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PHYSICO-CHEMICAL AND ANALYTICAL STUDIES OF SIDDHA HERBO MINERAL FORMULATION *KODIPAVALA CHUNNAM*

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ABSTRACT

Siddha medical science has been drawing global attention in recent times since they offer treatment possibilities for even degenerative disorders with no adverse or side effects. In particular the marine environment provides a remarkable source of many drugs including Corals (Pavalam), a calcareous substance with calcium carbonate, which is used in Siddha medicine for various ailments. Even though many therapeutic approaches mentioned in Siddha system of medicine for Coral, this system of medicine lacked systematic research on the physicochemical standardization based on modern science which restricts the development of the characteristics of this system. In order to overcome this obstacle and make it credible and acceptable to all, they should undergo detailed scientific study for their physical and chemical properties, quality standards, processing methods by using modern tools of standardization to ensure their safety and effectiveness for the better use. This present study attempted to provide valuable physico chemical standard of the Siddha drug made from Coral "*Kodipavala Chunnam".* This drug was prepared as per Siddha classical literature and then various physico chemical parameters such as organoleptic characters, solubility test, chemical and elemental analysis which include ICP-OES, SEM and FT-Raman were scanned. As there is no standard physico chemical profile available for *Kodipavala Chunnam*, the result of this work may be taken as standard for future research.

KEYWORDS: Siddha Medicine, Coral, Physico chemical standardization, *Kodipavala Chunnam*, Analytical study.

INTRODUCTION

Numerous single drug and compound drug formulations documented in classical Siddha literatures are highly efficacious and devoid of side effect or adverse effects and are useful to the mankind. Today we are only exploring their efficacy and usefulness through advanced scientific research tools and modern techniques. Siddha medical science has been drawing global attention in recent times since they offer treatment possibilities for even degenerative disorders with no or less adverse or side effects.

Drugs obtained from animal source and marine products are used extensively in Siddha system of medicine.[1,2] At present 15-20 percent of Siddha medicines is based on animal products.[3] It is well known that the annual global trade in animal-based medicinal products accounts for billions of dollars per year.[4] In particular the marine environment provides a remarkable source of many animal species including Corals (Pavalam), a calcareous substance with calcium carbonate[5], which is used in Siddha medicine for various ailments. Coral has Calcium and 74 other life-enhancing minerals.[6]

Coral has been recommended for its life enhancing properties, detoxification and as a cure for liver disorder.[7] Coral (Pavalam) is made into *Parpam, Chenduram, and Chunnam* used therapeutically for various disorders. It is used for severe fevers, respiratory diseases, polydipsia, cough, haemoptysis, hepatomegaly and dropsy conditions. They act as blood purifiers and as a general tonic[8] and are effective in the treatment of liver diseases and disorders of bile secretion.[9] They are useful as a prescription for longevity and improvement in blood circulation.[10]

Even though many therapeutic approaches mentioned in Siddha system of medicine for Coral, this system of medicine lacked systematic research on the physicochemical standardization and pharmacological studies based on modern science which restricts the development of the characteristics of this system. In order to overcome this obstacle and make it credible and acceptable to all, they should undergo detailed scientific study for their physical and chemical properties, quality standards, processing methods by using modern tools of standardization to ensure their safety and effectiveness for the better use. One of the Siddha drug made from

Coral is "*Kodipavala Chunnam"* which is yet remained unexplored for its exact chemical, pharmacological and clinical values in terms of scientific research. To fill these scientific lacunae, the first step was undertaken to evaluate the physicochemical standardization and instrumental analysis of *Kodipavala Chunnam* by using modern scientific methods.

MATERIALS AND METHODS

Collection and Authentication of Raw Materials

Kodipavalam (Red Coral) was purchased from the standard supplier in the Country drug market at Parrys Corner, Chennai and got authenticated by Gunapadam (Pharmacology) experts, Marine biologists and Chemists. Fresh *Keezha nelli* (*Phyllanthus amarus*) and Lemon fruits (*Citrus aurantifolia*) were purchased from the standard supplier in Koyembedu Market and got authenticated by Botanist of Govt.Siddha Medical College, Arumbakkam, Chennai. Honey was purchased from the tribal of Kolli Hills and got authenticated by Gunapadam experts and Chemists. The relevant classical authentication and chemical identification were carried out. Sample of each raw materials have been kept in the department for future reference.

