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STANDARDIZATION AND PHYTOCHEMICAL EVALUATION OF SIDDHA HERBAL FORMULATION ATHIMADHURA CHOORANAM AS PER INDIAN REGULATORY STANDARDS

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ABSTRACT

Siddha medicine pioneers the traditional practice since several centuries as it was practiced by ancient siddha physicians called siddhars. Siddha also holds a credential of having well optimized procedures and guidelines in preparing wide range of formulations such as mono herbal, poly herbal, herbomineral etc. Siddha formulations are devoid of major side effects as imposed in modern medicine which attracts the wide range of people towards this holistic practice. In recent times the public attraction towards the traditional health care system has been constantly rising due to its potential therapeutic benefits. But in order to gain global access siddha formulation should ascertain the quality and standards as per the regulatory guidelines. Hence the main aim of the present investigation is to systematically standardize the formulation Athimadhura chooranam (AC) as per PLIM guideline for siddha medicines and to explore the possible therapeutic phytocomponents present in the formulation that can able to render the maximum biological benefits upon clinical utilization. The results obtained from the physicochemical evaluation reveals that total ash value of AC was found to 8.66%. In which the acid insoluble ash was 0.36 %. Similarly, loss on drying value at 105oC was found to be 3.6 % respectively. The results of water soluble extractive of AC were 23.27% and similarly the alcohol soluble extractive value of AC were found to be 26.77 % w/w. The result of the phytochemical analysis indicates that the formulation AC shows the presence of alkaloids, steroids, triterpenoids, coumarin, phenols, tannins, saponins and sugars. From the data's obtained from the present investigation it was concluded that the siddha formulation Athimadhura chooranam confirms the regulatory standards.

KEY WORDS: ***Siddha medicine, Athimadhura Chooranam, Regulatory standards, Physicochemical, Phytochemical***

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1. Introduction

Standardization requires a lead/natural plant product to be authenticated at origin itself by adoption of good agricultural practices [1] collection strategies from wild and good manufacturing practices for extraction modes and related parameters [2-5]. Natural products, especially those derived from plants, have been used to help mankind sustain human health since the dawn of medicine. Traditional medicine has been in existence since time immemorial and has been well accepted and utilized by the people throughout history. Since ancient times, plants have been an exemplary source of medicines. Plant-derived medicinal products have attracted the attention of scientists around the world for many years due to their minimum side effects and positive effects on human health.

World Health Organization (WHO) has defined herbal medicines as finished labeled medicinal product that contain an active ingredient, aerial, or underground parts of the plant or other plant material or combinations. According to a report of WHO, about 80% of the world population is reported to rely on traditional medicine for their primary health care needs. Even in the developed countries, complementary or alternative medicine is gaining popularity. A report of a global survey on national policy on traditional medicine and regulation of herbal medicines indicated that about 50 countries including China, Japan, and Germany already have their national policy and laws on regulations of traditional medicines [6].

Herbal drugs possess a long history of its use and better patient tolerance. These are cheaper and easily available in countries like India due to rich agro culture conditions. However, reckless utilization of resources threatens the sustainability of several plant species. Traditional medicines are governed by the Drugs and Cosmetics Act of 1940 and the Drugs and Cosmetics Rules of 1945. In 1959, the Government of India amended the Drugs and Cosmetics Act to include drugs that are derived from traditional Indian medicine [7].

Plants are considered not only as dietary supplement to living organisms but also traditionally used for treating many health problems and the medicinal value of many plants still remains unexplored investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents [8]. Over 60% of the world human population, 80% in developing countries depends directly on plants for their medicinal purposes [9]. The main aim of the present investigation is to systematically standardize the formulation Athimadhura chooranam (AC) as per PLIM (protocol for testing of Indian medicine) guideline for siddha medicines and to explore the possible therapeutic phytocomponents present in the formulation that can able to render the maximum biological benefits upon clinical utilization.

2.Materials and Methods

2.1. Ingredients

The major ingredient present in the formulation Athimadhura chooranam is Glycyrrhiza glabra

2.2. Source and authentication of raw drug

Raw drugs were bought from Indigenous authentic country drug shop at Chennai, Tamil Nadu, India. Herb were identified and authenticated by the Botanist of Central Council for Research in Siddha, Tamil Nadu, India.

2.3. Method of preparation

The above mentioned raw drug were purified as per the standard operating procedure and powdered to make final formulation.

Figure 1: Siddha formulation Athimadhura chooranam

2.4. Physicochemical Evaluation [10,11]

Organoleptic evaluation of the drug was made with respect to state, appearance, nature and odor etc.

2.4.1.Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105oC for 5 hours and then weighed.

2.4.2.Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

2.4.3.Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

2.4.4.Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

2.4.5.Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

2.5.

2.5. Preliminary Phytochemical Investigation [12]

Test for alkaloids

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarin

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

Aanthocyanin

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100ºC. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

3.Results

3.1. Results of Physicochemical Analysis of AC

Organoleptic evaluation of the formulation AC indicates Solid crude material with pale brownish color and also possess mild characteristic odor which confirms the genunity of the drug. The results obtained from the physicochemical evaluation reveals that total ash value of AC was found to 8.66%. In which the acid

insoluble ash was 0.36 %. Similarly, loss on drying value at 105oC was found to be 3.6 % respectively. The results of water soluble extractive of AC were 23.27% and similarly the alcohol soluble extractive value of AC were found to be 26.77 % w/w. As shown Table 1.

