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PHYSICO CHEMICAL AND HEAVY METAL ANALYSIS OF SIDDHA FORMULATION VAAYU MATHIRAI

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ABSTRACT

Siddha system is a very ancient medicine in the world. It is mainly occur in South India and is considered to be one of the India's oldest systems of medicine. This system is based on a combination of ancient medicinal practices and spiritual disciplines as well as alchemy and mysticism. Medicinal plants have been a major source of treatment for human diseases. In human body joints are the important structure which helpful for normal stability, movement and activity. Arthritis is categorized as Vali or Vali Noigal in the ancient literature of Siddha. These trial drug formulations are documented for the treatment of different kinds of Vali Noigal. Among Arthritis "VALI AZHAL KEELVAYU" (Rheumatoid Arthritis) is most common type of arthritis. The aim of the study was qualitative analysis of trial drug *Vaayu mathirai* indicates the presence of Carbonate, chloride, calcium, iron, zinc, potassium, aluminium, ammonium, copper, sulphate, magnesium, potassium, alkaloids and Starch revealed the enhancement of therapeutic action in arthritis. Indian systems of medicine are frequently reported with heavy metal analysis content above permissible limits by the western science. The present study gains its own importance in the scientific society being focused on analysis of heavy metals content in siddha medicine Vaayu mathirai Using AAS (Atomic Absorption Spectrometry).

KEYWORDS: Siddha medicine, qualitative analysis, heavy metals, AAS, vaayu Mathirai, arthritis.

INTRODUCTION

The siddha system of medicine is a prestigious system belonging to South India. According to

siddha system, medicine is a substance that helps to alleviate or eradicate the diseases, gives strength to the body and normalizes the functions of the body.[1] This ancient system of herbal medicines is being utilized by Indians and has also gained attention worldwide due to its long-term benefits in terms of overall wellness with no side effects.[2] Normally, raw drugs are submitted to series of processes such as purification, trituration, incineration and calcination to get the end product.[3] The siddha system is based on a combination of ancient medicinal practices and spiritual disciplines as well as alchemy and mysticism.[4] Siddha medicine incorporates wide usage of herbs, metals, minerals and animal products were used to prepare medicine in treating lot of medical aliments. The scientific evaluation is needed to validates is preciousness. It helps to ensure safety to the public and effective traditional treatment for diseases.[5] Indian system of medicine frequently reported with heavy metal content above permissible limits by the western science. Siddha medicines is claimed to alleviate the root cause of the diseases by maintaining the ratio of Vatham, Pitham and Kabam. Siddhars use metals and minerals in medicine, because of their characters like longer shelf life. Smaller dose with greater efficacy and the therapeutic value is much higher compared to herbal formulations. Before getting into a prepared medicine siddhars follow numerous method of purification for every single metal and minerals used. Thereby it ensures the safety of medicines prepared in siddha system. Due to lack of standardization of the drugs in Siddha system of medicine.[6]

In siddha system of medicine a large number of formulations aremade up of metal and minerals. One such herbo mineral formulation is Vaayu Mathirai. According to siddha literatures vaayu mathirai is given Vaayu noigal, keelvayukal, kaikaail mudakku, soolai, linga putru, alkul putru, azhiranam and Kuzhiranam.[7] It is mystery to the scientific society to evaluate the exact action of herbo mineral formal formulation. Since modern society is against the usage of mercurial drug as medicine these study is more important for its own. Here, taken the opportunity to reveal the important of this medicinal preparation and its analysis of heavy metals with the help of AAS (ATOMIC ABSORPTION SPECTROMETRY).

On the basis of our siddha text Rheumatoid Arthritis is inter correlated with keelvayu and more often keelvayu comes under 80 types of Vadha diseases in Yugi Vaithiya Chinthamani-60 one among them is “VALI AZHAL KEELVAYU”. The drug from Siddha literature (Anuboga Vaithiya Navaneetham Part-IV) Vaayu mathirai is analyses for the biochemical

composition.[7]

MATERIALS AND METHODS

INGREDIENTS OF VAAYU MATHIRAI

1. Pooram (Calomel)
2. Nelikai Gandhagam (Sulphur)
3. Karisalnganni (Eclipta prostrata)

COLLECTION, IDENTIFICATION AND AUTHENTICATION OF THE DRUG

The required raw drugs for preparation of "VAAYU MATYIRAI" were purchased from a well reputed country shop at Chennai. The herbal drugs were authenticated by the Assistant Professor of Medicinal Botany, NIS, and mineral drug were authenticated by the Assistant professor of Gunapadam, NIS. After that the raw drug were purified separately. Then the trial drug was prepared in Gunapadam laboratory, National Institute of Siddha.