Purification process

The Red coral was purified as per the Siddha classical literature.[11] 500 gm coral was taken in a mud pot, broken into small pieces, and then cleaned thoroughly by rinsing repeatedly in lukewarm water to remove the adherent sand particles and organic debris. Then it was allowed to dry in a shady place. Cleaned coral were taken in a container and lime juice was added into that until the Coral got immersed and the mouth of the container was closed with other proper plate. It was kept idle for 24 hours and the next day coral was taken from the container and cleaned thoroughly with lukewarm water and allowed to dry well in a shadowed area. Then the dried coral stored in an air tight container. This processed coral was taken for *Chunnam* preparation.

Preparation of *Kodipavala Chunnam*

The *Kodipavala Chunnam* is prepared as per the Siddha literature.[12] Purified *Kodipavalam* (Red coral) is kept soaked in juice of *Phyllanthus amarus* for a day, exposed, to the sun to be completely dried, then honey is poured in it, mixed well, kept for a day, transferred to a suitable mud pot, mouth covered with mud plate, clay cloth seven layers to the margin are provided, dried and is subjected to heavy calcination process with 100 cow dung cakes in a pit in an airtight compartment. On next day, it is taken out and weighed.

Next, this is powered in Stone mortar and pestle, rubbed with *Phyllanthus amarus* juice for 3 days, small cakes made, dried, covering was made with the rubbed paste of whole plant of *Phyllanthus amarus*, dried and is subjected to heavy calcination process as before with about 350 cow dung cakes until it burnt completely. After this process of incineration, the cake was allowed to cool and it was opened by breaking carefully and the white ash material was collected and weighed. Then it was ground well in the stone mortar and pestle into a very fine powder and was labeled as KPC.

Organoleptic & Physicochemical Evaluation

KPC was subjected for the observations of organoleptic characters such as colour, odour, touch taste and appearance. Physicochemical parameters such as loss on drying at 105° C, moisture content, total ash, acid insoluble ash, water soluble and insoluble extractive, alcohol and water soluble extractives, solubility test, the presence of heavy/toxic metals and inorganic qualitative analysis were carried out as per AYUSH protocol.[13]

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES): In the Inductively coupled plasma optical emission spectroscopy (ICP-OES), a large amount of elements of the periodic table such as Ca, K, Na, Mg, P, Mn, Zn, Fe, Cu, Mo and S can be quantified at trace and ultra-trace concentration levels. Cd, Co, Ni, Sr, B, Y, Rb, Ni, Al, As, Ba, Cr, Pb, Se and other elements can be determined as well. In this plasma emission spectroscopy (OES), a sample solution is introduced into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000^{o}C. At this temperature all elements become thermally excited and emit light which is collected by the spectrometer, converted to an elemental concentration by comparison with calibration standards.

Scanning Electron Microscopy (SEM)

SEM is a type of electron microscope which helps to know about the sample's surface topography (external morphology), chemical composition, crystalline structure, particle size, orientation of materials and other properties such as electrical conductivity.[14]

Raman Spectroscopy

Fourier transformation Raman spectroscopy is suitable to investigate chemical composition and structural properties of solid and liquid (polarization measurements) samples. Raman can best be thought of as producing a precise spectral fingerprint, unique to a molecule or indeed and individual molecular structure.

RESULTS AND DISCUSSION

Physicochemical Analysis: The process of manufacturing *Kodipavala Chunnam*, the trial drug as per *Siddha* Research Pharmacopoeia was carried out in a standardized environment where in at the end of *pudam* (calcination) process, the sample was subjected for various analyses, which revealed the important characteristics. The observations of organoleptic characters such as colour, odour, touch, taste and appearance of KPC was presented in Table.1. From the result it was found that KPC was dull white in colour, odourless and alkaline in taste. It was appeared as amorphous in nature with soft and very fine in touch.

Table. 1. Showing Organoleptic Character of KPC.

Colour	Dull White
Odour	Odourless
Touch	Soft and very fine
Taste	Alkaline in taste
Appearance	Amorphous powder

Determination of physicochemical parameters include loss of drying at 105^0C, moisture content, total ash, acid insoluble ash, water soluble and insoluble extractives, pH, specific gravity and TLC of KPC was tabulated in Table.2. From the result total ash of KPC was 96.72%. The powdery nature of KPC was noted by the increased total ash values and decreased acid insoluble ash values (0.20 %).

A very low value of acid insoluble ash suggested that *Kodipavala Chunnam* did not contain any sand, dust, dirt, stones, etc. that get mixed during processing or are present in the parent material as contamination. The lower value indicated superior quality and high hygiene standards in the production process.[15]

The values of loss on drying (0.02%) and acid insoluble ash have got decreased in KPC which indicates that the sample on repeated processing will results with lesser particle size and the quality of the finished product is improved respectively. This shows that the finished product is highly hygienic in nature.

