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Research Article

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Biochemical, Physico chemical and Heavy metal standardization of Siddha herbo-mineral preparation Kasa Kulanthaga Mathirai

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Abstract

Owing to longstanding and time proven uses of herbo-mineral drug along with their safety margins, World Health Organisation (WHO) has taken necessary steps to ensure quality control with modern techniques and application of suitable standards for this purpose. The pharmacopoeias of different countries include monographs indicating quality parameters and standards for various herbal drugs and also their products. For the purpose of quality control of herbal drugs, W.H.O. has prepared accordingly the guidelines. The objectives put forth are provisions for recommended general test methods and also the general limits for contaminants for herbal drugs. In the literature of Kannusami Parambari Vaidhyam, there is a preparation; called “Kasakulanthaga mathirai”, which is exclusively indicated for Swasakasam (Bronchial asthma).The quality assessment of this herbo-mineral formulation is of paramount importance in order to justify their acceptability in modern system of medicine. Standardization of herbo-mineral formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, and Heavy metal analysis.

Keywords: siddha, standardization, kasakulanthaga mathirai

Introduction

Siddha system of medicine is one of the ancient systems contemporaneous with those of the submerged lands, Egyptian, Mesopotamian, Chinese, and Grecian medicines. The ancient siddhars knew the scientific truth by meditation and worked wonders through their mental force.

In siddha literature various herbal, herbomineral drugs are mentioned for the management of bronchial asthma. In the text kannusamiparam baraivaithyam “Kaasakulanthaga Mathirai” a siddha formulation has been specifically indicated for “Swaasakasam ’’ (Bronchial Asthma).

Nearly 80% of world populations rely on Herbal and herbo-mineral medicines, particularly in developing countries for primary health care needs. The herbo-mineral drugs described in siddha system have been the basic treatment of various human diseases. Standardization of herbo-mineral formulations is essential in order to assess the quality of the drugs, based on the concentration of their active principles, physical, chemical, and Heavy metal analysis. The quality assessment of this herbo-mineral formulation is of paramount importance in order to justify their acceptability in modern system of medicine. World Health Organization (WHO) encourage, recommend and promotes traditional remedies in natural health care programmes, because safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of herbo-mineral products by using modern techniques and applying suitable standards.

Materials and Methods

Standard operating procedure for "Kaasakulanthaga Mathirai":

Required raw drugs:

1. Lingam (Cinnabar)	- 8.75gms
2. Manosilai (Arsenic disulphide)	- 8.75gms
3. Karunabi (*Aconitum ferox*. Lin)	- 8.75gms
4. Milagu (*Piper nigrum*. Lin)	- 8.75gms
5. Thalagam (Arsenic trisulphide)	- 8.75gms
6. Vengaram (Borax)	- 8.75gms
7. Chukku (*Zingiber officinale*. Lin)	- 8.75gms
8. Thippili (*Piper longum*. Lin)	- 8.75gms
9. Ginger juice	- 1 ml

Source of raw drugs:

The above said raw drugs were purchased from a well reputed country shop at Chennai and Nagarcoil. The raw drugs were authenticated by Botanist of NIS, Chennai. The raw drugs were purified and the medicine were prepared as per SOP as in the Gunapadam Laboratory of NIS, Chennai.

Purification of raw drugs:

1. Chukku: At first the skin of the dried ginger was removed. Then it was covered with a paste of lime stone and dried under sunlight for three hours and washed in plain water.(5)
2. Milagu: In the fermented butter milk, pepper seed were soaked for 75 minutes and then dried.(5)
3. Thippili: Thippili was soaked in lime juice and then dried.(7)
4. Naabi: The root of naabi was soaked in cow's urine for three days and dried under sunlight. (4)
5. Vengaram: Vengaram was roasted well to a crispy consistency and powdered. (3)
6. Lingam: Lingam was first powdered in kalvam and then grounded for 6 hours by adding lemon juice little by little. Then it was made in to a ball and wrapped in a thin cloth and then suspended (Thulayanthiram) in a bowel containing lemon juice. About 250 ml of lemon juice was added for 35 gm. of cinnabar in the vessel. The knot of lingam laid 10 cm above the bottom of the vessel. Then lingam was collected after all the lemon juice was reduced and dried. Finally the lingam was powdered and stored in a porcelain vessel.(6)
7. Manosilai: Manosilai was grounded well in kalvam with ginger juice for three hours and then it was dried.(3)
8. Thalagam: Thalagam was kept in between the limestone and then showered with palm toddy. This procedure was repeated for 10 times.(3)

Method of preparation:

The above purified drugs were powdered separately and then added one by one in the kalvam and grounded with ginger juice for 12 hours and then it was taken in mezhugupatham (wax like consistency) then made into 130 mg pills (kundrialavu) and then dried.