PURIFICATION OF RAW DRUGS

The following drugs were purified as per the Siddha text.

1. Purification of Pooram (CALOMEL)

Take a mud vessel and make a decoction of piper bettle with Piper nigrum. Cover the pooram with a cloth (kizhi method) and dip in to the decoction. Pooram was soaked in to it for 3 days and dry it.

(Yakobu vaithiyam 300 pg.no-5)

2. PURIFCATION OF GANTHAGAM(SULPHUR)

The Gandhagam (sulphur) was melted with cow's ghee and poured into cow's milk and after cooling down Gandhagam (sulphur) were collected. These processes were repeated for 30 times with fresh milk.

(Anuboga Vaithiya Navaneetham Part-4Pg.no-8) PREPARATION OF VAYU MATHIRAI

INGREDIENTS

- *Purifiedpooraparpam(Calomel) - 1 palam (35 gm)*
- *PurifiedNellikaiGandhagam(Sulphur) - ¼ palam (8.75 gm)*
- *Juiceofkarisalankanni(Ecliptaprostrate) - required amount*

Method of preparation

The above drugs are made in to fine powder and ground with Eclipta prostrate juice and made in to 65-130mg tablet.[7]

BIO CHEMICAL ANALYSIS

Screening the drug of Vaayu Mathirai was identifying the Biochemical properties present in the ingredients.

CHEMICALS AND DRUGS

The chemicals used in this study were of analytical grade obtained from Department of Biochemistry, National Institute of Siddha, and Chennai – 47.

METHODOLOGY

Preparation of the drug for Qualitative Analysis

10 g of *Vaayu 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 then it is made up to 100 ml with distilled water. This fluid was taken for analysis.[8]

QUALITATIVE ANALYSIS

	PROCEDURE	**OBSERVATION**	**INFERENCE**
1.	Appearance of sample	Dark brown in Color	
2.	**Solubility test** **Test for Nitrate** Little of the sample was shaken well and mixed with distilled water.	Sparingly soluble	Absence of **Nitrate**
3.	**Action of heat** **Test for Carbonate and Nitrate** A small amount of the sample was taken in a dry test tube and heated gently at first and then strong.	No brown fumes White fumes appeared.	Absence of Nitrate Presence of Carbonate
4.	**Flame test** **Test for Copper** 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.	Bluish green flame appeared.	Presence of **Copper**
5.	**Ash test-Test for Sodium** A filter paper was soaked into a mixture of sample and cobalt nitrate solution introduced into the Bunsen flame and ignited.	No Yellow color flame is developed	Absence of **Sodium**

I. TEST FORACID RADICLES

1.	**Test for Sulphate** 2 ml of above prepared extract was taken in the test tube to this added2 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 till the 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 2ml of Ammonium molyb date Solution and 2 ml of Con. HNO_3.	Presence of 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 ofCon.HCL.		Absence of **Sulphide**
7.	**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.	Cloudy appearance absent.	Absence of **Fluride** and **Oxalate**
8.	**Test for Nitrite** 3drops of the extract was placed on the filter paper on that 2 drops of Acetic acid and 2 drops of Benzedine solution is placed.	No characteristic changes observed	Absence of **Nitrite**
9.	**Test of Borate** 2 pinches of the substances was made into paste by sulphuric acid alcohol (95%) and introduce into blue flame.	Bluish yellow colored flame not appeared.	Absence of **Borate**

II. TEST FOR BASIC RADICLES

1	**Test for Lead** 2 ml of extract was added with2 ml of Potassium iodide solution.	Yellow color 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.	Blue color flame appeared	Presence of **Copper**
3	**Test for Aluminum** To the 2 ml of the extract Sodium hydroxide was added in drops to excess.	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 color appear Blood red color 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.	Yellowish precipitate was obtained.	Presence of **Potassium**
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 color flame appeared.	Absence of **Sodium**
11	**Test for Mercury** 2ml of the extract was treated with 2ml of sodium hydroxide solution.	No Yellowish precipitate was obtained.	Absence of **Mercury**
12	**Test for Arsenic** 2ml of the extract was treated with 2ml of sodium hydroxide solution.	No Brownish red precipitate was obtained.	Absence of **Arsenic**

