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Physicochemical characterization for the quality assessment of a Siddha herbomineral pill – *Karpoora chindhamani mathirai*

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ABSTRACT

Karpoora Cinthamani Mathirai (KCM) is a traditional Siddha medicinal preparation using to treat Arthritis associated with fever narrated in the text *Anubhoga Vaithiya Navaneetham*. This formulation has the detoxified ingredients such as *Hydrargyrum subchloride* and *Croton tiglium* seeds. The aim was to establish a fingerprint to ensure the quality and safety of KCM. Physicochemical characterization of KCM was carried out using qualitative biochemical analysis and modern techniques such as Fourier transform infra-red spectroscopy, inductively coupled plasma analysis and scanning electron microscopy. Physical evaluation revealed that KCM is a light green colour pill, neutral nature and having solubility in water and HCl with stabilized particle size distribution of 3µ. A clearly identifiable fraction of KCM particles were below 50 nm. The presence of nano sized particles and functional groups carboxylic acids and nitrocompounds in KCM might impart the therapeutic property. Trace elemental analysis of KCM revealed that heavy metals like arsenic, cadmium, mercury and lead were below the deduction limit. Further, elemental analysis of KCM revealed the presence of minerals like calcium, iron, potassium, sodium, and phosphorus under acceptable limits at the prescribed dose of KCM.

INTRODUCTION

Siddha system of medicine is one among the Indian system of medicines has been practicing in Tamil Nadu, Kerala, Malaysia, Singapore, Srilanka, and other Indian Ocean countries. The unique nature of this system is its continuous services to humanity for more than 10000 years in combating diseases and in maintaining the physical, mental and moral health. Siddha medicine incorporates wide usage of heavy metals and minerals for curing chronic illness. The scientific evaluation is needed to validate its preciousness. The Siddha system has not only the curative and preventive effects on different diseases but also paves the way for longevity and immortality. WHO has also recognized Indian system of medicine has an effective alternative medicine in the place of conventional allopathic system of medicine. In spite of strong efficacy in Siddha system, it is facing crisis in getting appreciation among the mass. The western scientific community condemned the Indian system of medicine to market the drugs reporting the presence of heavy metals like Lead, Cadmium, Arsenic and Mercury. JAMA (Robert Saper *et al*., 2004) published an article reporting that the presence of heavy metals over the permissible limit of WHO in Ayurvedic drugs which were imported. This issue made the western countries bans the traditional drugs in the market. Department of AYUSH, Govt. of India made valuable effort in overcoming this issue and strongly incised to do characterization and toxicological studies in experimental animals for proving the safety of the traditional drugs. In view of this issue, we validated the safety and efficacy of a Siddha pill "*Karpoora Cinthamani Mathirai"* which is indicated for fever associated with arthritis by analyzing the physical, chemical and physico-chemical properties. *Karpoora Cinthamani Mathirai* has the toxic ingredients such as *Hydrargyrum subchloride* and the seeds of *Croton tiglium* (Abdhula, 2006). But, there is no scientific validation of safety profile behind this formulation. Now, we have established the fingerprint including metallic content data for this pill pave the way towards quality standard.

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MATERIALS AND METHODS

Collection of raw ingredients and chemicals

Pooram (Calomel – *Hydragyrum subchloride* – Hg_2cl_2) were procured from country drug store at Chennai and authenticated by the chemist of Siddha Central Research Institue, Chennai. *Sadhikkai* (*Myristica fragrans*), *Nervalam* seeds (*Croton tiglium* seeds), *Karuvelam pisin* (*Acacia arabica* gum), *Vetrilai* (Betel leaf), *Milagu* (Black Pepper – *Piper nigrum*) were procured from raw drug shop at Chennai.

Elumitchai pazam (Lemon - *Citrus limon*) were collected from local market, Chennai and all herbal drugs were authenticated by the botanist of Siddha Central Research Institute, Chennai. Concentrated cow's dung juice was obtained from Cow's dung mixing with water. Raw *Pooram* – Sample A was subjected to qualitative and elemental analysis through ICP-OES instrument at IIT, Chennai before purification of *Pooram.* Analytical grade chemicals were procured from Golchha Chemicals Ltd., Jamshedpur and Himedia laboratories, Mumbai.

