of alcohol in a closed flask for 24 hours (shaking frequently during first 6 hours and allowed to stand for 18 hours). Filter the extract, 25 ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish and weighed. The percentage of alcohol soluble extractive value was calculated with reference to air-dried sample.

Determination of water soluble extractive value

This is the amount of extract obtained from formulation with the help of water as solvent. Water is a good solvent for extraction of various active constituents like tannins, sugars, plant acids, mucilage, glycosides, etc. Add 5 ml of the KKS with 100ml of cholroform water in an air tight closed container for one day, shaking repeatedly for 6 hour and keeping motionless for 18 hrs, Filter the solvent after the above process, allowed to evaporate the 25ml of the filtrate at 105°C to form dry product. Calculate the percentage of alcohol-soluble extractive value with reference to air-dried sample.

III. Phytochemical Analysis[10]

Phyto-constituents are the contributors of pharmacological activities of a plant. The Polyherbal formulation as well as individual constituents used in the formulations are subjected to qualitative tests for identification of various plant constituents.

Test for Carbohydrates

I. **Molisch Test:** To the aqueous extract, 1ml of ∝-napthol solution was added and Conc. Sulphuric acid was added along the sides of the test tube. Purple or reddish violet colour at the junction between the two liquids indicates the presence of carbohydrates.

II. **Fehling Test:** To the aqueous extract, equal quantities of Fehling A&B were added .Upon heating gently, a brick red precipitate indicates the presence of carbohydrates.

III. **Benedict's test:** To 5ml of Benedict reagent, 8 drops of solution under test was added to the aqueous extract and mixed well. Then it was boiled vigorously for 2 minutes and cooled. Red precipitate indicates the presence of carbohydrates.

Test for Proteins

I. **Biuret Test:** To the aqueous extract, 1ml of 40%NaOH and 2drops of 1% copper sulphate solution was added. A violet colour indicates the presence of proteins.

II. **Xanthophoretic Test:** To the aqueous extract, 1ml of conc. Nitric acid was added. When a white precipitate was formed, it is boiled and cooled. Then 20% of NaOH or ammonia was added. Orange colour indicates the presence of aromatic acids.

III. **Lead acetate Test:** To the aqueous extract, 1ml of lead acetate solution was added. A white precipitate indicates the presence of proteins

Test for Amino acids

I. **Ninhydrin Test:** 2 drops of freshly prepared 0.2% ninhydrin reagent was added to the aqueous extract and heated. Development of blue colour indicates the presence of proteins, peptides or amino acids.

Test for Steroids

I. **Liebermann Burchard Test:** The aqueous extract was dissolved in 2ml chloroform in dry test tube. 10 drops of acetic anhydride and 2 drops of conc. sulphuric acid were added. The solution becomes red and then blue and finally bluish green in colour indicates the presence of steroids.

II. **Salkowaski Test:** The aqueous extract was dissolved in chloroform and equal volume of sulphuric acid was added to it. Bluish red to cherry red colour was observed in chloroform layer, whereas acid layer assumes marked green fluorescence indicates the presence of steroids.

Test for Cardiac glycosides

I. **Keller-killiani Test:** Test sample was dissolved in acetic acid containing traces of ferric chloride and transferred to the surface of conc. Sulphuric acid. At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presence of cardiac glycosides.

Test for Saponins

I. **Foam Test:** About 1ml of aqueous extract is diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins.

Test for Alkaloids

I. **Dragendroff's Test:** To the aqueous extract, add 1ml of Dragendroff's reagent. An orange red coloured precipitate indicates the presence of alkaloids.

II. **Wagner's Test:** To the aqueous extract, add 1ml of Wagner's reagent. Reddish brown coloured precipitate indicates the presence of alkaloids.

III. **Mayer's Test:** To the aqueous extract, add 1ml of Mayer's reagent. A dull white coloured precipitate indicates the presence of alkaloids.

Test for Phenolic compounds and Tannins

I. Small quantities of alcoholic and aqueous extracts in water were tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%), 1% solution of gelatin containing 10% sodium chloride, 10% lead acetate and bromine solutions. The respective observations may be deep blue black colour, white precipitate, white precipitate, decolouration of bromine water showing the presence of tannins and phenolic compounds.

Here in this study we compare all the standardisation parameters of marketed formuation of KKS with in lab preparation.

RESULTS AND DISCUSSION

The Ayurvedic formulation Kathakakhadiradhi kashayam were subjected to various standardization methods and the results obtained by all the test procedures reveal the genuineity of the formulation.

In all the evaluation we could observe comparable results to that of standard marketed formulation.

Table 2: Observed Oranoleptic evaluation reports of standard marketed as well as in lab Kathakakhadiradhi kashayam formulation

S. No	Parameter	Kathaka Khadiradhi Kashayam	
		In house preparation	Marketed sample
1	Colour	Brownish black	Brownish black
2	Odour	Agreeable odour	Agreeable odour
3	Taste	slightly Bitter-sour-astringent	slightly Bitter-sour-astringent
4	Description	fairly clear mobile liquid, slight sediment on storage	Mobile liquid slight sediment on storage

Table 3: Observed Physicochemical evaluation reports of standard marketed as well as in lab Kathakakhadiradhi kashayam formulation

