Asian Journal of Chemistry; Vol. 35, No. 8 (2023), 1793-1798

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.27712

Standardization and Surface Characterization Studies of a Siddha Formulation: *Chandamarutha centhuram*

SRIDHARAN SRIRAM, VELLINGIRI VADIVEL, PERUMAL RAJALAKSHMI* and PEMAIAH BRINDHA

Centre for Advanced Research in Indian Systems of Medicine, School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur-613401, India

*Corresponding author: E-mail: rajalakshmi@carism.sastra.edu

Received: 20 February 2023; *Accepted*: 29 May 2023; *Published online*: 31 July 2023; AJC-21309

Chandamarutha centhuram is an important and popular metal-based Siddha preparation and mainly used for all types of arthritis, lupus erythematosus, gout, infections, parkinsonism, hemiplegia, delirium, throat infection and all types of fever. In present work, three batches of *Chandamarutha centhuram* were procured from the same manufacturer and subjected to standardization and surface characterization studies. Elemental analysis was performed using XRF and surface characterization studies were done employing sophisticated instrumentation techniques like XRD and SEM, which revealed the size of the particle ranging from 6.92 µm to 55.46 µm. The FTIR studies demonstrated peaks in the region at 3851.62 cm^{-1} to 592.50 cm^{-1}. Particle size analysis and thermal stability studies were carried out using zeta sizer and TG-DTA. Data of physico-chemical analysis revealed the total ash content of the samples to be below 1.5%, which indicated purity of the sample. While Batch I of *C. centhuram* did not contain acid insoluble and water soluble ash, Batch II and III contained negligible amounts. The moisture content was below 3%. The XRF analysis showed the presence of mercury as the major constitutent (72.63-76.4%). The current information may prove helpful in establishing the physico-chemical criteria for the formulation and in deducing the surface characterisation of a chosen Siddha formulation, which hints at its nano-structure and nature.

Keywords: *Chandamarutha centhuram,* Physico-chemical parameters, Surface characterization.

INTRODUCTION

Siddha system of medicine is one of the ancient traditional health care systems, which flourished in South India. It has also been popular in Srilanka, Burma, Malaysia and other south East Asian countries [1]. In this system metals, minerals, plants and animal products are used to prepare medicines. Siddha Materia medica divided the medicines into 64 types of internal and external medicines [2] *Centhuram* is one among them. In the metallic preparation, special chemical process is involved to convert toxic metal and mineral into potent non-toxic therapeutic agents [3]. *Centhuram* are a category of medicines made from metals, minerals, arsenics or salts. The pharmaceutical processes adopted are grinding the metal with specified juices or distillates or extractives and subjecting the same to the metal used in the formulation process of calcinations or sublimation or burning or frying or exposing or incineration, till the characteristic reddening of the product is obtained. Generally, two methods are followed for *centhuram* preparations with some exceptions and variations [3,4]. Generally *centhurams* are red coloured powder having metal and mineral drugs and retain their effectiveness for 75 years [4].

Chandamarutha centhuram (CMC) is an important and popular metal-based medicine in Siddha system of medicine. It has five ingredients *viz.* (i) *Lingam* (red sulphide of mercury) 2 parts; (ii) *Pooram* (hydrargyrum subchloride) 1 part; (iii) *Veeram* (hydragyrum perchloride) half part; (iv) R*asa chenduram* (red oxide of mercury) half part and; (v) *Gandhagm* (elemental sulphur) half part [4-6]. It is a special medicine for all types of arthritis, which is proven in a clinical trial [7]. These ingredients are highly toxic, so they are used after subjecting to proper purification process using specific herbs. Medicine dosage is 30 to 60 mg administrated with honey for 5 days [4]. Food restriction such as exclusion of spice, chilly and tamarind and intake of only curd rice without salt is recommended during administration of *C. centhuram* [8].

In present study, the physico-chemical analysis of *C. centhuram* was performed in order to determine the quality standards of the formulation. Surface characterization studies were done using sophisticated instrument techniques like SEM, XRD, TG-DTA and Zeta sizer. The elemental composition was determined using XRF studies, while FTIR spectroscopy was used to determine if there were any interactions between the purifying agents and the starting metals.