Purification and detoxification of toxic ingredients

Pooram and *Nervalam* seeds were the toxic ingredients found in the preparation of KCM. So these raw drugs should be purified by the traditional Siddha method. Purification of *Pooram*: 50 g of sliced Betel leaves and 50 g of coarse powder of Black pepper were made into 250 ml hot decoction using 1000 ml of water. 5 g of *Pooram* was knotted in a cotton cloth and soaked in the decoction for 3 days. After 3 days, soaked *Pooram* was taken out and enveloped cloth was removed and purified *Pooram* – Sample B was obtained (Thiyagarajan, 2008).

Purification of Nervalam seeds

This was purified by boiling separately in equal proportion of Cow's dung juice, Cow's urine and juice of *Citrus limon* respectively for 1 h. Then washed with water and seed coat was removed and seeds were fried with Cow's ghee (Murugesa, 2008).

Preparation of extract for preliminary basic and acidic radicals studies

The sample A and B were subjected for qualitative analyses of cations and anions based on Asokan (2001) and Sofowora (1996). 5g of each sample was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then, it was boiled well for about 10 min and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water and used for the study.

Preparation of *Karpoora Chindhamani Mathirai* (KCM)

Ingredients

Purified *Pooram* (Calomel) - 21 g**,** *Sadhikkai* (*Myristica fragrans* seeds) - 21 g, Purified *Nervalam* (*Croton tiglium* seeds) - 42 g**,** *Sottrukatrazhai* (*Aloe barbadensis* juice- as required quantity for trituration and *Karuvelam pisin* (*Acacia Arabica* gum) - 4.2 g**.**

Method

Purified Pooram was taken in a *kalvam* (Stone mortar) and powdered well. *Sadhikkai* was powdered separately in a pulverizer. Purified *Nervalam* and *Sadhikkai* powder were added little by little respectively to the powdered Pooram and grounded well in the *kalvam* and triturated with the juice of *Katrazhai* for 6 h and made into a paste consistency form. Then, *Karuvelam pisin* was added to this paste and grounded well up to the nonsticky form of paste and rolled into pills (KCM) of 130 mg weight **–** Sample C (Abdhula, 2006).

Physico-chemical evaluation

KCM was subjected for the determination of physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, moisture content, foreign organic matter, alcohol and water soluble extractive and loss of weight at 105° C and limit tests for heavy metal contents according to the standard methods described in "The Ayurvedic Pharmacopoeia of India" (The Controller of Publications, India, 2007). The determination of Lead and Cadmium concentration were done by graphite oven method, for Arsenic by hydride method and for Mercury by cold absorption method using Atomic absorption spectrophotometer.

Fourier transform - Infra red (FTIR) spectroscopy study

IR data acquired with Perkin Elmer FT-IR Spectrometer carried out at SAIF, IIT Madras, Chennai-36. For sampling techniques, we follow KBr method (Price, 1972). The sample was grounded using an agate mortar and pestle to give a very fine powder. The finely powder sample was mixed with about 100mg dried KBr salt. The mixture was then pressed under hydraulic press using a die to yield a transparent disc (measure about 13mm diameter and 0.3mm in thickness), through which the beam of spectrometer passed.

Inductively coupled plasma optical emission spectrometry (ICP-OES) study

The experimental procedure was done at SAIF, IIT Madras, Chennai – 36 using Perkin Elmer Optima 5300 DV. The sample preparation for metal analyses was done by microwave digestion method (Charles, 1997). A 0.25 g of test sample was transferred into a liner provided with the instrument. To this, 9 ml of Nitric acid was added and mixed thoroughly and allowed reacting for few minutes.

Then, the liner was placed in the vessel jacket and the vessel was sealed and placed in the rotor and fixed in microwave. The vessel was heated up to 180°C for 5 minutes and held at 180°C for 10 minutes. The vessel was allowed to cool down below 60°C of vessel interior temperature and below 50°C of a vessel surface temperature and rotor was removed. The digested sample was made up to 100ml with millipore water. If visible insoluble particles exist, solution could be filtered through whatmann filter paper. The digested solution was transferred into plastic containers and properly labeled.