Internal medicine: Kaasakulanthaga Mathirai
Dosage: 1 tab (130 mg) two times a day (after food)
Adjuvant: Ginger juice
Duration of treatment: 5 days
Reference: Kannusami Parambaraivaithyam
Page .No:133
Edition: 5th edition, 2006
Edited By: Thirumakalvilasam
Published by: Rathinanayakar and sons

Results and Discussion

Biochemical analysis of kasakulanthaga mathirai

Preparation of extract:

10 g of Kasakulanthaga Mathirai was measured accurately and placed in 250 ml of clean beaker and added with 250 ml of distilled water. Then it is boiled well for 10 minutes. Then it was cooled and filtered in a 100 ml volumetric flask and made upto 100 ml with distilled water. (37)

Table1: Biochemical analysis

S. No	Experiment	Observation	Inference
1.	Appearance of sample	Dark brown in colour	
2.	**Solubility** Little of the sample was shaken well and mixed with distilled water.	Sparingly soluble	Absence of Nitrate
3.	**Action of heat:** A small amount of the sample was taken in a dry test tube and heated gently at first and then strong.	No brown fumes No white fumes Evolved.	Absence of Nitrate Absence of Carbonate
4.	**Flame test :** A small amount of sample was made into paste with con.HCL in a watch class and introduced into non luminous part of the Bunsen flame.	No bluish green flame appeared.	Absence of Copper
5.	**Ash test:** A filter paper was soaked into a mixture of sample and cobalt nitrate solution introduced into the Bunsen flame and ignited.	No Yellow coloured flame appeared	Absence of Sodium

Table 2: Test For acid Radicles

1.	**Test for Sulphate :** 2 ml of above prepared extract was taken in the test tube to this added 2 ml of 4% ammonium oxalate solution.	Cloudy appearance present	Presence of **Sulphate**
2.	**Test for Chloride :** 2 ml of the above preparedsolution was added with dil.HNO_3 tillthe effervescence ceases. Then 2 ml of Silver nitrate solution was added.	Cloudy appearance present	Presence of **Chloride**
3.	**Test for Phosphate :** 2 ml of the extract was treated with 2 ml of Ammonium molybdate Solution and 2 ml of Con. HNO_3.	Cloudy yellow appearance	Presence of **Phosphate**
4.	**Test for Carbonate:** 2 ml of the extract was treated with 2 ml of Magnesium sulphate Solution.	Cloudy appearance present	Presence of **Carbonate**
5.	**Test for Nitrate :** 1 drop of the substance was heated with Copper tunics and concentrated H_2SO_4 and viewed the test tube vertically down.	No characteristic changes of formed	Absence of Nitrate
6.	**Test for Sulphide:** 1 ml of substance was treated with 2 ml of Con.HCL.	Rotten egg smelling gas evolved	Presence of **Sulphide**
7.	**Test for Fluoride and Oxalate:** 2 ml of the extract was added with 2 ml of dis. Acetic acid and 2 ml Calcium chloride solution and heated.	No Cloudy appearance present	Absence of fluoride and Oxalate
8.	**Test for Fluride and Oxalate:** 2 ml of the extract was added with 2 ml of dis. Acetic acid and 2 ml Calcium Chloride solution and heated.	No Cloudy appearance present	Absence of Fluoride and Oxalate
9.	**Test for Nitrite:** 3drops of the extract was placed on the filter paper on that 2 drops of Acetic acid and 2 drops of Benzidine solution is placed.	No characteristic changes observed	Absence of Nitrite
10.	**Test of Borate:** 2 pinches of the substances were made into paste by sulphuric acid alcohol (95%) and introduce into blue flame.	Bluish yellow coloured flame not appeared.	Absence of Borate

