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Research Article

Physico Chemical Standardization of the Siddha Herbo Mineral Pill - Siromaniboopathy Kuligai

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Abstract

Siromani Boopathy Kuligai is a traditional Siddha herbomineral medicine. The aim of this study was carried out to standardize the drug Siromani Boopathy Kuligai by evaluating its Organoleptic, physico-chemical properties and modern scientific instruments like, Fourier Transform - Infra Red (FTIR) Spectroscopy, SEM (Scanning Electron Microscope) with Energy Dispersive X-ray Analysis (EDAX) and X-Ray Fluorescence Spectroscopy (XRF). The organoleptic characters, physico-chemical characters like ash values, pH value, and solubility were analyzed. The total ash value was found to be 9.24% w/w, acid insoluble ash value is 0.76% w/w, water soluble ash value is 6.3% w/w, and moisture content is 6.11% w/w. The pH value is 7.5 and water soluble extractives and alcohol soluble extractives were found to be 8.86% w/w and 5.89% w/w. The SEM analysis of the drug indicated the existence of nano and near nano particles. EDAX of the drug showed the content of carbon, magnesium, mercury, sulphur, chloride, potassium, and calcium. The FT-IR spectroscopy showed the presence of functional groups like FT-IR spectroscopy revealed the existence of functional groups like phenols and alcohols, alkanes, alkenes, primary amines. XRF analysis demonstrated the drug had mercury, sulphur, selenium, zinc, magnesium, manganese, sodium, calcium, and potassium.

1. INTRODUCTION

Siddha system is one of the ancient chemico-philosophical system of India existed separately in early times. Siddhars were the innovators in the use of metals and minerals and medicinal plants. The typical distinctive features of Siddha Pharmacology (Siddha Materia Medica) are utilization of metal and mineral based preparations to a greater extent in comparison with other traditional systems of medicine.

Herbomineral drugs (metals or minerals triturated with herbal juices or decoctions lead to reduction in the size of the particulate size even up to nano levels (less than 100 nm). These drugs are known to be effective even in low concentration with longer shelf life. It has been noted that metallic or mineral medicines can help tremendously in patients with chronic or degenerative diseases. Siddha medicine has immense faith in the miracles of mercurial drugs and in the prolongation of life through rejuvenating treatments and intense yogic practices¹.

For metal and mineral based medicines, standardization of the drugs for quality control is a mandatory process and it is achieved through the evaluation of organoleptic, physico-chemical characters and sophisticated analysis of instruments. Based on these specifications metal and mineral based drugs can be well standardized on scientific basis. In the present-day of globalization for the improvement of a world market for Siddha medicines research & development is important.

To enlighten the scientific basis for the use of a safe herbomineral drug Siromani Boopathy Kuligai for thrombolytic activity certain measures were to be adopted. There are many thrombolytic

medicines in Siddha system of medicine yet there is no appropriate scientific standardization of such medicines. This is a preliminary attempt to establish Siromani Boopathy Kuligai as a good thrombolytic drug in Siddha system of medicine.

The present study investigated to standardize its Organoleptic, Physico-chemical properties and Instrumental analysis like Fourier Transform - Infra Red (FTIR) Spectroscopy, SEM (Scanning Electron Microscope) with Energy Dispersive X-ray Analysis (EDAX) and X-Ray Fluorescence Spectroscopy (XRF).

2. MATERIALS AND METHODS

2.1 Selection of drug

The drug Siromani Boopathy Kuligai was collected from the classical Siddha literature².

2.2 Collection and authentication of the drug

The raw materials included in the formulation are Cinnabar, Sulphur, *Santalum album* and *Syzygium aromaticum*, *Michelia champaca* were procured from the country drug shop at Thanjavur, Tamilnadu. Fresh raw Ginger (*Zingiber officinale*) was procured from Koyambedu market at Chennai, Tamilnadu. They were identified and authenticated by the PG Gunapadam experts, Govt. Siddha Medical College, Arumbakkam, Chennai-106. The specimen sample of each ingredient was labeled separately and kept in the lab for future reference.

