Research Article

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STANDARDIZATION OF A SIDDHA HERBOMINERAL FORMULATION KUMARA VEERIYA KANTHA CHENDURAM

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ABSTRACT

The Siddha system of medicine is a traditional healing system, one of India's oldest holistic systems. This system comprises a large source of drugs of four types, namely herbals, metals, minerals and animals. Lack of standardization and validation is a lacuna in these herbo mineral products which causes concern about their safety and effectiveness. There has been substantial work in recent years into traditional medicines based on these aspects of validity and standardization. The aim of the study is to standardize the Siddha herbomineral formulation Kumara veeriya kantha chenduram. Kumara veeriya kantha chenduram is one the herbomineral formulation mentioned in Anuboga Vaidya Navaneetham part 1. This medicine has been standardized on the basis of organoleptic characters, biochemical analysis, preliminary phytochemical analysis, physico-chemical properties, TLC and HPTLC. A primary phytochemical and biochemical analysis conducted on Kumara veeriya Kantha chenduram revealed that the presence of bio active molecules like Iron, Zinc, Calcium, Ammonium, Starch, Alkaloids, Tannic acids, Phenols, Tannins, Saponins were detected. HPTLC finger printing analysis revealed the presence of two prominent peaks corresponds to presence of five versatile phytocomponents present within it. This research article will report safety and efficacy data for the herbomineral formulation Kumara veeriya kantha chenduram through standardization.

Keywords: Iron deficiency anemia, Standardization, Siddha drug, Kumara veeriya kantha chenduram

INTRODUCTION

Drug Standardization is an essential factor for herbal formulation in order to assess the quality of the drugs based on the concentration of their active principle and to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic action.[1] In Indian System of Medicines (ISM) majority of the remedies are based on plants and plant products. The plant materials generally work on human systems to improve the immunity, resistance and there by cure the disease.[2] Siddhars has recognized many herbals and minerals like karisalai, ponnankanni, aththi, nellikkai, maathulai, iyam, kaantham, annabedhi, manduram etc., which can be used effectively to treat anemia. In that Theriyar mentions, “Irumbinum kaantham maenmai” which means Kaantham (Magnetic oxide of iron) is better than Iyam (Iron). One of such typical kaantham formulation is ‘Kumaara veeriya kaantha chenduram’, mentioned in Anuboga vaidhya navaneetham part 1[3] According to its name, Kumara (Young), veeriya (Potency) it can be correlated that it has rejuvenating power to keep humans younger and gives longevity. Kumara veeriya kantha chenduram includes two ingredients i.e. kantham (Magnetic Oxide of iron) and thara leaves (*Mollugo oppositifolia* Linn.) For ensuring its quality and efficacy standardization is essential. In this study the parameters for quality assessment have been followed as per CCRAS guidelines for analytical specification of chenduram.[4]

MATERIALS AND METHODS

Standard operating procedure for Kumara veeriya kantha chenduram.

Ingredients

- Purified Kantham (Magnetic Oxide of iron)
- Thara leaves (*Mollugo oppositifolia* Linn.)

Source of raw drugs and authentication

The required raw drugs for preparation of Kumara veeriya kantha chenduram were purchased from a well reputed country shop at Chennai. The raw drugs were authenticated by the Botanist, National Institute of Siddha and Associate Professor, Department of Gunapadam, National Institute of Siddha, Chennai. (Voucher No NISMB4002019) The raw drugs were purified, and medicine was prepared at Gunapadam lab of National Institute of siddha.

Method of purification

Kantham is grounded into powdered form then tied in a piece of cloth and boiled with vinegar and horse gram decoction and then washed and dried well [5].

Method of preparation

Step 1

Purified Kantham (280 g) was grounded with sufficient quantity of Thara leaf juice (Approximately 1.5 L) for 4 saamam (12 hours) then made into small pellets and dried.

Step 2

All the small dried pellets were put into agal and surrounded by seelai man then processed to kaja pudam (1000 varatti).

Step 3

Next day removed the agal and Kumara veeriya kantha chenduram was collected and stored in airtight container.[3]

Analytical study

Organoleptic characters, preliminary phytochemical screening, physicochemical evaluation, TLC and HPTLC test were done by following the standard procedure in Noble Research solutions, Chennai and the biochemical analysis were done in National institute of Siddha, Chennai.

Biochemical analysis of trial drug

Test for silicate

A little sample was shaken well with distilled water and then with con.HCL/con.H_2SO_4, insoluble indicated the absence of silicates.

Test for Carbonate

A small amount of the sample was taken in a dry test tube and heated gently at first and then strongly, formation of white fumes indicated the presence of carbonate.

Test for Sulphate

2 ml of the extract was taken in a test tube add 2 ml of 4% Ammonium Oxalate solution, formation of cloudy appearance indicated the presence of sulphate.

Test for Chloride

2 ml of the extract was treated with 2 ml of dilute HNO_3, until the effervescence ceases off. Then 2 ml of silver nitrate was added, no formation of cloudy appearance indicated the absence of chloride.