Scanned electron microscopy analysis (SEM)

To evaluate grain size, particle size distributions, material homogeneity and inter metallic distributions. The SEM was carried out by using FEI-Quanta FEG 200-High Resolution Instrument done at SAIF, IIT Madras, Chennai-36

RESULTS AND INFERENCES

Qualitative analysis of *Pooram* before and after purification

The result of table 1 shows that the sample A (before purification) contains Sodium, Magnesium, Calcium, Aluminium, Potassium, Ammonium and Ferrous iron. It also contains toxic metals such as Arsenic, Mercury, Lead and Zinc. But after purification of sample A, the toxic metals such as Arsenic, Mercury and Lead were removed. Only the trace metals such as Ammonium, Sodium, Aluminium, Potassium, Calcium, Ferrous iron and Zinc were found in sample B (after purification). From the table 2, we infer that the sample B was free from Sulphate and had the presence of Chloride, Phosphate, Flouride, Oxalate and Nitrate.

Finished form of test drug - *Karpoora Cinthamani Mathirai* (KCM – Sample C)

The KCM was prepared following the literature containing the knowledge of Siddha system using sample B and other ingredients. The finished KCM gave positive results to all traditional quality tests (Table 3) for *Mathirai* as mentioned in Siddha Gunapadam literature.

Physico Chemical Parameters

The KCM looks light green colour under normal vision and ultraviolet rays in solid nature having weak basic nature. The table 4 depicts the standard quality of KCM will be followed in future for quality validation. All the values noted in the table 4 should not be exceeding in future preparation of KCM. The total ash value is 9.54% w/w, acid insoluble ash value is 0.96% w/w, water soluble ash is 7.3% w/w, moisture content is 7.11% w/w, foreign organic matter 6% w/w and alcohol soluble extractive value is 6.89% w/w and, water soluble extract is 9.86% w/w and, loss of weight at 105° C is 7% w/w. The limit tests for heavy metals (Table 5) depicts that Lead, Cadmium and Arsenic were within the permissible limit laid down by WHO rather mercurial concentration was 3.115 ppm above the permissible limit.

Fourier transform – Infra red spectroscopic analysis

The result of table 6 and figure 1 shows that KCM constitutes alcohols, phenols, aromatics, nitro compounds, alkanes, alkenes, carboxylic acids, primary amines, aliphatic amines and alkyl halides as functional groups.

Inductively coupled plasma optical emission spectrometric analysis

The result of table 7 shows that the Sample B has the presence of Mercury as major constituent but its concentration was decreased while comparing with raw *Pooram*. 150 ppm of mercury was eliminated from raw *Pooram* after purification. The amount of Sodium, Potassium, Calcium, phosphorus and Sulphur were also decreased after purification. The analyses of toxic heavy metals and ICP-OES result reveal KCM was free from Arsenic, Lead and Cadmium apart from Mercury. The presence of trace metals such as Calcium, Ferrous Iron, Potassium, Sodium and Phosphorous were in optimum concentration. Obviously, the concentrations of metals were decreased during processing of KCM while comparing with raw ingredients.

Scanned Electron Microscopic analysis

The figure 2 shows that the particles were all in stabilized form having irregular morphology and distributed in near nano range. KCM has the particle size range from 1 to 4 nm.