2.3 Purification of the drug

The purification process was done according to the procedures mentioned in the classical Siddha literature³. For the purification of Cinnabar, lime juice, cow's milk and the Indian *Acalypha* juice were mixed together in equal proportion and allowed to fuse with cinnabar so as to get it in consolidated purified potential state. Sulphur was placed in an iron pan. Sufficient quantity of cow's butter was added and the pan was heated till the butter melts. This

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mixture was immersed in inclined position in cow's milk to get purified sulfur. Fresh milk was used every time and the process was repeated for 29 times. Ginger, Cloves, Sandal wood, and the Golden champak flowers were purified by the procedures mentioned in the classical Siddha texts⁴.

2.4 Preparation of the drug

2.4.1 Preparation of decoction

105 grams of Powder of sandal wood, 35 grams of Cloves and 26 ½ grams of Golden champak flowers were taken and mixed with water (1.3 liters) and condensed by 1/8 part, then filtered.

2.4.2 Preparation of pill

Purified 70 g of Cinnabar and 35 g of Sulphur were ground for half an hour and then 175 g of ginger juice was added to it little by little and again ground for 3 hours. The ground material was put in an iron vessel and heated in coal fire till the remaining juice is evaporated. This process repeated again by grinding it for 9 hours with using decoction of sandal wood, then made into pills are rolled in 130 mg size and dried in shade. The pills were stored in clean airtight container and named as SBK.

2.5 Organoleptic and Physicochemical Evaluation

The drug Siromani Boopathy Kuligai was subjected for the determination of organoleptic characters like colour, odour, taste, texture, hardness and physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, moisture content, alcohol and water soluble extractives, loss of weight at 105° C, tablet disintegration test, solubility test, and they were evaluated according to the standard methods described in the texts⁵.

2.6 Preparation of extract for preliminary basic and acidic studies

The drug Siromani Boopathy Kuligai was subjected for qualitative analyses of cations and anions based on the texts mentioned⁶⁻⁷.

2.7 Instrumental analysis

2.7.1 FTIR (Fourier Transform - Infra red Spectroscopy)

IR statistics acquired with Spectrum one: FT-IR Spectrometer with scan range MIR450-4000 cm⁻¹ was carried out at Anna University, Chennai. Kerr Pellet method was adopted in the analysis⁸. 50 mg of the drug was stuck by the agate mortar and pestle to provide a very fine powder. The finely powdered drug was assorted with 100mg of dried Kerr salt. This mixture was then pressed under hydraulic press using a die to produce a translucent disc of about 13mm diameter and 0.3mm in thickness through which the beam of spectrometer passed and the results were interpreted in a graphical form.

2.7.2 SEM (Scanning Electron Microscope)

To evaluate the size of the particle, surface topography SEM analysis was carried out using S-3400n SEM-Hitachi at a magnification range of 12 X to 1,00,000X at Anna University, Chennai⁹. The drug was fixed on specimen stub, placed inside the microscope's vacuum column evaporator and a beam of electrons passed from an electron gun, travelled through a series of magnetic lenses. The electrons are counted by the detector and the signals are sent to the amplifier. The number of electrons dispersed from each spot of the drug builds up the resultant image. The micrographs got sufficient statistics about the structure of the drug.

2.7.3 Energy Dispersive X-ray Analysis(EDAX)

The SEM device prepared with EDAX allowed the instrument to find the composition of elemental investigation of the drug Siromani Boopathy Kuligai.

2.7.4 X- Ray Fluorescence spectroscopy (XRF)

2 grams of boric acid was filled in the aluminium cups. 1 gram of test drug was uniformly covered over the boric acid. about 34mm diameter of aluminium cup was made using 25 tons of hydraulic press. Then drug was analyzed by using XRF spectrometer (Bruker S8 Tiger) in Centre for Advanced Research in Indian System of Medicine (CARISM) SASTRA University, Thanjavur. X-rays must be generated using 4KW, Rh anode X-ray tube.

3. RESULTS

3.1 Organoleptic & physicochemical evaluation

The drug SBK appears brick red in colour, having characteristic odour, solid in nature, hard consistency and spherical in shape. The drug is weakly basic in nature having a pH of about 7.5 and the size of the pill is 4.364 mm. The values were noted in the Table no.1 and Table no.2. The total ash value is 9.24% w/w, acid insoluble ash value is 0.76% w/w, water soluble ash is 6.3% w/w, moisture content is 6.11% w/w, and alcohol soluble extractive value is 5.89% w/w and, water soluble extract is 8.86% and, loss of weight at 105° C is 6% w/w and soluble in most of the solvents.