EXPERIMENTAL

Chandamarutha centhuram was procured in three different batches from the local Siddha medicine dealer of Trichy city, India. It contains four forms of mercury drugs namely *Lingam* (red sulphide of mercury), *Pooram* (hydrargyrum subchloride), *Veeram* (hydragyrum perchloride) and *rasa chenduram* (red oxide of mercury) and elemental sulphur. Raw drugs used in *Chandamarutha centhuram* are presented in Fig. 1a-f.

Physico-chemical analysis: Physico-chemical analysis was performed three times according to standard procedures given in Ayurvedic Pharmacopoeia of India [9] and quality control methods for medicinal plant materials [10]. Loss on drying (LOD) was evaluated by placing 1 g of *C. centhuram* in a pre-weighed plate and kept in the hot air oven at 105 ºC and the LOD was measured. Total ash content of *C. centhuram* was estimated by adopting the standard procedure of Joshi & Aeri [11].

Characterization: The composition of three batches of *C. centhuram* were analyzed by XRF spectro-meter (S8 Tiger, Bruker AXS, Germany) using a 4 kW rhodium anode X-ray pipe. To carry out FTIR spectroscopic analysis (Spectrum100, Perkin-Elmer, USA), the samples were mixed with KBr powder and pelleted. The SEM (Scanning electron micrograph) analysis was performed utilizing a JSM6701F, Jeol, Japan instrument. The SEM image obtained can help in understanding the surface characters and its nano forms and size. The thermogravimetric analysis of the sample was done using TG-DTA instrument (SDT-Q600, TA Instruments, USA). Samples were heated at a temperature varying from 40 ºC to 830 ºC at 20 ºC/min in a nitrogen atmosphere. To estimate the crystalline structure at ambient temperature, the X-ray Diffractometer (D8 Focus, Bruker, Germany) was equipped with a Cu tube for generating Cu$K\alpha$ radiation (λ = 1.5406 Å), as an incident beam in the 2θ mode, with voltage 40 KV and current 30 mA.

Zeta sizer: The particle size and zeta potential were measured with Malvern Instruments' Zeta sizer, which covers the range of 0.6 nm to 6000 nm. The information gathered from the zeta sizer will be useful in identifying any aggregates of particles.

RESULTS AND DISCUSSION

Physico-chemical studies: Table-1 shows the physico-chemical parameters of three batches of *C. centhuram*. Its appearance was brick red coloured fine powder and odour slightly aromatic [12]. The total ash content of all three batch samples were below 1.5%, which indicated the purity of the medicine. While Batch I of *C. centhuram* did not contain acid insoluble and water soluble ash, Batch II and III contained negligible amounts. The moisture content was below 3%. Thus, it is evident that the preparation was free from moisture and unwanted chemical molecules.

XRF studies: Three batches of *C. centhuram* were analyzed for their elemental composition (Table-2). There were no major differences in the elemental concentration in all the three Batches except those 3 new elements (Mg, V and Ni) were found in Batch II. However, it was observed that mercury was present as the major