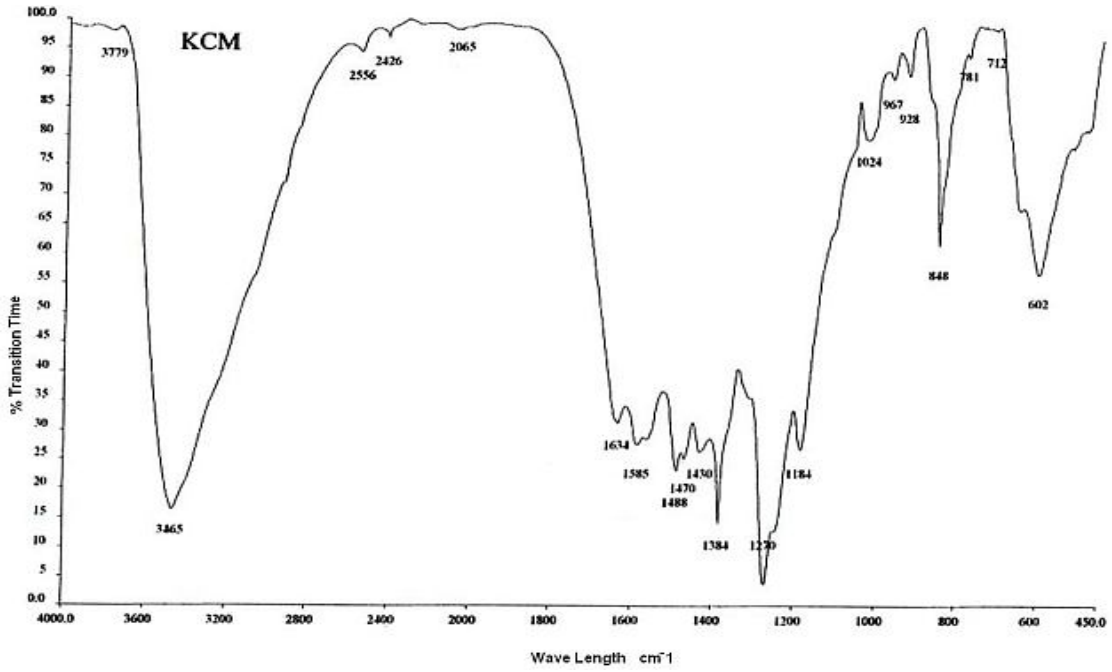
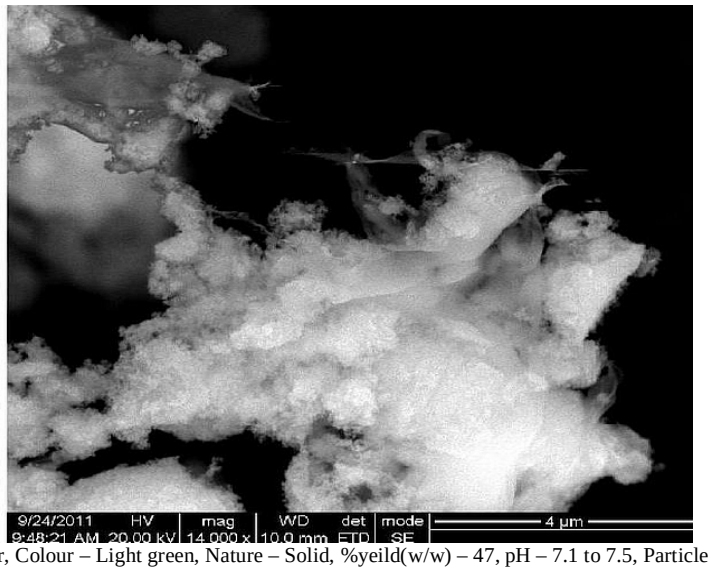


Fig. 1: FT-IR spectrm of KCM.



Extract solvent - Water, Colour – Light green, Nature – Solid, %yeild(w/w) – 47, pH – 7.1 to 7.5, Particle size – 1 to 4 micron

Fig. 2: SEM image of KCM.

Table 1: Test for Basic radicals.

Test	Reagents used	Positive observation appeared	Sample A	Sample B
Ammonium	1. Nessler's reagent, NaOH	Brown precipitate	+	+
	2. NaOH, Δ	Ammonia gas smell	+	+
Sodium	Pasted in $Co(NO_3)_2$, Δ in Bunsen flame	Yellow colour flame	+	+
Aluminium	Nessler's reagent	No purple precipitate	+	+
Potassium	NaOH, $Co(NO_3)_2$, 30% glacial acetic acid	Yellow precipitate	+	+
Calcium	1. K_2CrO_4	No precipitate	+	+
	2.NH_4Cl, NH_4OH, Ammonium oxalate	White precipitate -Insoluble in Acetic acid	+	+
Ferrous iron	$K_3[Fe(CN)_6]$	Blue precipitate	+	+
Zinc	1. NaOH	White precipitate	+	+
	2. $K_4[Fe(CN)_6]$	White precipitate -Soluble in NaOH		
Arsenic	Pure Zn, dil. H_2SO_4, $AgNO_3$ soaked filter paper	Paper turns yellow then black	+	-
Mercury	1. dil. HCl, boil with water, NaOH,	No precipitate	+	-
	2. dil. HCl, H_2S gas, boil with dil. HNO_3, $SnCl_2$/KI	Grey/Red precipitate	+	-
Lead	1. K_2CrO_4	Yellow precipitate	+	-
	2. KI / Hot H_2O	Yellow precipitate / glittered like gold	+	-

Sample A - Raw Pooram before purification, Sample B - Pooram after purification. All other test for basic radicals gave negative inference in both samples. Here, only positive inferences were tabulated.

Table. 2: Test for Acidic radicals.