Table 1: Organoleptic characters of Siromani Boopathy Kuligai

S. No	Parameter	Results
1	Colour	Brick red
2	Odour	Characteristic odour
3	State of matter	Solid
4	Consistency	Hard
5	Shape	Spherical
6	Size	4.364mm

Table 2: Physicochemical characterization of Siromani BoopathyKuligai

1	Solubility <ul style="list-style-type: none"> ▪ Distilled water ▪ Ethanol ▪ Petroleum ether ▪ Propylene glycol ▪ Benzene ▪ Toluene ▪ Chloroform ▪ Ethyl alcohol ▪ Xylene ▪ Carbon tetrachloride 	Soluble in water <ul style="list-style-type: none"> ▪ Soluble ▪ Soluble ▪ Sparingly soluble ▪ Soluble ▪ Soluble ▪ Soluble ▪ Soluble ▪ Soluble ▪ Sparingly soluble ▪ Sparingly soluble
2	pH	7.5
3	Total Ash	9.24%w/w
4	Acid insoluble ash	0.76%w/w
5	Water Soluble Ash	6.3%w/w
6	Moisture content	6.11 % w /w
7	Water Soluble Extractive	8.86 % w/w
8	Alcohol soluble Extractive	5.89%w/w
9	Disintegration Time	35 min

3.2 Traditional test for pill

3.2.1 Character Inference

Non sticky on rolling +
No cracks over the surface after drying +
Shall be rolled uniformly over the plane surface+

3.3 Results of basic and acidic radical studies

From the basic and acidic radical studies, the drug Siromani Boopathy Kuligai had Potassium, Magnesium, Mercury and Sulphate which were shown below in the Table no.3.

Table 3: Results of basic and acidic radical studies

Parameter	Result
Test for Potassium	+
Test For Magnesium	+
Test For Mercury	+
Test for Sulphate	+

3.4 Fourier Transform – Infra Red (FTIR) Spectroscopy

The results of Table no.4 and Figure no.1 demonstrated that the drug Siromani Boopathy Kuligai contains phenols and alcohols, alkanes, alkenes, primary amines, nitro groups, ester and poly sulfides as functional groups. These functional groups are responsible for thrombolytic activity.

Table 4: Results of FTIR analysis of SBK

Absorption peak cm-1	Stretch	Functional Group
3410.8	Hydrogen-bonded O-H stretch	Phenols and alcohols
2928.1	H-C-H Asymmetric and Symmetric stretch	Alkanes
2370.1	-	Amines
2345.1	-	Amines
1646.7	C-C≡C Symmetric stretch	Alkenes
1560.0	N-H bend	Amines primary
1383.1	N=O bend	Nitro groups
1035.4		Ester
775.5	C-F Stretch	Aliphatic Fluoro compounds
605.1	C-Cl Stretch	Aliphatic Chloro compounds
458.5	S-S Stretch	Poly Sulfides