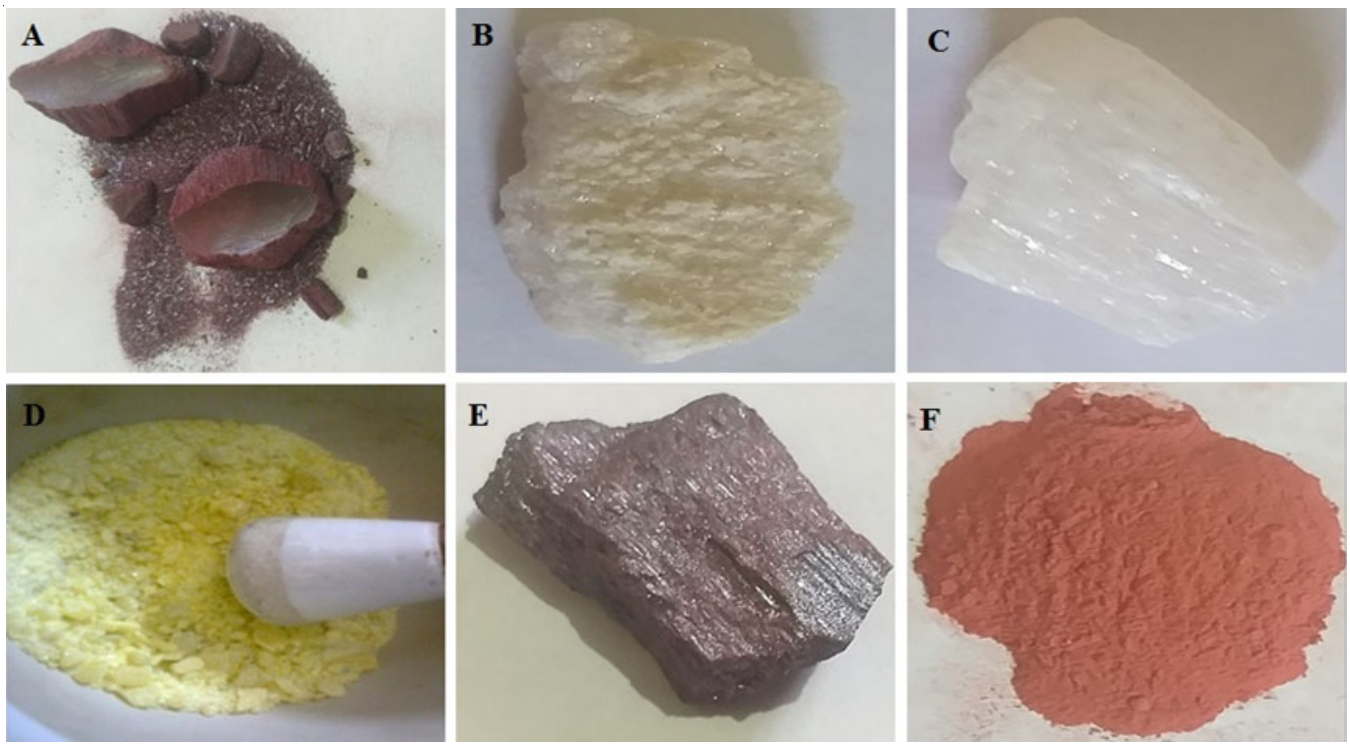

Fig. 1. Images of Lingam (A), Pooram (B), Veeram (C), Gandhagm (D), *Rasa chenduram* (E) and *Chandamarutha centhuram* (F)

TABLE-1
PHYSICO-CHEMICAL PARAMETERS OF ALL THREE BATCHES OF *Chandamarutha centhuram*

Standards determined (%)	Batch-1	Batch-2	Batch-3
Appearance	Light brick red coloured fine powder	Brick red coloured fine powder	Brick red coloured fine powder
pH (1%w/v suspension)	3.53 ± 0.01	4.95 ± 0.04	5.68 ± 0.02
Loss on drying (%, w/w)	1.498 ± 0.0125	2.047 ± 0.05	2.915 ± 0.07
Total ash (%, w/w)	1.152 ± 0.1980	0.459 ± 0.02	0.807 ± 0.003
Acid insoluble ash (%, w/w)	Nil	0.0592 ± 0.01	0.1642 ± 0.01
Water soluble ash	Nil	0.5623 ± 0.02	0.3199 ± 0.02

TABLE-2
XRF RESULTS OF ALL THREE BATCHES OF *Chandamarutha centhuram*

Components in oxide form				Components in elemental form			
Formula	Batch I	Batch II	Batch III	Formula	Batch I	Batch II	Batch III
Hg	52.38	48.23	45.74	Hg	76.4	74.73	72.63
SO_3	38.88	42.64	44.30	S	15.57	17.08	17.74
Cl	6.03	5.62	4.90	Cl	6.03	5.62	4.90
As_2O_3	1.39	1.72	3.54	As	1.06	1.30	2.68
Al_2O_3	0.30	–	0.44	Na	0.17	0.19	0.14
Na_2O	0.22	0.25	0.19	Al	0.16	–	0.23
MnO	0.15	0.15	0.13	Pb	0.12	0.14	0.28
Fe_2O_3	0.14	0.48	0.1	Mn	0.11	0.11	0.10
PbO	0.13	0.15	0.30	Tl	0.11	0.09	0.03
Tl	0.11	0.09	0.03	Fe	0.10	0.34	0.08
SiO_2	0.09	0.10	0.18	Se	0.06	0.05	–
SeO_2	0.08	0.08	–	Ca	0.05	–	0.09
CaO	0.06	–	0.12	Si	0.04	0.05	0.08
CuO	0.02	0.03	0.02	Cu	0.02	0.02	0.02
MgO	–	0.27	–	Mg	–	0.16	–
V_2O_5	–	0.18	–	V	–	0.10	–
NiO	–	0.02	–	Ni	–	0.01	–