The moisture content is very low in KPC (0.08%). Thus stability and longer shelf life period of the drug is determined by this very low moisture content. The maximal colloidal and minimal crystalline nature of the KPC was also noted by the increased water insoluble ash values and decreased water soluble ash values which indicated that KPC is more absorbable/ get easy assimilated. Hence this solubility tests and water soluble and insoluble ash values indicates the Maximal Colloidal (Immiscible) nature of the drug and Minimal Miscible nature of the drug.

The specific gravity was 0.998 indicates the weightlessness of the drug and its size, density which was brought to low level to make the drug more assimilable to the body. The organic content of the drug is nil in KPC as indicated by no spots in TLC.

The alkalinity/basicity of the KPC is denoted by the pH evaluation of the drug and pH is 8.95 which are within the admissible limit. This is emphasized by the presence of calcium hydroxide ($Ca(OH)_2$) when dissolved in aqueous solution as calcium carbonate of coral which on heating oxidized to its oxide form rendering basicity of the drug obviously.

Table. 2. Showing physical constants of *Kodipavala Chunnam*.

Physical Constants	**KPC**
Loss on Drying at 105^0 C	0.02 %
Moisture Content	0.08 %
Total Ash	99.72 %
Acid Insoluble Ash	0.20 %
Water Soluble extractive	9.30 %
Water insoluble extractive	90.42%
pH	8.95
Specific gravity	0.998
Calcium	36.436 %
TLC	No Spots

Solubility tests: KPC was subjected to solubility test in different solvents. KPC was sparingly soluble in distilled water, ethanol, methanol and propylene glycol, but insoluble in petroleum ether, benzene, xylene, toluene, chloroform and carbon tetra chloride (Table.3).

Table. 3. Showing Solubility tests of *Kodipavala Chunnam*.

Sparingly soluble	**Not soluble**
Distilled water, Ethanol, Methanol, Propylene glycol	Benzene, Carbon tetra chloride, Chloroform, Petroleum ether, Toluene and Xylene

Chemical Analysis of *Kodipavala Chunnam:* The qualitative chemical analysis of *Kodipavala Chunnam* was done as per the standard procedure which was presented in Table 4. From the result it was showed that the presence of the basic radicals such as calcium, zinc, magnesium, iron, copper, sodium and potassium. It should be noted that the carbonate, sulphate, phosphate moieties in the KPC which offers the alkalinity to the drug are the actual entities of the body pH buffer systems to control the acid – base balance. The alkaline environment of the drug *Kodipavala Chunnam* facilitates their absorption in intestine. A high alkaline level within the body also makes it easier for cells to discard waste and toxins. By maintaining an alkaline pH, will be helping to protect the body's cells in addition to discouraging the development and growth of cancerous cells.[16]

Table. 4. Showing the presence of Acid radicals in *Kodipavala Chunnam*.

Acid radicals	**Basic radicals**
Carbonate, Sulphate and Phosphate	Calcium, Copper, Iron, Zinc, Magnesium, Potassium and Sodium

Elemental Analysis

ICP-OES: Analytical reports of KPC shown that the presence of Ca, Mg, Mn, Fe, K, Na, Zn, Se, Si, P, S, Cr and Cu. (Table 5). This may be due to presence of these trace elements in *Phyllanthus amarus* used for triturating process during drug preparation. The heavy toxic metals

like As, Cd, Hg and Pb were in below detectable level which inferred that that they are not present in the trial drug.

Table. 5. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Analyte	Frequency	Mean (mg/L)	Analyte	Frequency	Mean (mg/L)
As	193.696	BDL	**P**	213.617	26.221
Ca	317.933	380.154	**Pb**	230.204	BDL
Cd	226.502	BDL	**S**	181.975	14.555
Hg	253.652	BDL	**Se**	196.026	1.665
Fe	238.204	1.798	**Si**	251.611	24.421
K	766.490	17.845	**Zn**	213.856	1.977
Mg	285.213	87.298	**Cu**	324.754	48.463
Na	589.592	162.754	**Mn**	279.482	60.243

BDL – Below Detectable Limit.