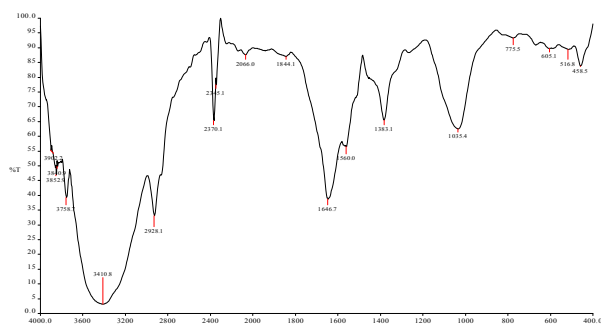


Fig. 1: showing the graph of FTIR spectroscopy
Full scale counts: 10964

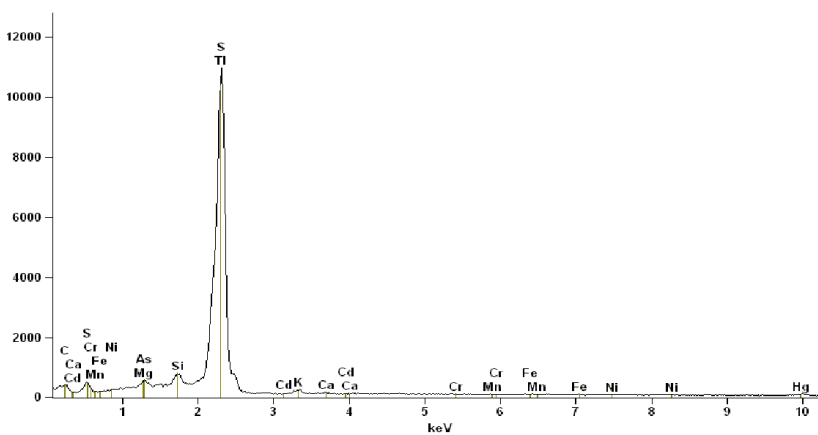


Fig. 3: Showing the graph of EDAX

Table 5: Results of SBK by EDAX analysis

Element	Net Counts	Weight %	Atom %
C	1454	6.72	33.72
Mg	397	0.08	0.20
Si	1574	0.31	0.66
S	82086	22.56	42.41
K	1739	0.71	1.09
Ca	408	0.18	0.28
Cr	43	0.03	0.04
Mn	25	0.02	0.03
Fe	227	0.24	0.26
Ni	78	0.12	0.12
Cd	686	0.45	0.24
Hg	2351	55.65	16.72
Tl	30591	12.12	3.57
Total		100.00	100.00

3.5 Scanning Electron Microscopy (SEM)

SEM analysis of the drug Siromani Boopathy Kuligai revealed the presence of nano and near nano particles of size 79.4 nm, 89.3 nm, 121 nm and 139 nm. The Figure no.2 showed the particles had irregular morphology.

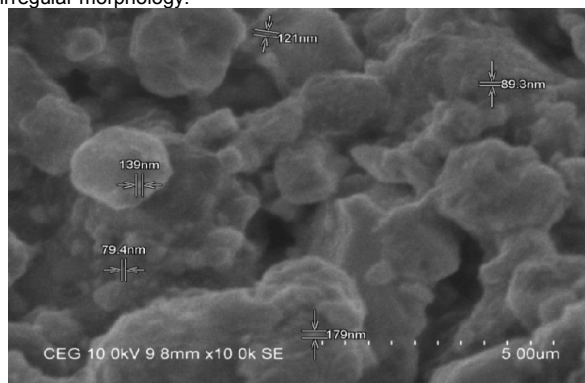


Fig. 2: SEM picture showing nano and near nano particles

3.6 EDAX Analysis

Composition of elements of the drug was detected by EDAX analysis and the results were tabulated in the Table no.5. From this analysis it was confirmed that the drug Siromani Boopathy Kuligai had 16.72 % of mercury and 42.41 % of sulphur and magnesium, calcium in traces. SEM and EDAX provide good estimate of the concentration of main elements in the drug. Furthermore, it provides useful information in the distribution of the elements forming the drug and their sample chemical form.

3.7 XRF Analysis

From the analysis of the Table no.6 mentioned below, the drug revealed the presence of various elements in oxide form and elemental form.

Table 6: Showing the Quantitative results of SBK by XRF analysis Element in oxide form Element form

Formula	Concentration (%)	Formula	Concentration (%)
S	77.81	SO ₃	77.85
Hg	19.32	Hg	19.32
Zn	0.54	ZnO	0.54
Se	0.52	Se ₂ O ₃	0.50
K	0.44	K ₂ O	0.47
Na	0.24	Na ₂ O	0.25
Cl	0.18	Cl	0.24
Mg	0.22	MgO	0.26
P	0.06	P ₂ O ₅	0.11
Al	0.06	Al ₂ O ₃	0.06
Fe	0.05	Fe ₂ O ₃	0.08
Mn	0.05	MnO	0.08
Ca	0.07	CaO	0.11
Pb	0.04	PbO	0.04
Tl	0.04	As ₂ O ₃	0.03
As	0.03	Tl	0.04
Si	0.02	SiO ₂	0.03

4. DISCUSSION

Siromani Boopathy Kuligai is indicated for Thrombolytic activity in classical Siddha literature. The preparation stages of SBK were followed strictly for maintaining the safety, quality and efficacy as well as purification and detoxification of ingredients such as Cinnabar and Sulphur. During repeated trituration with Ginger juice along with decoction of Sandal produce a well blinded, solid, soluble brick red and stable pill.