Table 1: physicochemical evaluation of *Athimadhura Chooranam*

S.No	Parameter	Mean (n=3) SD
1.	***Loss on Drying at 105 °C (%)***	3.6 ± 0.2
2.	***Total Ash (%)***	8.667 ± 0.4163
3.	***Acid insoluble Ash (%)***	0.36 ± 0.06
5.	***Alcohol Soluble Extractive (%)***	26.77 ± 2.554
6.	***Water soluble Extractive (%)***	23.27 ± 3.595

3.2. Qualitative Phytochemical evaluation of AC

The result of the qualitative phytochemical analysis indicates that the formulation AC shows the presence of biologically significant phytochemicals such as alkaloids, steroids, triterpenoids, coumarin, phenols, tannins, saponins and sugars. The results were tabulated in Table 02 and shown in figure 2.

.**Table 2: Preliminary phytochemical analysis of Athimadhura chooranam**

S.NO	TEST	OBSERVATION
1	ALKALOIDS	+
2	FLAVANOIDS	-
3	GLYCOSIDES	-
4	STEROIDS	+
5	TRITERPENOIDS	+
6	COUMARIN	+
7	PHENOL	+
8	TANIN	+
9	PROTEIN	-
10	SAPONINS	+
11	SUGAR	+
12	ANTHOCYANIN	-
13	BETACYANIN	-

+ -> Indicates Positive and - -> Indicates Negative

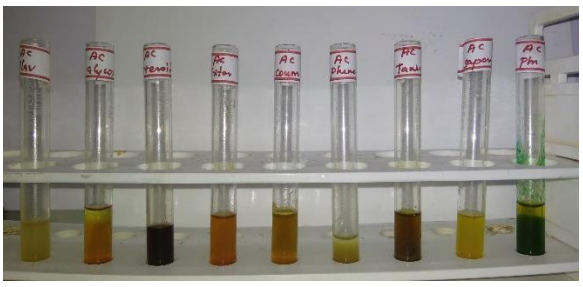

Figure 2: Preliminary phytochemical observation of Athimadhura Chooranam

4.Discussion

Herbal therapy is a holistic therapy, integrating emotional, mental and spiritual levels. Life style, emotional, mental and spiritual considerations are part of any naturopathic approach. The use of herbs does not generally involve "drug" actions or adverse effects. Of course, informed knowledge of the effects of medicinal plants as well as doing a clinical trial to understand the appropriate medical application is necessary. It has been suggested that we use the terms indications and contraindications for using a herb instead of "side effects" [13-17]. Plant phytocomponents have a proven track record of becoming an ailment for several infective and degenerative disorders. Traditional herbal supplement's believed to have possess high therapeutic efficacy with low or no side effects [18].

The regulatory approvals to ascertain consistent chemical profile and biological activity of future drug candidate [19] includes a) quality assurance by determining adulterants, pesticides residue, aflatoxin content, bacterial/fungal growth and heavy metals contamination etc. [20]; b)prevention of adverse reactions by evaluating pharmacodynamics, pharmacokinetics, dosage, stability, self-life and toxicity (acute/ chronic) etc. [21]; c) reproducibility by repetitive testing using different batches to control batch-to-batch variation and development of standard assay markers [22] and; d) chemiinformatic approaches to ensure that pharmacological profiles matches with the activity profiles of active constituents of drug itself.

The major demand for the standardization relies more on ensuring the quality and genunity of the drugs and finished formulations. Regulation of siddha formulation complies with the method of purification of the raw materials, detoxification procedure as listed in the vedic literatures and further in recent times myth on contaminant in the siddha formulation gaining more attention. Hence PLIM guideline enforces the quality on profiling each siddha formulation which should be free from heavy metals, pesticide, toxins and other infective pathogens like E-coli, staphylococcus etc. Organoleptic evaluation of the formulation AC indicates Solid crude material with pale brownish color and also possess mild characteristic odor which confirms the genunity of the drug. The results obtained

from the physicochemical evaluation reveals that total ash value of AC was found to 8.66%. In which the acid insoluble ash was 0.36 %. Similarly, loss on drying value at 105oC was found to be 3.6 % respectively. The results of water soluble extractive of AC were 23.27% and similarly the alcohol soluble extractive value of AC were found to be 26.77 % w/w.

Herbal medicines, containing active ingredients in complex chemical mixtures developed as crude fractions, extracted from aerial or underground parts of plant or other plant material or combination thereof, are widely used in health-care or as dietary supplements. One of the major drawbacks of these medicines is limited bioavailability, being poorly absorbed if taken orally [23]. According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for fulfilling their primary health care needs [24]. It is essential to understand that its effectiveness may vary and it might interact with other drugs leading to contraindications. Safety considerations regarding toxicological analysis, pre-clinical and clinical trials are essential prior to adoption of any herbal medicine. At present, herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens [25].

Identification and validation of the phytocomponents are the primary responsibility of the researcher to elaborate the predicted mechanism of the study drug. Hence standardization specifically helps to quantify the individual components present in the trial drug along with the structural and functional moieties responsible for the expected pharmacological action. The result of the qualitative phytochemical analysis indicates that the formulation AC shows the presence of biologically significant phytochemicals such as alkaloids, steroids, triterpenoids, coumarin, phenols, tannins, saponins and sugars.

5.Conclusion

India, with pluralistic health care system, having extensive expertise in modern medicine, Indian systems of medicine, and life & pharmaceutical sciences with an approach towards observational therapeutics is an antecedent path towards reverse pharmacology in natural drug development as being proposed. It was concluded from the result of the present investigation that the siddha formulation Athimadhura Chooranam shows the presence of wide range of phytocomponents and also confirms the prescribed regulatory standards for Indian medicine

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