Test for carbonate

2 ml extract was treated with 2 ml of Magnesium Sulphate solution. Formation of cloudy appearance indicated the presence of carbonate.

Test for Nitrate

3 drops of the extract was placed on a filter paper, on those 2 drops of acetic acid and 2 drops of Benzidine solution was placed. No characteristic change was observed, indicating the absence of nitrate.

Test for Sulphide

1 gm of the extract was treated with 2 ml of conc. HCL; no rotten egg smelling gas indicated the absence of sulphide.

Test for Lead

2 ml of the extract was added with 2 ml of Potassium Iodide solution. No yellow precipitate was formed, indicating the absence of Lead.

Test for Copper

2 ml of extract was added with excess of ammonia solution. No blue precipitate was formed, indicating the absence of Copper.

Test for Aluminium

To the 2 ml of the extract, Sodium Hydroxide was added in drops to excess. No characteristic change was observed, indicating the absence of Aluminium.

Test for Iron

2 ml extract was treated with 2 ml of Ammonium Thiocynate solution and 2 ml of Conc. HNO_3 was added. The formation of blood red colour indicated the presence of Iron.

Test for Zinc

To the 2 ml of the extract Sodium Hydroxide solution was added in drops to excess. White precipitate was formed, indicating the presence of Zinc.

Test for Magnesium

To the 2 ml of the extract Sodium Hydroxide solution was added in drops to excess. No white precipitate was formed, indicating the absence of Magnesium.

Test for Calcium

2 ml of extract was taken in a clean test tube and 2 ml of 4% Ammonium Oxalate solution was added. White precipitate was formed, indicating the presence of Calcium.

Test for Ammonium

To 2 ml of extract 2 ml of Nessler's reagent and excess of NaOH solution were added. The appearance of Brown colour indicated the presence of Ammonium.

Test for Potassium

A pinch of substance was treated with 2 ml of sodium nitrate solution and then treated with 2 ml of cobalt nitrate in 30% glacial acetic acid. No yellowish precipitate was formed, indicating the absence of Potassium.

Test for Sodium

2 pinches of the substance were made into paste by using HCL and introduced into the blue flame of Bunsen burner. No yellow colour flame was formed, indicating the absence of Sodium.

Test for Mercury

2 ml extract was treated with 2 ml of NaOH solution. No yellowish precipitate was formed, indicating the absence of Mercury.

Test for Arsenic

2 ml of the extract was treated with 2 ml of Sodium Hydroxide solution. No brownish red precipitate was formed, indicating the absence of Arsenic.

Test for Starch

2 ml extract is treated with weak Iodine solution. The formation of blue colour indicated the presence of starch.

Test for reducing sugar

5 ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and 8-10 drops of the extract was added and again boil it for 2 minutes. The colour change is noted. No brick red colour was developed, indicating the absence of reducing sugar.

Test for Alkaloids

2 ml extract was treated with 2 ml of Potassium Iodide solution and 2 ml Picric acid was added. Yellow colour was developed indicating the presence of Alkaloids.

Test for Tannic acid

2 ml of the extract was treated with 2 ml of Ferric Chloride solution. Black precipitate was formed indicating the presence of Tannic acid.

Test for Amino acid

2 drops of the extract was placed on a filter paper and dried well. No violet colour was developed, indicating the absence of Amino acid.[6]

Preliminary phytochemical tests

Test for alkaloids (Mayer's Test)

To the test sample, 2 ml 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 was 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 green wash black color showed the presence of tannins.

Test for glycosides (Bontrager's Test)

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

Test for flavonoids

To the test sample about 5 ml of dilute ammonia solution where 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, 2 ml of chloroform was added with few drops of conc. Sulphuric acid (3 ml) 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.

Test for 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 (Anthocyanin)

To the test sample, 1 ml of 2 N sodium hydroxide was added and heated for 5 min at 100°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 was 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 color indicates the presence of proteins.[7]

Physicochemical evaluation

Solubility of the Kumara veeriya kantha chenduram

S. No.	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	Hexane	Insoluble
6	DMSO	Soluble[8,9]

Determination of 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 was boiled with 25 ml of dilute hydrochloric acid for 6 min. Then the insoluble matter was collected in crucible and washed with hot water and ignited to constant weight. Percentage of acid insoluble ash was 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 for 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.

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 for 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.

TLC and HPTLC profile

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7 X 6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was 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 254 nm and light long-wave UV light 365 nm.[10,11]

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. 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 Phyto therapeutics.

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 366 nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic fingerprint was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

RESULT

Organoleptic characters

The organoleptic characters of Kumara veeriya kantha chenduram are tabulated as Table 1.

Biochemical analysis

The biochemical analysis result of Kumara veeriya kantha chenduram is tabulated as Table 2.

Preliminary phytochemical analysis

Preliminary phytochemical analysis results of Kumara veeriya kantha chenduram are tabulated as Table 3.