components. Batch one contained 76.4% of mercury besides sulphur, chloride and arsenic whereas second batch consisted 74.73% mercury. In the third batch, mercury percentage was 72.63%. Aluminium was present in trace quantity in first and third batch but absent in second batch. Variations obtained in this elemental analysis may be attributed to the time and force applied during the purification process.

FTIR studies: In the first batch, the spectroscopic peaks were observed in the region of 3851.62 cm^{-1} to 601.58 cm^{-1}. Totally 7 peaks were obtained which included three hydrogen stretching regions, one triple bond region, one double bond region, two fingerprint region peaks and one peak in an unknown region (Fig. 2). In 2nd batch, the spectroscopic peaks were observed in the region of 3410.27 cm^{-1} to 604.26 cm^{-1} about eight peaks were obtained which included two hydrogen stretching regions, one triple bond region one double bond region, two fingerprint regional peaks and one peak in an unknown region. In 3rd batch, the spectroscopic peaks were observed in the region 3375.45 cm^{-1} to 592.50 cm^{-1}. Totally 10 peaks were obtained, which included two hydrogen stretching regions, two triple bond regions, one double bond region, three fingerprint regional peaks and one peak in an unknown region. This is in agreement with the work carried out previously on other herbo-metallic preparations [13]. A region at 2924 cm^{-1} indicated the presence of fatty acids dominated by the stretching of CH_3, CH_2 and CH. The peaks at 1800 and at 1680 cm^{-1} suggested the probable interaction with fat [14]. Similarly, the plant materials interaction was evidenced from the peaks obtained, which were almost comparable to that of amine, phenols and aliphatic amines present in the purifying agents [15]. These groups must have come from the primary/secondary metabolites present in the purifying agents and contributed in the complex formation.

Zeta sizer: In three batches of *C. centhuram*, the particles were present within the range of 478.9 nm, 356.7 nm and 570.2 nm in first, second and third batches, respectively (Fig. 3). The particle size distribution in second batch was comparatively very less (Table-3). Same technique was employed to a mercury based medicine of *Rasasindura* [16]. In all the three batches the particles were found to be aggregates of much smaller particles. The particle size of Batch II and Batch III *C. centhuram* were similar. These preparations generate a suspension of hydrophobic, negatively charged particles when mixed with water. The hydrophobic nature of these particles causes them to stick together, forming larger aggregates [17].

SEM studies: In the present study, SEM images of three batches of *C. centhuram* suggested the presence of large irregular nano-structural spherical particles (Fig. 4). In first batch, the particles were in a size ranging from 9.22 µm to 55.4 µm. Particles with a size ranging from 6.92 µm to 41.5 µm were observed in the second batch. In the third batch, particles were similar in size to second batch.

Thermal studies: TG-DTA curves of the three batches of *C. centhuram* are presented in Fig. 5. The thermal analysis of three different batches showed the endothermic peaks in

