

Physico-chemical characterization of Annapavala Chenduram - A Siddha herbo marine formulation

Research Article

**Sabari Girija N^{1*}, Sakthimanipriya L², Sinekha MA², Prithiviraj K³,
Shanmugapriya P⁴, Madhavan R⁴**

1. Resident Medical Officer, 2. PG Scholar (Alumini), 4. Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamilnadu. India.
3. Lecturer, Excel Siddha Medical College and Research centre, Pallakapalayam, Tamilnadu. India.

Abstract

Annapavala Chenduram (APC) is the herbo – marine formulation in Siddha. *Annabedhi* (Green vitriol) is one of the *Uparasam* (Hydro chemicals) and *Kodipavalam* (Coral) is one of the *Navamanigal* (Nine gems). These both are the main ingredients of APC. Present day demand of Siddha formulations has been raised globally due to increased response towards Siddha system of medicine. Therefore, certain things like standardization, quality control, and safety became essential requirements for Siddha formulations. The test drug APC was prepared at *Gunapadam* (Pharmacology) lab of National Institute of Siddha, Chennai as per traditional method (incineration) mentioned in the classical texts. Physico-chemical and instrumental analysis including Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) was performed as per PLIM (Pharmacopeia Laboratory of Indian Medicine) guidelines. The pH of test drug APC was 8.34. It contains 0.56% acid insoluble ash and Solubility in the water was 6.75%. Quantitative measures of chloride, sulphate, carbonate, iron, calcium, sodium and potassium was 0.63%, 1.21%, 11.84%, 19.56%, 14.85%, 2.2%, 3.5% respectively. Scanning Electron Microscopy analysis revealed that particle size of APC about 200nm. This study suggests that quality specifications of APC can be developed using parameters described in Siddha along with analytical tools available today.

Key Words: *Annapavala Chenduram*, Physico – chemical analysis, *Siddha*, Standardisation, Spectroscopic analysis.

Introduction

Indian System of Medicine is of great antiquity. Among the various traditional system of practicing medicine, Siddha is one of the predominate medicine without much of side effects. In Vedic period, Siddhars are considered to be scientists those who dealt with metallurgy. In addition, they had a deep understanding in the physical and chemical properties of know metals and minerals that is being used in the Siddha medicine system. (1) In olden days, most of the Indian medicine system were incorporating plants, minerals and the animals as the main ingredients in the drugs to cure various ailments. Efficacy of raw drug very much depend on the quality and quantity of the proportion weighted in the therapeutical medicine. In general, Siddha medicine is classified into 32 internal and 32 external medicines. *Chendhuram* is one of the potential formulations among the 32 internal medicines in Siddha, which retains the potency for 75 years mentioned in Siddha literature. (2)

Rising popularity and long-term stability have increased the present demand of Siddha formulations globally, due to the clear understanding of the mechanism via recent research. Hence, commercialization of Siddha drug manufacturing has attracted locally and internationally. To ascertain the essential standardization, quality control, and safety became necessity for Siddha formulations. It is the need of the time to present, understand, and implement these things into Siddha formulations more accurately for globalization of Siddha. Thus, the production of standard, effective, genuine, safe drugs of utmost quality should be given prime importance by the processing units of Siddha drugs. Even though some research studies have been conducted on APC, but there is no physico – chemical analysis carried out on the APC.

Annapavala Chendhuram is the herbo – marine formulation in Siddha. *Annabedhi* (green vitriol) is one of the *Uparasam* (hydro chemicals) and *kodipavalam* (Coral) is one of the *Navamanigal* (Nine gems). These both are the main ingredients of APC. (3) It is widely prescribed for thyroid dysfunction by Siddha practitioners. Here, in the present study, test drug was prepared and its physicochemical analysis, spectroscopic characterization was carried out by using Scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) as per PLIM guidelines.

* Corresponding Author:

Sabari Girija N

Resident Medical Officer,
National Institute of Siddha, Tambaram
Sanatorium, Chennai, Tamilnadu. India.
Email Id: drgirijakrish@gmail.com

Materials and Methods

Procurement of the raw drug

The raw drug *Annabedhi* and *kodipavalam* were procured from the authenticated raw drug shop at Chennai. Lemon was purchased from the Vegetable market, Tambaram, Chennai.

Authentication of the raw drugs

Department of Geology, University of Madras, Chennai, authenticated the *Kodipavalam* and *Annabedhi* samples by investigating the microscopical characters and literature sources.

Purification process of raw drugs

Annabedhi

Annabedhi was soaked in Lemon juice for 3 days and made it dry under the dark in earthen vessel.