Table 3: Test For basic radicles

1.	**Test for Lead:** 2 ml of extract was added with2 ml of Pottasium iodide solution.	Yellow coloured precipitate was not obtained.	Absence of Lead.
2.	**Test for Copper:** One pinch of substance was made into paste with Con.HCL in a watch glass and introduced into the non- luminous part of the flame.	No blue coloured flame appeared	Absence of Copper
3.	**Test for Aluminum:** To the 2 ml of the extract Sodium hydroxide was added in drops to excess.	No characteristic changes observed	Presence of **Aluminium**
4.	**Test for Iron:** a) To the 2ml of extract add 2ml of ammonium thiocynate solution. b) To the 2ml of extract add 2ml ammonium thiocynate solution and 2ml of con HNO_3 is added.	Mild red colour appear Blood red colour appears.	Presence of **Iron**
5.	**Test for Zinc:** To 2ml of the extract sodium hydroxide solution was added in drops to excess.	White precipitate was appeared.	Presence of **Zinc**
6.	**Test for Calcium :** 2ml of the extract was added with 2ml of 4% ammonium oxalate solution.	cloudy appearance present	Presence of **Calcium.**
7.	**Test for Magnesium:** To 2ml of extract sodium hydroxide solution is added in drops to excess.	White precipitate was appeared.	Presence of **Magnesium**
8.	**Test for Ammonium :** To 2ml of extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added.	Brown colour appeared	Presence of **Ammonium**

9.	**Test for Potassium:** 1ml of substance was treated with 2ml of sodium and then treated with 2ml of cobalt nitrate in 30% glacial acetic acid.	No yellowish precipitate was obtained.	Absence of Pottasium
10.	**Test for Sodium:** 2 pinches of the substance was made into paste by using HCL and introduced into the blue flame of Bunsen burner.	No yellow colour flame appeared.	Absence of Sodium
11.	**Test for Mercury:** 2ml of the extract was treated with 2ml of sodium hydroxide solution.	Yellowish precipitate was obtained.	Presence of **Mercury**
12.	**Test for Arsenic:** 2ml of the extract was treated with 2ml of sodium hydroxide solution.	Brownish red precipitate was obtained.	Presence of **Arsenic**

Table 4: Miscellaneous

1.	**Test for Starch:** 2ml of the extract was treated with weak iodine solution.	Blue colour developed.	Presence of **Starch.**
2.	**Test for reducing sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for two minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The color is noted.	No brick red colour developed.	Absence of Reducing sugar.
3.	**Test for alkaloids :** 2ml of extract was treated with 2ml of picric acid.	Yellow colour developed.	Presence of Alkaloid
4.	**Test for Tannic acid:** 2ml of extract was treated with 2ml of ferric chloride solution.	No Black colour Precipitate is appeared.	Absence of Tannic acid.
5.	**Test for Unsaturated compounds :** To the 2ml of extract 2ml of potassium permanganate solution was added.	Potassium permanganate is not decoloured.	Absence of Unsaturated compounds
6.	**Test for Amino acids:** 2 drops of the extract was placed on a filter paper and dried well.	No violet colour developed.	Absence of Amino acids
7.	**Test for type of compound:** 2ml of the extract is treated 2ml of ferric chloride solution.	No green colour developed.	Absence of Oxyquinole epinephrine and pyrocatechol. Anti pyrine, Aliphatic amino acid and meconic

		No red colour developed. No violet colour developed. No blue colour developed.	acid absent. Apomorphine, Salicylate and Resorcinol are absent. Morphine, Phenol cresol and Hydro quinine are absent.

Table 5: Interpretation of Biochemical Analysis

Sl. No	Presence	Absence
1.	Sulphate	Copper
2.	Chloride	Tannic Acid
3.	Carbonate	Sulphide
4.	Aluminium	Nitrite
5.	Iron	Lead
6.	Zinc	Reducing Sugars
7.	Calcium	Amino acids
8.	Magnesium	
9.	Ammonium	
10.	Starch	
11.	Alkaloid	
12.	Phosphate	
13.	Mercury	
14.	Arsenic	

Physicochemical Analysis

Project ID	NRS/AS/0527/03/2020
Name and Address of the Researcher	Dr. Hitha Shyam M.S National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamil Nadu, India
Sample –ID	Kasha Kulanthaga Mathirai – KKM