II. MISCELLANEOUS

1.	**Test for Starch** 2ml of the extract was treated with weak iodine solution.	Blue color 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 color developed.	Absence of Reducing sugar.
3.	**Test for alkaloids** 2ml of extract was treated with 2ml of picric acid.	Yellow color developed.	Presence of **Alkaloid**
4.	**Test for Tannic acid** 2ml of extract was treated with 2ml of ferric chloride solution.	Black color Precipitate is not 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 discolored.	Absence of Unsaturated compounds
6.	**Test for Aminoacids** 2 drops of the extract was placed on a filter paper anddried well.	No violet color developed.	Absence of **Amino acids**
7.	**Test for type of compound** 2ml of the extract is treated 2ml of ferric chloridesolution.	No green color developed. No red color developed.	Absence of **Oxyquinole Epinephrine** and **pyrocatechol**.

		No violet color developed. No blue color developed.	**Anti pyrine, Aliphatic amino acid** and **meconic acid** absent. **Apomorphine, Salicylate** and **Resorcinol** are absent. **Morphine, Phenol cresol** and **Hydro quinine** are absent**.**

RESULTS OF BIOCHEMICAL ANALYSIS

S.N O	PRESENCE
1.	Ammonium
2.	Magnesium
3.	Zinc
4.	Carbonate
5.	Sulphate
6.	Aluminum
7.	Iron
8.	Calcium
9.	Starch
10.	Chloride
11.	Alkaloid
12.	Sulphate
13.	Phosphate
14.	Potassium
15.	Copper

DISCUSSION

The Bio chemical analysis of the trial drug Vaayu Mathirai contains, Sulphate, Calcium, Iron, Potassium, Phosphate, Ammonium, Magnesium, Aluminium, Copper, Magnesium, Zinc, Carbonate, Chloride, Alkaloid and Starch.

CARBONATE

In various parts of human body, PH and acid balance are regulated by the carbonate, as bicarbonate Ions.[9]

CHLORIDE

Chloride needed for fluid regulation and electrolyte balance. Electrolyte imbalance can lead to irregular heartbeat, confusion, muscle spasms, numbness, fatigue and Nerve (or) bone disorders. Chloride is an essential electrolyte located in all body fluids responsible for maintaining acid / base balance, transmitting impulse and regulating fluid in out of cell.[10]

CALCIUM

The calcium maintains strong bones and decreased the risk of Cardio Vascular Diseases and stroke. It helps during pregnancy reduce the risk of Pre Eclampsia.[11]

In Juvenile Arthritis patient who take calcium supplements will have at least a 10% greater increase in total body bone mineral density compound to patients who take the placebo.[12]

IRON

Iron plays a vital function in the body, including general energy and focus Immune system. Iron plays a vital role for normal functioning of the Central Nervous System.[13]

POTASSIUM

Potassium has an important role in regulating muscular activity, maintaining water and electrolyte balance and acid base balance and also essential for neuronal activity.[14]

ZINC

Zinc is an essential trace element for plant growth and also plays an important role in various cell processes and also zinc is important for the production of Insulin hormone and carbonic anhydrase in the body.[15]

Zinc helps in regulating immune function, Perm production and fetus development.

AMMONIUM

Ammonia plays an important role in protein synthesis in the human body and also maintains the body's PH balance.[16]

MAGNESIUM

Magnesium is an essential mineral for optimal metabolic function. Magnesium is the fourth most abundant essential mineral in the body. It is distributed approximately one half in the bone and one half of the muscle and soft tissue and less than one percent in the blood.[17]

Magnesium is associated with maintaining (or) improving bone mineral density as a dietary component in combination with Potassium, fruits and vegetables (or) as an oral supplement.[18,19]

Magnesium was more effective than placebo for pain relief.[19]

COPPER

Copper is as essential trace mineral necessary for survival. It is found in all body tissues and plays a role in making red blood cells and maintaining nerve cells and the immune system.

Copper is an essential nutrient for the body.