Scanning Electron Microscopy (SEM)

In this present study SEM analysis of *Kodipavala Chunnam* showed the nano particle sizes in a 7000 x magnification within a small area of 5 micron. (Fig.1). Due to the wet grinding of the red coral with *Phyllanthus amarus* juice and consequent heat treatment in the form of *pudam* may be responsible for reduction in this particle size. The KPC showed size from 0.1 -1.5µ. This showed that the particles of KPC Ranges from 100-1500 nm. Particle size of drug substances has an influence on chemical and physical behavior. It is one of the factors which will affect dissolution, absorption of drug and bioavailability.[17-18]

Size analyses of these products are essential to achieving a homogeneous product, while optimal particle size and shape is product dependent. Smaller the drug particle size larger the surface area, leads to faster dissolution and provides for better and more rapid absorption and allows them to penetrate cells and interact with cellular molecules.[19-21]

The trial drug KPC possess the particle size ranges from 100-1200 ensure the inorganic colloidal with nano particle size. Hence the maximal colloidal nature of the drug favours steady absorption, distribution, and other pharmacokinetic principles of the drug. These help cure people faster and without the side effects that the ancient system of *Siddha* medicine have.

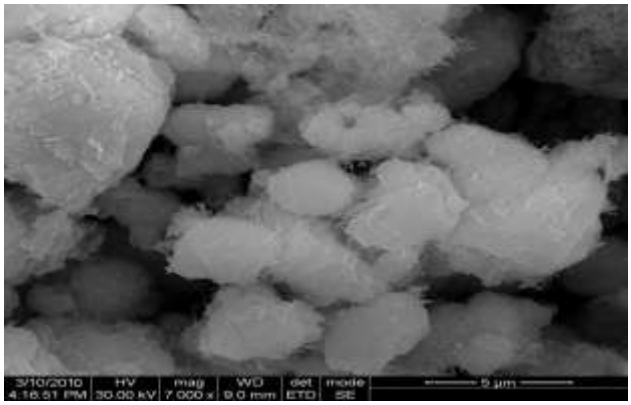

Fig. 1. SEM picture of KPC.

FT-Raman Spectroscopy: The *Kodipavala Chunnam* showed 9 peaks which is presented in Fig.2 and Table.6. The drug obtained after calcinations process as per standard *Siddha* -SOP shows effective carbon linkages with Sulphur, Carbon, Nitrogen, Oxygen, Chlorine {halo-carbon linkages}, X-Metal oxide functional regions. These bond natures might be Ionic, (X-Metal Oxide), Covalent, Coordinate Covalent (C–Cl bond linkage, C=C bond linkage, C≡N bond linkage, N=C=S bond linkage). These favour the presence of the alkoxide nature of KPC. As mentioned earlier the *Pudam* process convert the heterogeneous material into homogeneous substance and creating new complex compounds of various bonds. This electronegativity/nucleophilic of the *Chunnam* makes free radicals to get attracted by this electron clouds and some of the new bonding make hold the metabolites from damaging the cells and detoxificating them and also this characters favours the phenomenon of electron transport chain and maintain the cellular metabolism properly and ensures the Anti-Oxidant property.[22-23]

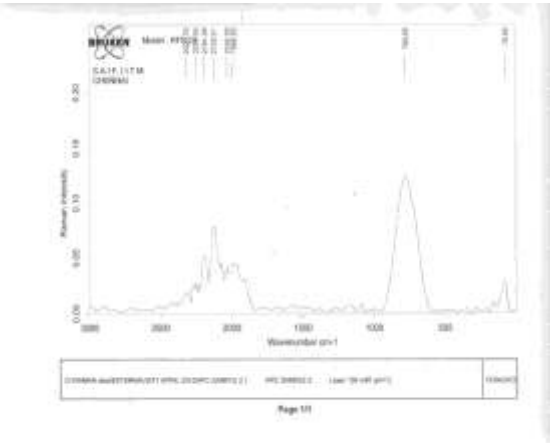

Fig. 2. Raman Spectroscopy of KPC.

Table. 6. Raman Spectroscopy results of KPC.

Raman wavenumber	Functional vibration/groups	Inference
78	Lattice vibration	Ionic lattice vibration
782	C–Cl linkage	All these shows that the Chunnam might undergone inorganic chemical bonding with carbon moiety of the coral. These functionally active regions in Chunnam might provide the active site of action.
1995	C=C linkage	
2032	N=C=S linkage	
2129	C≡N linkage	
2191	C≅C linkage	
2250	C≅N linkage	
2320	P–H linkage	
150 – 430	= X Metal Oxide	

CONCLUSION

The *Kodipavala Chunnam* samples were evaluated organoleptically and with classical parameters and confirmed its perfect method of purification and preparation. *Siddha* parameters and Physico-chemical like, Ash value, extractive values, moisture content, chemical parameters like acid and basic radicals, sophisticated analytical instrumental parameters like ICP-OES, SEM, and RAMAN Spectroscopy were found to be informative. As there is no standard physico chemical profile available for *Kodipavala Chunnam*, the results obtained for the various physicochemical parameters of *Kodipavala Chunnam* may be taken as standard parameters for future reference

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