Picture 1: Kaasakulanthaga Maathirai

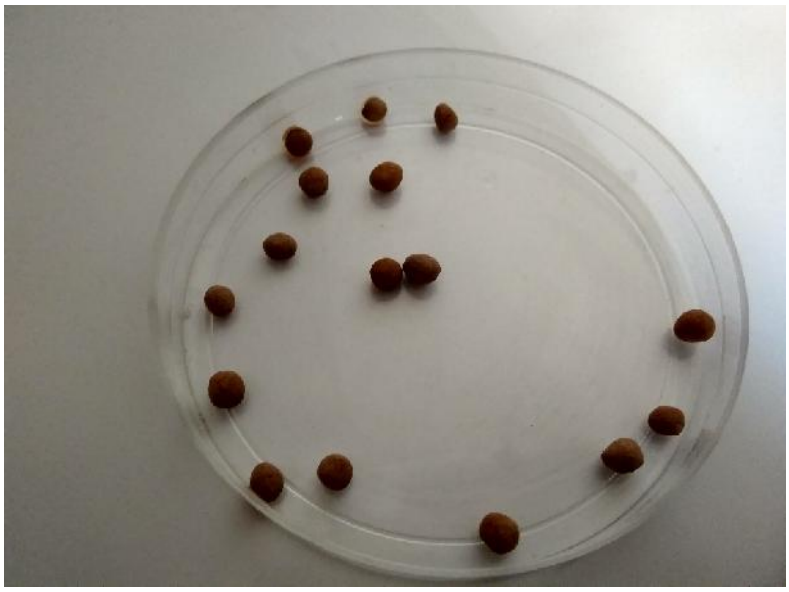

Table 6: Physical state of Kaasakulanthaga Maathirai

State	Solid
Nature	Hard solid with smooth
Odor	Mild
Touch	Rigid
Flow Property	Free flowing
Appearance	Brownish Orange

Table 7: Solubility Profile of Kasha Kulanthaga Mathirai – KKM

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

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace at temperature of 400 ^{o}C 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 6 mins. 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 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 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.

Table 8: Final Test report of physicochemical Analysis

S.No	Parameter	**Mean (n=3) SD**
1.	Loss on Drying at 105 °C (%)	0.8333 ± 0.3055
2.	Total Ash (%)	7.6 ± 2.163
3.	Acid insoluble Ash (%)	0.32 ± 0.08718
4.	Water soluble Extractive (%)	19.7 ± 0.3464
5.	Alcohol Soluble Extractive (%)	10.63 ± 0.7572

Heavy Metal Analysis

Table 9: Heavy Metal Analysis

Project ID	**NRS/AS/0527/03/2020**
Name and Address of the Researcher	Dr. Hitha Shyam M.S National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047,Tamilnadu, India
Parameter Requested for Analysis	Heavy Metal analysis by AAS
Sample Received	In Person
Sample –ID	Kasha Kulanthaga Mathirai - KKM
Description of the Sample	Solid
Method of Analysis Instrument Extraction Solvent	Model: AA 240 Series HCl and HNO_3
Analysis Type	Third Party Analysis
Date of Analysis	20/03/2020
Result of Analysis	Test Report Attached as Annexure

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 samplewas performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determine 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

Table 10: Test Report of Heavy Metal Analysis

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Mercury	253.7 nm	0.10	1 ppm
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	0.85	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL- Below Detection Limit Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metals such as Lead and Cadmium. Whereas thesample shows the presence of heavy metal Arsenic at 0.85 ppm and Mercury at 0.10 ppm which may be less than the recommended limit.

Conclusion

Bio chemical analysis of Kaasakulanthaga Maathirai shows the presence of Sulphate, Chloride, Carbonate, Aluminium, Iron, Zinc, Calcium, Magnesium, Ammonium, Starch, Alkaloid, Phosphate, Mercury, Arsenic. In Physico-chemical analysis shows, all the parameters are within acceptable range. So it can be stored for a long period and would not easily be attacked by microbes. Water soluble extract shows, water is a better solvent of extraction for the formulation. Disintegration time shows, quickly disintegrate with water. Water can be used as adjuvant for KSK Mathirai. Heavy metal analysis of sample shows the presence of heavy metal Arsenic at 0.85 ppm and Mercury at 0.10 ppm which may be less than the recommended limit.

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