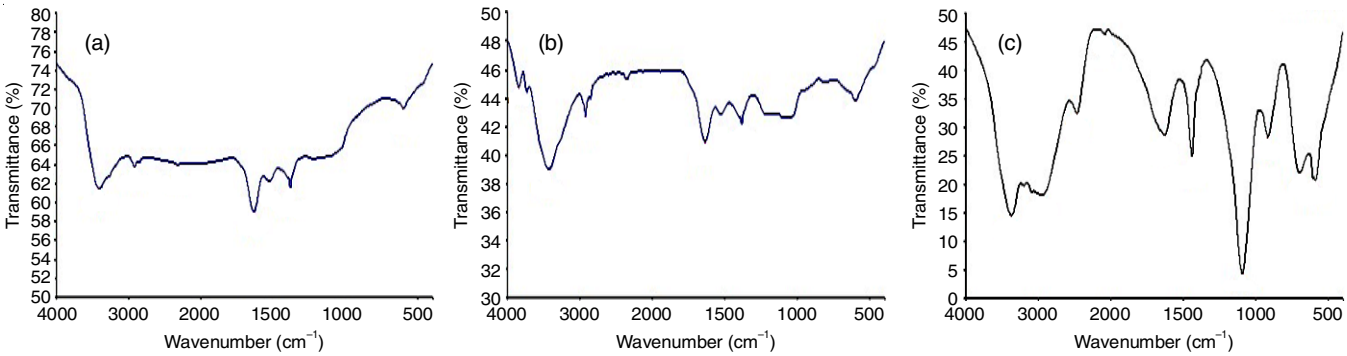

Fig. 2. FTIR spectrum of *Chandamarutha centhuram* (CMC) prepared from batch-1 (a), batch-2 (b) and batch-3 (c)

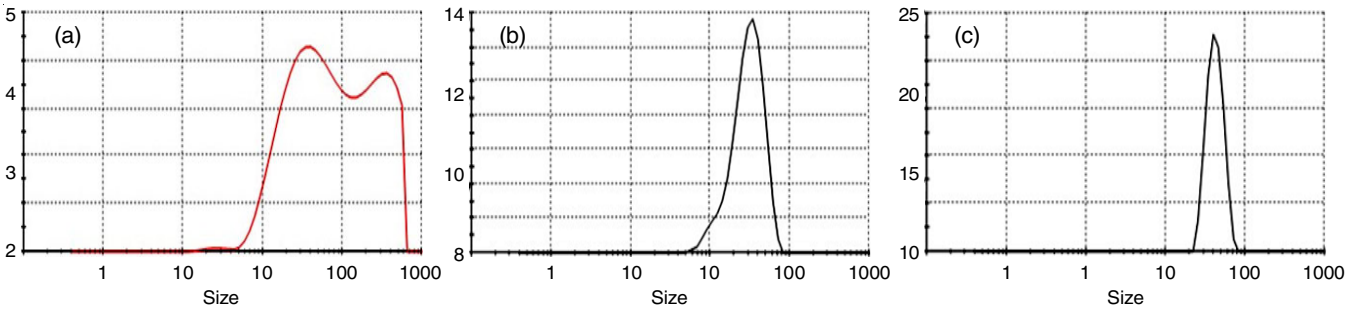

Fig. 3. Zeta sizer of *Chandamarutha centhuram* (CMC) from batch-1 with 478.9 nm diameter (a), batch-2 with 356.7 nm diameter (b) and batch-3 with 570.2 nm diameter (c)