It helps maintain bones, blood vessels, nerves and immune function. Copper may help prevent or delay Arthritis.[20]

ALKALOIDS

The alkaloids and their derivatives are used as anti – spasmodic, analgesic and anti – bacterial drugs.[9]

This finding supported in treating Thozh Piningal (Skin Diseases), Vali (Arthritis), Veri (Psichiatric disorder) and also prevent the formation of Infection.

This study revealed the presence of effective minerals like *calcium, iron, Ammonium, Magnesium, Zinc, Carbonate, Aluminium, Copper, Chloride, Potassium, Sulphate, Starch, Alkaloids and Phosphate.*

PHYSICO CHEMICAL ANALYSIS OF VAAYU MATHIRAI

ORGANOLEPTIC CHARACTERS OF VAAYU MATHIRAI

State	Solid
Nature	Hard solid and dense
Odor	Characteristic
Touch	Non sticky
Flow Property	Free
Appearance	Pinkish gray

PHYSICO CHEMICAL ANALYSIS

1. 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.

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 colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

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.

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 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.

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 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.[22]

RESULTS

S. No	Parameter	Mean (n=3) SD
1.	**Loss on Drying at 105 °C (%)**	5.667 ± 2.354
2.	**Total Ash (%)**	0.5767 ± 0.254
3.	**Acid insoluble Ash (%)**	0.073 ± 0.07
4.	**Water soluble Extractive (%)**	19.83 ± 1.25
5.	**Alcohol Soluble Extractive (%)**	15.67 ± 4.315

DISCUSSION

ASH VALUE

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus ash value is a validity parameter describe and to assess the degree of purity of a given drug.

TOTAL ASH

Total Ash value of plant material indicated the amount of minerals and earthy materials present in the material. Present in the drug is measured through the Total Ash value is 0.5767

± 0.254 for Vaayu Mathirai.

ACID INSOLUBLE ASH

The acid insoluble Ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble ash value is low. It is 0.073 ± 0.07 for Vaayu Mathirai.

EXTRACTIVE VALUES

These are indicating the approximate measure of chemical constituents of crude drug.

The percentage of soluble maters present in the drug is determined by the values of water extractive and ethanol extractive.

Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which will correlate with the metabolism reactions.

Water soluble extractive value plays an important role in evaluation of crude drugs.

The alcohol soluble extractive value was also indicative for the same purpose as the water soluble extractive value.

LOSS ON DRYING

The total of volatile content and moisture present in the drug was established in loss on drying.

Moisture content of the drug reveals the stability and its shelf – life.

High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life.

METHODS OF ATOMIC ABSORPTION SPECTROMETRY (AAS)

To determine the heavy metals such as Mercury, Lead, Arsenic and Cadmium in the test drug VAAYU MATHIRAI.

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 HNO3.

Standard Preparation

As & Hg-100 ppm sample in 1mol/ HCL Cd &Pb-100 ppm sample in 1mol/L HNO_3.

RESULT

Name of the Heavy Metal	Absorption Max A max	Result Analysis	Maximum Limit
Mercury	253.7 nm	BDL	1 ppm
Lead	217.0 nm	0.88	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL – Below Detection Limit, ppm- parts per million

DISCUSSION

Results of the Present investigations have clearly shows that the sample has no traces of heavy metals such as Mercury, Arsenic and Cadmium. Whereas the sample shows the presence of heavy metal Lead at 0.88ppm which may be less than there commended limit. This indicates that all heavy metals in Vaayu mathirai were within normal range and near to permissible limit. This study clearly showed Vaayu Mathirai is safe for human consumption.[23]

CONCLUSION

The Vaayu Mathirai is a Siddha drug taken from Siddha literature used in the treatment of Rheumatoid Arthritis. The drug is screened for its Bio chemical, physic chemical and Heavy metal properties. The present study evaluated the physico chemical properties of the traditional Indian medicine vaayu mathirai. This study hesitated by other countries due to poor standardization and lack of quality. This study is an earnest attempt of bio active principles present in the drug in relation with their actions a making appropriate scientific

validation of metal based ancient Siddha medicine using authentic scientific techniques. The present AAS (Atomic Absorption Spectrometry) study reveals that Vaayu Mathirai was non-toxic and the heavy metal content was below detection limits. The medicine is safe with trace of heavy metals which are within their permissible limits without losing their therapeutic efficacy.

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