Test	Reagents used	Positive observation appeared	Sample A	Sample B
Sulphate	dil. H_2SO_4, $BaCl_2$	Effervescence, White precipitate	+	-
Chloride	Chromyl chloride test	Yellow precipitate	+	+
Phosphate	1. Ammonium molybdate test	White precipitate	+	+
	2. Magnesia mixture test	White crystalline	+	+
Flouride	Boron trifluoride test	Green flame	+	+
Oxalate	dil. H_2SO_4, MnO_2	Colourless gas turning lime water milky	+	+
Nitrate	Brown ring test	Brow ring at junction	+	+

Sample A - Raw Pooram before purification, Sample B - Pooram after purification. All other test for acidic radicals gave negative inference in both samples. Here, only positive inferences were tabulated.

Table. 3: Traditional test for pill.

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

Table. 4: Physicochemical properties of KCM.

Parameters	Values obtained (%w/w)
Total ash value	9.54
Acid insoluble ash	0.96
Water soluble ash	7.3
Moisture content	7.11
Foreign organic matter	6
Alcohol soluble extractive	6.89
Water soluble extractive	9.86
Loss of weight at 105^0 C	7

Table. 5: Limit tests for KCM.

Heavy metal contents	Permissible limits	Amount found
Lead	10 ppm	BDL
Cadmium	0.3 ppm	BDL
Mercury	1 ppm	3.115 ppm
Arsenic	3 ppm	BDL

BDL – Below detection limit, ppm – Parts per million

Table. 6: FT-IR characterization of KCM.

Peak value	Group frequency (cm^{-1})	Bond	Compound type
3779 cm^{-1}	-	-	Unknown compound
3465 cm^{-1}	3500-3200	O–H stretch	Hydrogen bonded -alcohols, phenols
2556 cm^{-1}	3400-2400	O–H stretch	carboxylic acids
2426 cm^{-1}	-	-	Unknown compound
2065 cm^{-1}	-	-	Unknown compound
1634 cm^{-1}	1650-1580	N-H bend	Primary amines
1585 cm^{-1}	1600-1530	-NO2	Aliphatic nitro groups
1488 cm^{-1}	1500-1400	C-C stretch (in ring)	aromatics
1470 cm^{-1}	1470-1350	C-H bend	alkanes
1430 cm^{-1}	1470-1350	C-H bend	alkanes
1384 cm^{-1}	1390-1260	NO2 symmetrical stretch	Aliphatic nitro compounds
1220 cm^{-1}	1260-1000	C-O stretch	Alcohols, ethers, carboxylic acids, esters
1184 cm^{-1}	1260-1000	C-O stretch	Alcohols, ethers, carboxylic acids, esters
1024 cm^{-1}	1250-1020	C-N stretch	Aliphatic amines
967 cm^{-1}	1000-650	=C-H bend	alkenes
928 cm^{-1}	950-910	O-H bend	Carboxylic acids
848 cm^{-1}	870-675	C-H bend	Phenyl ring substitution bands
781 cm^{-1}	850-550	C-Cl stretch	Alkyl halides
712 cm^{-1}	715-685	C-H bend (mono)	aromatics
602 cm^{-1}	650-600	acetylenic C-H bend	alkynes

Table. 7: ICP- OES analyses.

Elements	Wave number (nm)	Sample A (ppm)	Sample B (ppm)	Sample C (mg/L)
Arsenic	193.696	BDL	BDL	BDL
Mercury	253.652	241.59	100.23	3.115
Lead	230.204	BDL	BDL	BDL
Cadmium	226.502	BDL	BDL	BDL
Ferrum	238.204	-	-	2.365
Sodium	589.592	3.29	1.24	20.234
Potassium	766.490	17.13	12.16	52.985
Calcium	317.933	23.70	20.26	16.152
Phosphorus	213.617	8.12	7.16	11.748
Sulphur	181.975	14.21	10.51	-

Sample A - Raw Pooram before purification, Sample B - Pooram after purification, Sample C - KCM