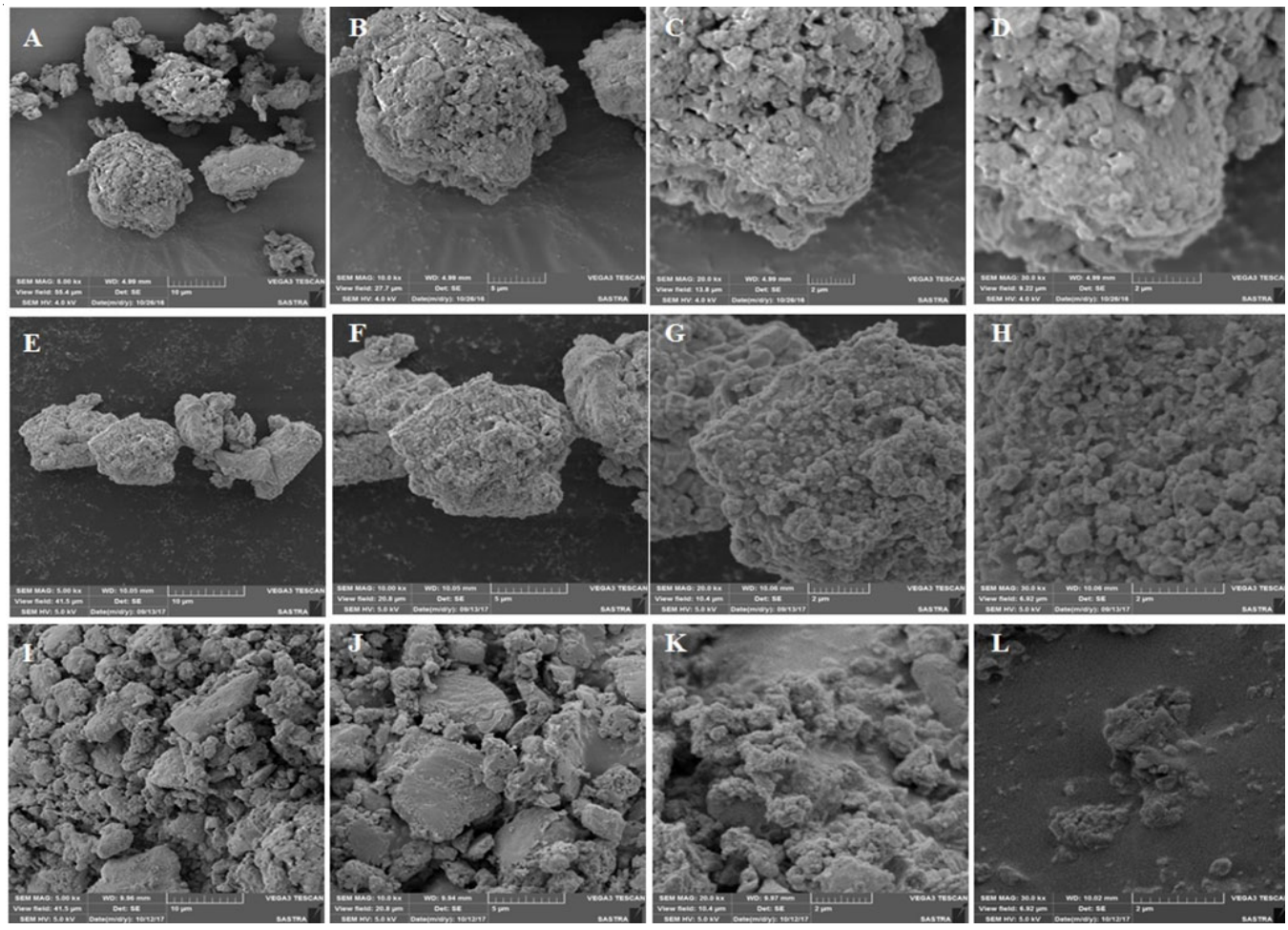

Fig. 4. SEM images of *Chandamarutha centhuram* (CMC) form batch-1 at 5 kx (A), 10 kx (B), 20 kx (C), 30 kx (D), batch-2 at 5 kx (E), 10 kx (F), 20 kx (G), 30 kx (H) and batch-3 at 5 kx (I), 10 kx (J), 20 kx (K), 30 kx (L)

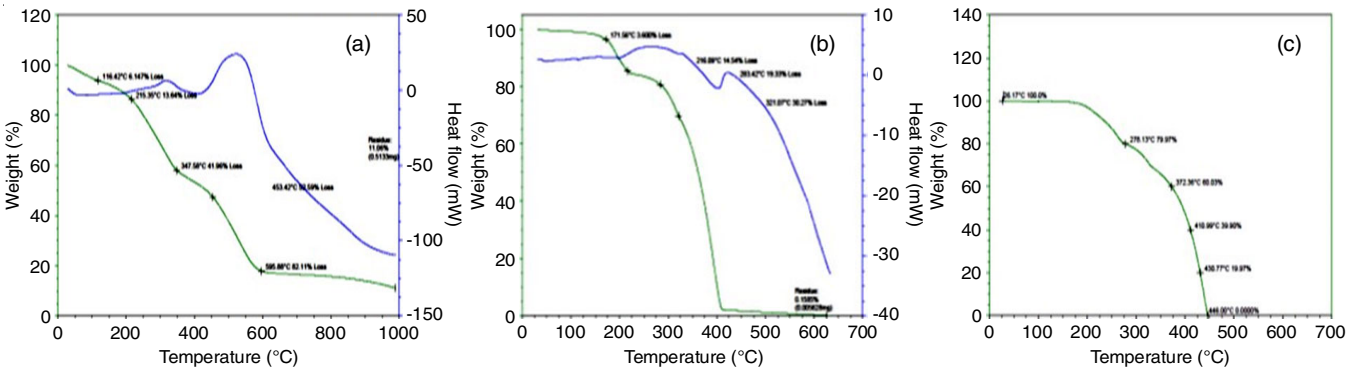

Fig. 5. TG-DTA spectrum of *Chandamarutha centhuram* (CMC) from batch-1 (a), batch-2 (b) and batch-3 (c)