Physicochemical evaluation

Physicochemical evaluation results of Kumara veeriya kantha chenduram are tabulated as Table 4.

TLC and HPTLC

HPTLC finger printing analysis of the Kumara veeriya kantha chenduram sample reveals the presence of two prominent peaks corresponds to presence of five versatile phytocomponents present with in it. Rf value of the peak's ranges from 0.02 to 0.58. Peak Table of Kumara veeriya kantha chenduram are tabulated as Table 5.

Table 1: Organoleptic characters of Kumara veeriya kantha chenduram

S. No.	Organoleptic Characters	Observation
1	State	Solid
2	Nature	Very fine
3	Odor	Mild
4	Touch	Soft
5	Flow Property	Free flowing
6	Appearance	Reddish orange

Table 2: Biochemical evaluation of Kumara veeriya kantha chenduram

S. No.	Test	Inference
1	Silicate	Absence of silicate
2	Sulphate	**Presence of Sulphate**
3	Chloride	Absence of Chloride
4	Phosphate	**Presence of Phosphate**
5	Carbonate	**Presence of Carbonate**
6	Nitrate	Absence of Nitrate
7	Sulphide	Absence of Sulphide
8	Nitrite	Absence of Nitrite
9	Borate	Absence of Borate
10	Lead	Absence of Lead
11	Copper	Absence ofCopper
12	Aluminum	Absence of Aluminium
13	Iron	**Presence of Iron**
14	Zinc	**Presence of Zinc**
15	Calcium	**Presence of Calcium**
16	Magnesium	Absence of Magnesium
17	Ammonium	**Presence of Ammonium**
18	Potassium	Absence of Potassium
19	Mercury	Absence of Mercury
20	Arsenic	Absence of Arsenic
21	Starch	**Presence of Starch**
22	Reducing sugar	Absenceof Reducingsugar
23	Alkaloids	**Presence ofAlkaloids**
24	Tannic acid	**Presence of Tannic acid**
25	Amino acid	Absenceof Amino acid

Table 3: Phytochemical evaluation of Kumara veeriya kantha chenduram

S. No.	Test	Observation
1.	Alkaloids	Negative
2.	Flavonoids	Negative
3.	Glycosides	Negative
4.	Steroids	Negative
5.	Triterpenoids	Negative
6.	Coumarin	Negative
7.	Phenols	**Positive**
8.	Tannins	**Positive**
9.	Protein	Negative
10.	Saponins	**Positive**
11.	Sugar	Negative
12.	Anthocyanin	Negative
13.	Betacyanin	Negative

Table 4: Physicochemical evaluation of Kumara veeriya kantha chenduram

S. No.	Parameter	Mean (n = 3) SD
1.	Loss on Drying at 105 °C (%)	12.82 ± 1.56
2.	Total Ash (%)	9.5 ± 0.3606
3.	Acid insoluble Ash (%)	0.3933 ± 0.2501
4.	Water soluble Extractive (%)	0.3467 ± 0.3564
5.	Alcohol Soluble Extractive (%)	0.09 ± 0.07

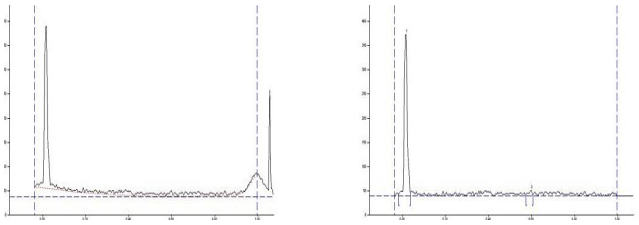

Figure 1: HPTLC finger printing of Kumara veeriya kantha chenduram

Table 5: Peak Table of Kumara veeriya kantha chenduram

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	4.2	0.02	334.5	96.46	0.04	7.5	3542.2	96.05
2	0.58	0.1	0.60	12.3	3.54	0.61	2.7	145.5	3.95

Figure 2: TLC Visualization of KVKC - TLC plate visualization at 366 nm

CONCLUSION

In biochemical analysis of Kumara veeriya kantha chenduram, the presence of carbonate, sulphate, Iron, Zinc, Calcium, Ammonium, Starch, Alkaloids and Tannic acids were detected. In phyto chemical analysis of Kumara veeriya kantha chenduram, Phenols, Tannins, Saponins were detected. The physicochemical evaluation like loss on drying at 105^0C, Ash, Acid insoluble Ash, Water soluble extractive, Alcohol soluble extractive and pH of Kumara veeriya kantha chenduram were 12.82 ± 1.56%, 9.5 ± 0.3606%, 0.3933 ± 0.2501%, 0.3467 ± 0.3564%, 0.09 ± 0.07% respectively. HPTLC finger printing analysis of the Kumara veeriya kantha chenduram sample reveals the presence of two prominent peaks corresponds to presence of five versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.02 to 0.58. Further the peak 1 (Rf value 0.02) occupies the major percentage of area of 96.05 which denotes the abundant existence of such compound.

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