DISCUSSION

The *Karpoora Chindhamani Mathirai* (KCM) is used as an anti inflammatory drug in the treatment of arthritis associated with fever under Siddha medicine (Abdhula, 2006). The preparation of KCM is a very complex process has been followed strictly for maintaining the safety, quality and efficacy including purification and detoxification of toxic ingredients such as *Pooram* - *Hydrargyrum subchloride* and *Nervalam* seeds - *Croton tiglium*. *Pooram* possess germicide, diuretic, sialagogue, alterative, cholagogue and purgative properties (Thiyagarajan, 2008). The purified *Pooram* could be administered up to the dose of 200 g along with sugarcane jaggery for 7 days cures all types of painful disorders (e.g. cancer and abdominal pleural pain). Ingestion of improper purified *Pooram* might produce serious adverse events such as an erosion of mucous membrane of intestinal tract, bloody watery stools, glomerular infiltration and liver congestion (Narayan, 2010). The other toxic ingredient, *Croton tiglium* seeds are known to be purgative croton contains fatty fixed oil, tigilinic acid, crotonic acid and croton oil. Crotonoleic acid, an active principle in the croton oil internally act as purgative. Under desirable amount, croton seed oil has therapeutic value such as a good anti inflammatory agent against rheumatism (Nadkarni, 2010). The other ingredients *Acacia arabica* gum, *Myristica fragrans* seed and *Aloe barbadensis* juice have synergistic therapeutic effects while adding with the above purified toxic compounds. All the ingredients have rationale of good anti inflammatory properties.

During the purification of *Croton tiglium* seeds, boiling with cow's dung juice and urine and lemon juice removes the higher concentration of crotonoleic acid to make the Croton seeds suitable for ingestion. The betel leaf and black pepper are known to be a good detoxifying agent used in the purification of *Pooram* (Calomel). The decoction of betel leaf and pepper detoxify the raw *Pooram* by reducing the trace and toxic heavy metals concentration to desirable quantity and paves the *Pooram* suitable for the usage in various preparations of Siddha formulations.

During repeated trituration with *Aloe barbadensis* juice along with *Acacia arabica* gums and *Myristica fragrans* seeds produce a well blinded, solid, soluble, light green and stable pill KCM due to the presence of mucilage, starch and tannin in the ingredient. The foreign organic matters present in the KCM should not be more than 6%. The loss on drying test at 105°C indicates that only 0.35 g (7%) of water and volatile component have been lost when 1g of KCM kept at 105°C. This moisture content helps to prevent degradation of efficacy and disintegration of KCM. So, the shelf life has been validated up to one year as mentioned in Siddha literature. The pH value at 25°C found to be 7.1 to 7.5 leading to state that it is in weak basic nature might be due to the presence of aleosone, aloesine and glucose content which have been derived from *Aloe barbadensis* juice. The ash values and foreign organic matters quantity are useful to judge the identity and purity of ingredients. The acid insoluble ash value should not be more than 0.96% w/w indicates lesser amount of siliceous matter present in KCM. The water and alcohol soluble extractive values indicate the presence of lower concentration of Organic compounds in KCM. The FT – IR analysis of KCM was done and the functional groups associated were determined. The FT-IR spectrum of KCM was obtained twenty effective peaks lying between 4000 cm^{-1} – 450 cm^{-1} This FT-IR fingerprint for KCM can be used as standard to ensure the quality and batch to batch consistency by comparing the frequency peaks of among the different sample. This analysis demonstrated the existence of nitrocompounds, carboxylic acids, alcoholic and phenolic compounds as the key components of KCM. The quantitative ICP analyses reveal heavy metals like Arsenic, Cadmium and Lead in KCM were below the detection limit and, Sodium, Potassium, Calcium, Phosphorous and Sulphur were in essential nutritional concentration. Only the quantity of Mercury was in above 100 ppm (above the permissible limit laid by WHO). But in the clinical practice, KCM does not produce any toxic manifestations on prescribed dose and duration as per Siddha text. While, continuous administration of KCM beyond 40 days and at high dosage beyond 130 mg pill per time may produce adverse reactions due to ingestion of higher concentration of Hg. So, we have to do future work related to the form of mercury present in KCM and also to justify the safety of KCM in animal. The scanning electron microscope study (Plate 1) revealed the size stabilization of particles on process and the presence of nano sized particles. Nanosized particles can attach with the cell surface and diffuse readily inside the cells. Thus, the size of particle is able to influence the efficacy at the targeted sites escape from the hepatic and renal pathway alters its plasma half time period.

CONCLUSION

Inspite of long usage of *Karpoora Chindhamani Mathirai* in Indian system, the confirmation of nano particles and nil content of heavy metals rather than Mercury favor the KCM as a safe drug under Siddha system.

The purification and repeated trituration process of mentioned period definitely impart specific physicochemical characters to *Karpoora Chindhamani Maathirai* which might be responsible for the safety and potent therapeutic activity of this pill. But in future work, we are in position to prove its safety in animal model because of the presence of Hg beyond WHO permissible limit and analyze the mercurial form.

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