Kodipavalam

Kodipavalam was soaked in the lemon juice for 3 days in an earthen vessel. It was kept in the sunlight for 3 days. Then 4th day *kodipavalam* was washed with the water and wiped with cloth. (4)

Preparation method of APC

The trial drug APC was prepared in the *Gunapadam* (pharmacology) Laboratory, National Institute of Siddha, Chennai. The purified *Kodipavalam* and *Annabedhi* were crushed into fine powder separately and both powders were placed in *kalvam* (Mortar). It was triturated well with lemon juice for six hours. Then it was made in to *villai* (pellets) and dried in sunlight. The pellets were stacked into an earthen vessel which was closed with another vessel and 7 clay cloth made to the margin of earthen vessels and the set up was dried in sunlight for one day. After that, it was placed in a deep pit and *pudam* process (incineration process) was done with 300 dried cow dung cakes. Once it reached [A1] room temperature, the closed pot was opened and the pellets were taken out. The pellets were collected after cooling, weighed, finely powdered and stored in a clean, dry, airtight glass container. (3)

Organoleptic Characters

The analytical specification of *chenduram* was done according to the protocol given by Pharmacopoeial Laboratory for Indian Medicine (PLIM), Department of AYUSH, Ministry of Health and Family Welfare, New Delhi [A2]. As per PLIM guidelines, APC was evaluated for the organoleptic characters like colour, odour, appearance, taste and solubility. (5)

Physico – chemical analysis

Physico chemical analysis of APC such as pH, solubility, total organic carbon, chloride, sulphate, carbonate, iron, calcium, sodium, potassium was carried out by Official method of analysis (AOAC international) (6) and crude protein, ether extract, crude fibre, total carbohydrate, acid insoluble ash was done by Indian standard procedure (7).

Spectroscopic analysis

Spectroscopic analysis for the characterisation of APC such as SEM, XRD, FTIR was carried out.

Scanning Electron Microscopic (SEM) analysis

The morphological features were recorded by FESEM (TESCAN MIRA 3) with an accelerating voltage of ~50 kV and an elemental composition was obtained using an energy-dispersive X-ray spectrometer. It is a fully PC-controlled scanning electron microscopes with a tungsten heated filament.

Energy Dispersive X – ray analysis (EDAX)

Energy Dispersive X-Ray Spectroscopy (EDS or EDAX) is a chemical microanalysis technique used in conjunction with scanning electron microscopy (SEM). SEM uses energy dispersive X-ray spectroscopy (EDS) in the production of elemental maps, which accurately represent the distribution of elements within the samples. The most typical use is elemental analysis, mineral orientation, morphology and contrast.

X- Ray diffraction measurement (XRD)

The sample was characterized by X'Pert PRO; PANalytical X-ray diffractometer using Cu K α as the radiation source ($\lambda = 1.5406 \text{ \AA}$) operated at 30 kV. The Sample was scanned from 10° to 80° at a scan rate of 1°s⁻¹ at 2 θ position.

Fourier Transform Infrared Spectroscopy (FTIR)

IR Affinity-1 is a compact Fourier transform infrared spectrophotometer that is housed within an elegant form. FTIR spectrometer (SHIMADZU) was used to analysis samples. The prepared powder samples were mixed with KBr in the ratio of 1:100, and then, the pellets were prepared by subjecting it to a load of 5 tons cm³ and also a pellet-maker (hydraulic pellet pressure; Kimaya Engineers, India). The spectra were collected from wavelength of 4000 cm⁻¹ to 400 cm⁻¹ with 4cm⁻¹ resolution over 40 scans.

Results

Organoleptic characters of APC

Organoleptic characters of APC mentioned in Table 1. This indicates APC was brownish red in colour, odourless, tasteless, smooth powder in nature.

Table 1: Organoleptic characters of APC

Parameter	APC
Colour	Brownish red
Odour	No odour
Taste	No taste
Touch	Smooth
Appearance	Powder

Siddha specification of APC

APC complied with all the classical parameters and various tests given in classical Siddha texts to confirm the quality of the APC can be interpreted as given in Table 2.

Table 2: Siddha standardization of APC

No	Parameter	APC
1	Irreversibility	Irreversible
2	Luster	Lusterless
3	Smoke (heating)	Smokeless
4	Weight (sprinkle test)	Floats in the water
5	Fineness (Furrow filling)	Enter into the finger lines

Physico – Chemical analysis of APC:

The pH of APC was 8.34. It contains 0.56% acid insoluble ash. Solubility of APC in the water was 6.75%. Crude protein and Crude fibre present in the sample were 0.32% and 5.34% respectively. Energy in APC was 30.8 Kcal. Ether extract of APC was 1.28%. Total carbohydrate and Total organic carbon were 25.40% and 2.14% respectively. (Table 3) Quantitative measures of chloride, sulphate, carbonate, iron, calcium, sodium and potassium was 0.63%, 1.21%, 11.84%, 19.56%, 14.85%, 2.2%, 3.5% respectively. It has tabulated in Table 4.