This drug was soluble in almost all the solvents, sparingly soluble in some of the solvents thereby proving its efficiency of solubility in the stomach and indirectly the bio-availability. The ash values were beneficial in evaluating the identity and purity of ingredients. The total ash was found to be 9.24% indicated a high amount of organic matter and a significant amount of minerals¹⁰. The acid insoluble ash value was 0.76% w/w indicating presence of low siliceous matter present in SBK. The water and alcohol soluble extractive values showed the occurrence of concentration of organic compounds in SBK. The loss on drying at 105°C indicates that only 0.30 g (6%) of water and volatile components have been lost when 1g of SBK kept at 105°C. This moisture content helped to prevent deprivation of efficacy and disintegration of SBK¹¹. The pH value at 25°C was found to be 7.1 to 7.5 indicating weak basic nature of the drug.

The results of preliminary basic radicals revealed the presence of potassium, magnesium, calcium. Potassium acts as a vasodilator, and brings blood flow freely in body, avoid the formation of clot and reduce the cause of stroke. Magnesium enhances the function of the endothelium, which is the inner most layer of blood vessels. On inhibition of calcium, it regulates the coagulation process. The transportation of calcium is regulated thus protecting calcium overload, so it is important to maintain the equilibrium of circulatory system as Ca promotes the blood clot formation and Mg promotes its destruction. The ratio of Ca and Mg must be balanced below 4 to 1 to maintain the healthy formation and destruction of blood clots. If it is above this ratio it forms Pathological blood clot. It inhibits the aggregation of platelets.

FTIR analysis of SBK showed 18 peaks. The results showed the existence of functional groups like poly phenols and alcohols, esters, alkanes, aromatic amines. Polyphenols Gingerol (Ginger) inhibited thromboxane-mediated platelet aggregation¹². Epidemiological studies suggest that high dietary intake of polyphenols is associated with decreased risk of a range of diseases including cardiovascular disease (CVD). In particular, a group of polyphenols known as flavonoids have been strongly linked with beneficial effects in many human, animals and in vitro studies. With respect to cardiovascular health, flavonoids may inhibit platelet aggregation¹³. Decrease vascular cell adhesion molecule expression; improve endothelial function¹⁴⁻¹⁵.

SEM analysis of the drug Siromani Boopathy Kuligai revealed the presence of nano particles of size 79.4 nm, 89.3 nm, 121 nm and 139 nm. The particles of size nano and near nano size showed that the drug may easily enter the cells at the molecular level to treat the

disease rapidly and increase the therapeutic effect. Nano medicine has its benefit in the treatment for the diseases of cardio vascular system, cancer and nervous system etc. The advantage of the nano particle is its smaller size which enhances the solubility, bioavailability of the drug and avoids macrophage clearance. It reduces possibly of side effects.

XRF studies the drug SBK demonstrated that Selenium (Se) helps to prevent abnormal Blood clotting (by facilitating the endogenous production of Prostacyclin). Manganese (Mn) is functioning with vitamin K, manganese plays a role in the regulation of blood clotting. Zinc (Zn) has been used in traditional medicine for everything from healing wounds to preventing blood clots. It can also help increase the blood flow. It has anti-oxidant properties that protect the cells from damage. It can produce healthy veins and arteries that enhance the blood circulation.

5. CONCLUSION

In the present study it is concluded that the organoleptic characters and physico-chemical parameters such as the total ash value (9.24%w/w), acid insoluble ash value (0.76%w/w), water soluble ash value (6.3%w/w), Moisture content (6.11%w/w), pH value (7.5) can be efficiently used for standardization of herbomineral formulation. The water soluble extractives and alcohol soluble extractives (8.86% w/w) and (5.89 % w/w) also helps in standardization. The sophisticated analysis of instruments like FT-IR spectroscopy revealed the existence of functional groups like phenols and alcohols, alkanes, alkenes, primary amines, nitro groups, ester and poly sulfides are primarily responsible for Thrombolytic activity. SEM analysis of the drug revealed the presence of nano and near nano particles. nano and near nano particle sizes of SBK may allow better absorption. EDAX analysis validated that SBK sample had minerals like carbon, magnesium, mercury, sulphur, chloride, potassium, calcium which may be responsible for thrombolytic activity. XRF analysis demonstrated the drug had mercury, sulphur, selenium, zinc, magnesium, manganese, sodium, calcium, and potassium. These elements may be responsible for thrombolytic activity.

The present study evaluated the Physico chemical properties of Siddha herbo mineral formulation Siromani Boopathy Kuligai. The results of FTIR, SEM with EDAX, XRF studies can be used as outstanding finger prints for physico chemical validation of the medicine. This study is earnest attempt to making appropriate scientific validation of Siddha herbo mineral medicine.

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