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Review article

Standardization of siddha poly herbal formulation ''Karisalankanni Chooranam''

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ABSTRACT

Karisalankanni Chooranam is a poly herbal formulation mentioned in the Siddha text, which is indicated for Jaundice, Anemia and dropsy. All the ingredients were purified as per the Siddha literature, after which the formulation was prepared. The prepared drug was subjected to analysis for standardization. For the standardization of this drug, Organoleptic Properties, Phytochemical Screening, Fluorescence Analysis, Heavy Metal Analysis, Physic Chemical Parameters Such As Moisture Content, Ash Values, Extractability in Water and ethanol were carried out. TLC and HPTLC fingerprints of Karisalankanni chooranam were also prepared to evaluate authencity of Karisalankanni chooranam and can be used as reference standards for the preparation of a standardization pharmaceutical product and further quality control researches.

Keywords:

Standardization, Poly herbal formulation, Siddha medicine, *Karisalankanni chooranam*.

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INTRODUCTION

Siddha is the oldest healing system of medicine and it has fundamental aspects for drug formulation. Major formulations used in Siddha are based on herbs. The medicinal herbs are used as decoctions, infusions and powder, etc. There is a global resurgence in the use of these medicines along with a growing scientific interest in them as a source of new drugs. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing up to technical standards, specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular medicines. Hence standardization is a tool in the quality control process. Thus the present study deals with standardization of Siddha poly herbal formulation *Karisalankanni Chooranam,* which is mentioned in the Siddha literature for the treatment of Jaundice, Anemia and Dropsy.

MATERIALS AND METHODS

SOURCE OF RAW DRUGS:

The raw drugs are purchased from a well reputed country shop .The raw drugs were authenticated by the Head of the department of Medicinal Botany, at Govt. Siddha medical college, Chennai. The raw drugs were purified and the medicine is prepared in Gunapadam laboratory of GSMC, Chennai. The prepared medicine is again authenticated by the Head of the department of Gunapadam.

PURIFICATION OF THE RAW DRUGS:

All the herbal drugs were purified as mentioned in the text Chikitcha rathna deepam vaithiya nool(2).

INGREDIENTS:

S.No	NAME	BOTANICAL NAME	PARTS USED	QUANTITY
1	*Karisalankanni*	*Eclipta prostrata*	Dried whole plant	4 thola
2	*Mookirattai*	*Boerhaevia diffusa*	Dried whole plant	1 thola
3	*Chukku*	*Zingiber officinale*	Dried rhizome	1 thola
4	*Milagu*	*Piper nigrum*	Dried seed	1 thola
5	*Thippili*	*Piper longum*	Dried fruit	1 thola
6	*Kadukkaai*	*Terminalia chebula*	Dried fruit coat	1 thola
7	*Nellikkaai*	*Phyllanthus emblica*	Dried fruit	1 thola
8	*Thandrikkaai*	*Terminalia bellerica*	Dried fruit coat	1 thola
9	*Maramanjal*	*Ciscinium fenestratum*	Dried wood	1 thola
10	*Thaniya*	*Coriandrum sativum*	Dried fruit	1 thola
11	*Athimathuram*	*Glycyrrhiza glabra*	Dried root	1 thola
12	*Karunseeragam*	*Nigella sativa*	Dried seed	1 thola
13	*Thalisapathiri*	*Abies spectabilis*	Dried leaves	1 thola
14	*Elam*	*Elettaria cardamomum*	Dried seed	1 thola
15	*Seeragam*	*Cuminum cyminum*	Dried seed	1 thola

PHYSICO CHEMICAL ANALYSIS

State	Solid
Appearance	Brownish
Nature	Fine powder
Odour	Strongly Aromatic
Flow property	Non- Free flowing

Organoleptic Evaluation

Solubility Profile of Karisalankanni Chooranam

Percentage Loss on Drying

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

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.

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.

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 tarred 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.

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 tarred 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.

PHYTOCHEMICAL ANALYSIS

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 coumarins:

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 hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydro lysate 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% ammonia solution is added to it. Pink color 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

A. Aanthocyanin: To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 1000 C. 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 Benedict'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

HEAVY METAL ANALYSIS BY AAS

Standard: Hg, As, Pb and Cd – Sigma

Methodology: Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item. Sample Digestion Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly for the determination of lead and cadmium the sample were digested with 1mol/L of HNO_3. Standard reparation As & Hg- 100 ppm sample in 1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO_3.

TLC AND HPTLC ANALYSIS:

TLC ANALYSIS

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system after the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm

High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High

performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantitation of Nano grams level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials.