Table 3: Physicochemical analysis of APC

Parameter	APC
pH	8.34
Acid insoluble ash	0.56%
Solubility in water	6.75%
Crude protein	0.32%
Crude fiber	5.34%
Energy	30.8 Kcal
Ether extract (Total fat)	1.28%
Total carbohydrate	25.40%
Total organic carbon	2.14%

Table 4: Quantitative Acid basic Radicals of APC

Parameter	APC
Chloride	0.63%
Sulphate	1.21%
Carbonate	11.84%
Iron	19.56%
Calcium	14.85%
Sodium	2.2%
Potassium	3.5%

Spectroscopic analysis of APC:

Scanning Electron Microscopy (SEM) of APC:

The FESEM micrographs of APC showed the presence of uniform distribution of particles in the lower magnifications. In higher magnification of SEM, the particles are agglomerated and form big particles. The particle size is estimated to about 200nm. There was no distinct features or shape is observed in the SEM image. (Figure 1).

Energy Dispersive X – ray analysis (EDAX) of APC:

In EDAX analysis revealed the APC possess the mass percentage of carbon, oxygen, iron, magnesium, Sulphur, Chlorine, Calcium is 5.41, 39.75, 5.43, 2.21, 12.29, 0.86, 34.05 respectively shown in Table 5.

X- Ray diffraction (XRD) characterization of APC:

The XRD spectra depicts the structural composition present in the prepared APC. (Graph 1)

Figure 1: Scanning electron microscopy images of APC

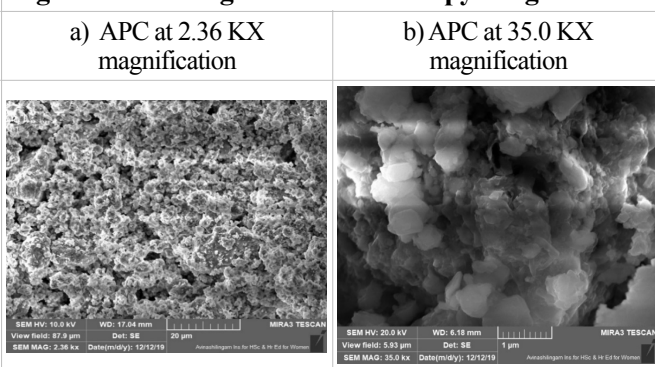
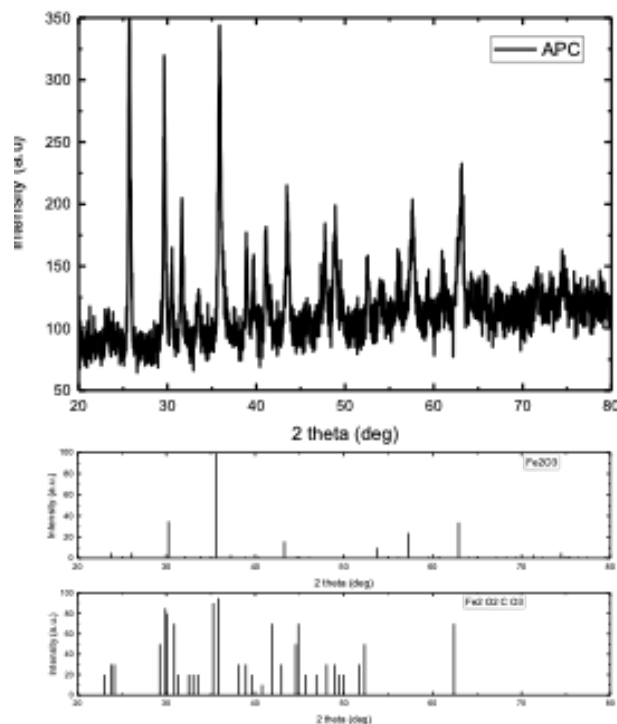


Table 5: Elemental composition of APC (EDAX)

Element	Weight%	Atomic%	Net Intensity	Error%
Ca	34.05	19.39	93.18	4.54
O	39.75	56.72	204.16	9.34
C	5.41	10.29	25.25	11.15
S	12.29	8.75	78.96	4.29
Fe	5.43	2.22	10.89	13.24
CO	0.00	0.00	0.01	99.99
Mg	2.21	2.08	19.77	8.78
Cl	0.86	0.55	4.41	29.77

The peaks are determined and indexed based on the Fe₂O₃ phases extracted from the JCPDS-card-no.00-039-1346 and JCPDS-card-no. 00-033-0665. The dominating peaks are quite visible and attributed to the Fe₂O₃ and indexed as (040) and (311). The phases such as (211), (102) (511) and (441) are extracted from the Fe₂O₂CO₃ and some of the other peaks could also indexed in the form of Fe based oxide with and without silica.