S. No	Parameter	Kathaka Khadiradhi Kashayam	
		In house preparation	Marketed sample
1	Loss on drying	5.64%w/v	6.74%w/v
2	p^H	5.45	5.35
3	Total dissolved solids	12.54g/L	10.74g/L
4	Total ash	5.8%w/v	6.64%w/v
5	Acid insoluble ash	1.56%w/v	1.26%w/v
6	Water soluble ash	2.52%w/v	2.6%w/v
7	Sulphated ash	4.34%w/v	4.46%w/v
8	Alcohol soluble Extractive	5.90%w/v	6.55%w/v
9	Water soluble extractive	6.56%w/v	6.74%w/v

Table 4: Observed Phytochemical screening reports of standard marketed as well as in lab Kathakakhadiradhi kashayam formulation

S. No	Test	Kathaka Khadiradhi Kashayam	
		In house preparation	Marketed sample
1	Alkaloids	-	-
2	Carbohydrates	+	+
3	Proteins	+	+
4	Amino acids	+	+
5	Flavonoids	+	+
6	Steroids	-	-
7	Cardiac glycosides	-	-
8	Saponins	+	+
9	Phenol and Tannins	+	+

Standardization of drugs means confirmation of its identity and quality determination and purity. It is an important step for the establishment of a consistent biological activity and chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs. However, it is an important aspect for maintaining and assessing the quality and safety of polyherbal formulations since these are combinations of more than one herb to attain the desire therapeutic effect. In the standardization of kathaka khadiradi kashayam we consider factors like organoleptic characters, Physico-chemical properties (Ash values,

extractive values, loss on drying, P^H) and phytochemical analysis.

Organoleptic evaluation provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. Here simple sensory characters like colour, odour and taste were analysed. Considering the Physicochemical properties we go for analysing the ash values, extractive values, loss on drying, P^H mainly. Ash value represents percentage of the residue remaining after incineration is the ash contents of the drug which simply represent inorganic salts, naturally occurring in the drug, adhering to it, or deliberately added to it in the form of adulterant. Extractive values is indicative of approximate measure of phytoconstituents and loss on drying represents the amount of moisture contents.

Plants are the treasure houses of structurally diverse bioactive molecules. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Phytochemical composition shows the physiological and the therapeutic character of the plant. Preliminary qualitative phytochemical analysis shows the presence of carbohydrates, proteins, amino acids, flavonoids, saponins, phenols and tannins. These constituents may be possibly responsible for the biological activity of polyherbal formulation.

This is a period of herbal renaissance all around the globe. The present era, standardisation of herbal drugs and products has become essential and vital for several reasons. According to a survey (1993) of World Health Organization (WHO), the practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh.[11] Standardization is an important measure for knowing the quality, purity and for sample identification. Standardisation aims in giving an identity to a herbal drugs, drug products or formulations. This is a preliminary level study and the reports will be useful in performing pharmacological, toxicological and therapeutic screenings in future.

CONCLUSION

The present work was an effort to standardize the polyherbal formulation KKS in accordance to WHO norms and standard laboratory procedures. Formulation was investigated for their organoleptic characters, physicochemical parameters and phytochemical parameters. All these parameters are compared with standard marketed as well as in freshly prepared sample and they were found to be analogous. The investigational outcomes of the standardization can be used for evaluating the quality and purity of the formulation future.

REFERENCES

1. Sandhya Wakdikar, Global health care challenge: Indian experiences and new prescriptions, Electronic Journal of Biotechnology, 7, 2004, 214-220.
2. Manisha Modak, Priyanjali Dixit, Jayant Londhe, Saroj Ghaskadbi, and Thomas Paul A. Devasagayam, Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes, Journal of Clinical Biochemistry and Nutrition. Vol.40, 2007, 163–173.
3. M. Umadevi, K.P Sampath Kumar, Debjit Bhowmik, S. Duraivel, Traditionally Used Anticancer Herbs In India. Journal of Medicinal Plants Studies, Vol.1, 2013, 56-74.
4. Thillaivanan S, Samraj K, Challenges, Constraints and Opportunities in Herbal Medicines – A Review, International Journal of Herbal Medicine, Vol.2, 2014, 21-24.
5. Siddiqui, Hakim MA, Format for the Pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, (appendix) New Delhi: Central Council for Research in Unani Medicine (CCRUM); 1995.
6. A Radha, Jessy Sebastian, Manjima Prabhakaran and D. Suresh Kumar, Observations on the Quality of commercially manufactured Ayurvedic Decoction, Maharasnadi Kvatha. Hygeia Journal of Drugs and Medicine, Vol.6, 2014, 74-80.
7. James Chacko, Sivabalaji, Ashwini, Preliminary Analytical Study of Rasnasapthakam Kashayam - An Ayurvedic Polyherbal Formulation, International Journal of Medicinal Plants and Natural Products, Vol.1, 2015, 4-7.
8. Rajalakshmy MR, Sindhu A, Geetha G, Phytochemical investigation on withania somnifera root marc generated in ayurvedic industry, International Research Journal of Pharmacy, Vol.4, 2013, 223-227.
9. The Ayurvedic Pharmacopoeia of India. Part 2-formulations, Vol.2, 1st edition. Appendices 1 to 5, New Delhi, 2008, 160-169.
10. Chitra R. Kamath, Bhavini Shah, Phytochemical Screening and Standardization of Polyherbal Formulation: Maharishi Amrit Kalash 5, International Journal of Pharmacy and Pharmaceutical Sciences, Vol.6, 2014, 96-98.
11. Sitansu Kumar Verma and Ajay Kumar, Therapeutic Uses of Withania Somnifera (Ashwagandha) with a note on Withanolides and its Pharmacological Actions, Asian Journal of Pharmaceutical and Clinical Research, Vol.4, 2011, 1-4.

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