TABLE-3
PARTICLE SIZE OF ALL THREE BATCHES
OF *Chandamarutha centhuram*

Test parameters	Batches	Result	Limit of detection or specification
Particle size	I	478.9 nm	NA
Particle size	II	356.7 nm	NA
Particle size	III	570.2 nm	NA

the range of 116 ºC and 126 ºC, indicating the decomposition of water molecules in first and third batches [15]. Temperature at 116 ºC may be due to the melting point of elemental sulphur. The sharp peaks indicated the presence of mercury sulphide at 321 ºC to 372 ºC in three batches and a peak at 354 ºC also corresponded to melting temperature of mercury sulphide and also indicated a weight loss of nearly 40% to 60% [18].

XRD studies: The 2θ values of the XRD peaks of all the three batches of *C. centhuram* samples were more or less the same (Fig. 6). Peaks of 25º, 26.5º, 28º, 31º, 38º, 43º, 45º and 53º corresponded to the 100, 101, 102, 103, 110, 104 and 113 planes, respectively of mercuric sulphide in accordance with the JCPDS card no. 06-0256. The results matched with the Ayurvedic mercury based medicine *Rasasindura* [19]. Additionally, the peaks at 20º, 23º, 33º, 35º, 41º, 47º and 55º corresponded to 030, 202, 223, 241, 143, 161 and 420 planes, respectively of sulphur. These findings corroborated the existence of mercuric sulphide and sulphur in the XRD data of *C. centhuram* after purification. The elemental analysis of *C. centhuram* also revealed the presence of mercury. It is also interesting to note that the toxic mercuric chloride was transformed into mercuric sulphide, which is a non-toxic form of mercury [17].

Conclusion

In present work, the physico-chemical parameters of *Chandamarutha centhuram* were determined as per AYUSH protocols and the surface characterization studies were carried out for the formulation using sophisticated instrumentation techniques like XRF, Zeta sizer, FTIR, XRD, SEM and TG-DTA. The physico-chemical data suggested the total ash content of the samples was below 1.5%. *C. centhuram* either did not contain acid insoluble and water soluble ash (Batch I) or contained them in negligible amounts (Batch II and III). The XRF analysis revealed that mercury was present in high concentration in all the three samples. The SEM images revealed the nanoparticle size ranging from 6.92 to 55.4 µm, whereas the Zeta sizer showed the particle size within the range of 356.7 nm to 570.2 nm. The reason for the increase in particle size may be due to the tendency of the particles to aggregate together to form larger particles when dispersed in an aqueous medium. The present data could be useful in determining the physico-chemical standards for the formulation as well as in understanding the chemical transformation of toxic metallic mercury into a non-toxic sulphide form due to purification treatment with organic agents such as milk and lemon juice.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the funding provided by the Department of AYUSH (Z.15015/1/2010-COE), India, Drugs and Pharmaceutical Research (VI-D&P/267/08/09/TDT), Department of Science & Technology (DST), India and SASTRA University for this work. The funding from Nano Mission Council