Chromatogram Development:

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning: Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

AFLOTOXIN ASSAY

Solvent

Standard samples was dissolved in a mixture of chloroform and acetonitrile (9.8 : 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Test solution: Concentration 1 µg per ml

Procedure:

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of2.5 µL, 5µL, 7.5 µL and 10 µL. Similarly the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85 : 10 : 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

RESULTS

SOLUBILITY

S.No.	Solvent Used	Solubility/Dispersibility
1	Chloroform	Soluble
2	Ethanol	Soluble
3	Water	Soluble
4	Hexane	Insoluble
5	Ethyl acetate	Soluble
6	DMSO	Partially Soluble

Table 1: Results of Solubility Profile of Karisalankanni Chooranam

PHYSICO – CHEMICAL ANALYSIS

S.No	Parameter	Mean (N=3) SD
1.	Loss on Drying at 105 °C (%)	1.433 ± 0.30
2.	Total Ash (%)	1.6 ± 0.7
3.	Acid insoluble Ash (%)	1.26 ± 0.034

4.	Alcohol Soluble Extractive (%)	12 ± 1.55
5.	Water soluble Extractive (%)	26± 3.606

Table 2: Results of physico-chemical analysis of karisalankanni chooranam

PHYTOCHEMICAL ANALYSIS

S.NO	TEST	OBSERVATION
1.	Alkaloids	+
2.	Flavanoids	+
3.	Glycosides	-
4.	Steroids	+
5.	Triterpenoids	+
6.	Coumarin	+
7.	Phenol	+
8.	Tannin	+
9.	Protein	-
10.	Saponins	+
11.	Sugar	+
12.	Anthocyanin	-
13.	Betacyanin	+

Table 3: Results of phyto-chemical analysis of karisalankanni chooranam

HEAVY METAL ANALYSIS

Name of Heavy Metals	Absorption Max Λ Max	Result Analysis	Maximum Limit
Mercury	253.7 nm	0.3	1 ppm
Lead	217.0 nm	0.5	10 ppm
Arsenic	193.7 nm	0.25	3 ppm
Cadmium	228.8 nm	0.22	0.3ppm

Table 4: Results of Heavy metal analysis of karisalankanni chooranam

TLC AND HPTLC ANALYSIS

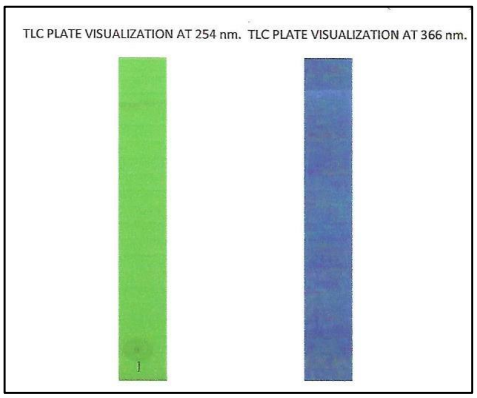

Figure 1: HPTLC finger printing of sample Karisalankanni Chooranam

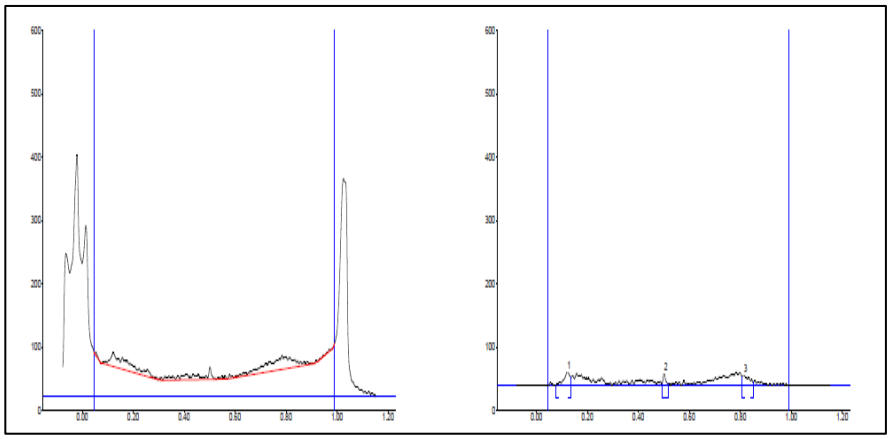

Figure 2: HPTLC finger printing of sample Karisalankanni Chooranam

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.08	0.5	0.12	21.9	37.64	0.15	12.5	378.9	44.89
2	0.49	2.8	0.5	19.6	33.56	0.52	1.2	136.6	16.18
3	0.81	13.2	0.82	16.8	28.8	0.85	3.8	328.5	38.92

Table 5: Peak table of HPTLC finger printing

HPTLC finger printing analysis of the sample reveals the presence of three prominent peaks corresponds to presence of three versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.08 to 0.81. Further the peak 1 and 3 occupies the major percentage of area of 44.89 and 38.92% which denotes the abundant existence of such compound.

AFLO TOXIN ASSAY

Aflotoxin	Sample KC	AYUSH Specification Limit
B1	Not detected -Absent	0.5ppm
B2	Not detected -Absent	0.1ppm
G1	Not detected -Absent	0.5ppm
G2	Not detected -Absent	0.1ppm

Table 6: Results of aflotoxin assay

The results shown that there was no spots were been identified in the test sample loaded on TLC plates when compare to the standard, which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

CONCLUSION

Qualitative analysis of the ***Karisalankanni chooranam*** presence of iron, calcium, starch, chloride and Reducing sugar. Phytochemical analysis of the trial drug shows that presence of Glycosides, carbohydrates, coumarins, phenol. Physico chemical analysis of the trail drug shows the pH 5, Total ash value 17.6% shows the safe and effectiveness of the trial drug. In physico chemical Analysis iron was found to be present as effective ingredients in treating anaemia. TLC and HPTLC fingerprints of *Karisalankanni chooranam* were also showed authencity of the trial drug.

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