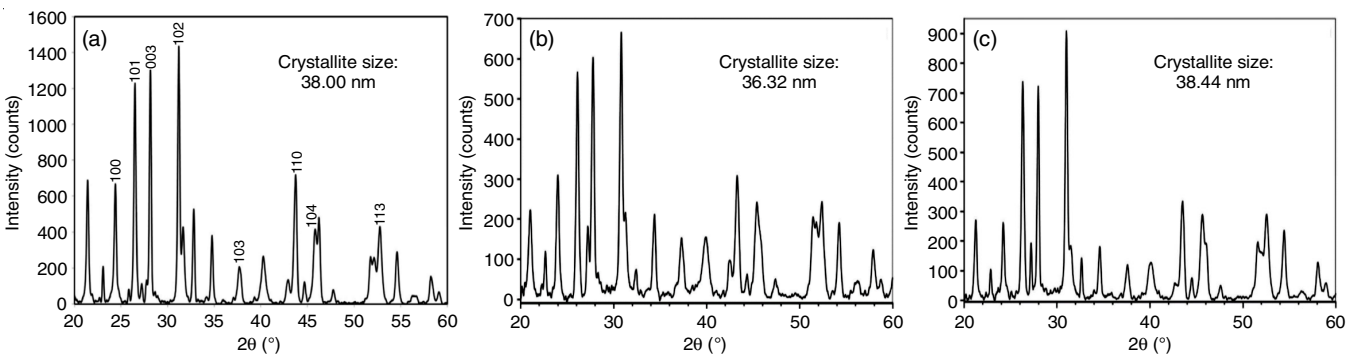

Fig. 6. Powder X-ray diffraction pattern of *Cantamarutac centuram* batch-1 (a), batch-2 (b) and batch-3 (c)

(SR/S5/NM-07/2006 and SR/NM/PG-16/2007), DST, India for SEM and XRD analysis is also gratefully acknowledged.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. T.V. Sambasivam Pillai, Tamil Dictionary, Tamilnadu Siddha Medical Council, Chennai, India, Edn. 4, vol. 1 (1992).
2. R. Thyagarajan, *Gunapadam Thaathu Jeeva Vaguppu*, Department of Indian Medicine and Homeopathy, Chennai, India, Edn. 4 (2004).
3. The Siddha Formulary of India, Ministry of Health and Family Welfare, Department of AYUSH, Government of India (1992).
4. Formulary of Siddha Medicines. Indian Medical Practitioners' Cooperative Pharmacy and Stores Ltd., Adyar, Madras, India (1989).
5. P. Rajalakshmi, R.G. Sudeer, V. Vadivel, C.S. Sahayam and P. Brindha, *Indian J. Pharm. Sci.*, **79**, 987 (2017); https://doi.org/10.4172/pharmaceutical-sciences.1000316
6. I. Sorrnamariyammal, *Bogharelayirathil siddha maruthuvakanimangal*, Published by Ulaga Siddha Arakkattalai, Chennai, India (2016).
7. R. Madhavan and K. Manickavasakam, *Int. J. Pharm. Pharm. Sci.*, **4**, 154 (2012).
8. K.N. Kuppusamy Mudaliyar and K.S. Uthamarayan, *Siddha vaithiya thirattu*, Tamilnadu Siddha Medical Council, Chennai, India (1998).
9. The Ayurvedic Pharmacopoeia of India, Ministry of Health and Family Welfare, Department of AYUSH, New Delhi, Part I, Vol. IV (2004).
10. Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva (1988).
11. S. Joshi and V. Aeri, Practical Pharmacognosy, Frank Bros & Co. (Publishers) Ltd., New Delhi, Edn. 1, pp. 290-293 (2009).
12. Pharmacopoeial Standards for Ayurvedic Formulations, Central Council for Research in Ayurveda and *Siddha* (India). Ministry of Health and Family Welfare, New Delhi (1987).
13. V. Charde, K. Patel, R. Gokarn, D. Rajput, B. Patgiri and P.K. Prajapati, *J. Res. Trad. Medicine*, **2**, 94 (2016); https://doi.org/10.21276/jrtm.2016/170
14. D. Lefier, R. Grappin and S. Pochet, *J. AOAC Int*., 79, 711 (1996); https://doi.org/10.1093/jaoac/79.3.711
15. S.K. Umate, V.R. Marathe and P.V. Pawar, *Adv. Zool. Botany*, **8**, 116 (2020); https://doi.org/10.13189/azb.2020.080306
16. S.U. Kamath, B. Pemiah, K.S. Rajan, S. Krishnaswamy, S. Sethuraman and U.M. Krishnan, *Indian J. Pharm. Sci.*, **76**, 495 (2014); https://doi.org/10.4103/0250-474X.147224
17. W. Paul and C.P Sharma, *Trends Biomater. Artif. Organs*, **25**, 124 (2012).
18. S.K. Singh, A. Chaudhary, D.K. Rai and S.K. Rai, *Int. J. Tradition Knowledge*, **8**, 346 (2009).
19. B. Amartya, D. Krishanu and S. Vikas, *Int. J. Pharm. Res. Biosci.* , **1**, 96 (2012).