Graph 1: X- Ray diffraction analysis of APC



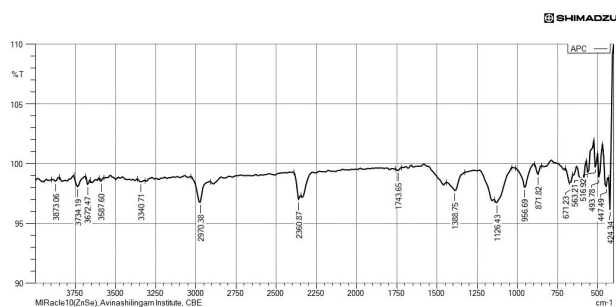
Fourier Transform Infrared Spectroscopy (FTIR) analysis of APC:

In the FTIR spectra analysis, APC exhibits the peaks at wavenumber of 3587 and 3672, 3340, 2360, 2970, 1743, 1388, 1126, 956, 871, 671, 563, 516, 493, 447, 424cm⁻¹ having the O-H stretch, N-H stretch, C-CH₃ stretch, C=O stretch, C-H stretch, C-O-C stretch and Fe – O stretch. The expanded region of the FTIR spectra between 4000 - 1500 cm⁻¹ looks complex as there are many peaks present in this region. (Table 6)

Table 6: FTIR Analysis of APC

FTIR peaks (cm ⁻¹)	Vibrational modes	Functional group
3587, 3672	O-H stretch	Alcohol group
3340	N-H stretch	Amine group
2360, 2970	C-H stretch	Alkane group
1743	C=O stretch	Carbonyl group
1388	C-H stretch	Alkane group
1126	C-O-C stretch	Esters
871 – 671	Fe – O stretch	Alkyl halides
563 – 424	Fe – O stretch	Alkyl halides

Graph 2: FTIR analysis of APC



Discussion

Standardisation of traditional medicine is the process of prescribing a set of standards and constant parameters that carry an assurance of quality, efficacy, safety and reproducibility of the drugs. (8) It is challenging for the Siddha pharmaceuticals to produce the standard, genuine and safe drug in required quantity with utmost quality. Raw drug quality plays main role in the quality of preparatory medicines. (9) After the purification with lemon juice, *Annabedhi* and *kodipavalam* loses its classical green and pink colour respectively. Acidic nature of lemon juice removes the impurity in raw materials and loss of water. (10) In organoleptic evaluation, APC was brownish red in colour with no specific odour and taste. Touch indicates physical properties such as smoothness, softness and fineness of Chendhuram. All the Siddha classical analytical parameters have definite significance. Sprinkle test of the drug APC on the surface of the water indicated the weightless of the drug and in furrow filling test, *Chendhuram* enters into the creases of fingers showed the micro fineness of the drug.

In physico – chemical analysis, pH value represents alkalinity and acidic nature of formulation. It plays main role in the drug absorption. pH of APC was

8.34 (weak base). The weak base is absorbed at a faster rate from the intestine (pH 7.50 – 8), this is because the basic substances can't be ionized in basic medium. So, the uncharged substances can be passed easily due to its lipid solubility. This alkaline nature revealed that it would be absorbed well in the intestine. (11) A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the medicine. Acid insoluble ash in APC was 0.56%. This result gives an inference that the drug was prepared without any external contamination as this may affect the absorption of drug. (12) Crude protein and crude fibre present in the APC were 0.32% & 5.34% respectively which indicated the protein and fibre amount in the APC.

Presence of acid radicals in APC was mentioned in Table 4. Chloride plays an important role for regulation of water balance, osmotic pressure, as well as acid base equilibrium. (13) sulphate is a major contributor to the ionic strength of urine and alters the equilibrium constants governing saturation and precipitation of calcium salts. (14) Carbonate ions in the APC revealed that, pH and acid balance are regulated by the carbonate, as bicarbonate ions. (15) Presence of basic radicals in APC were calcium, iron, sodium and potassium. Sodium and potassium regulate the acid – base balance of the body fluids. Potassium ions are essential for contraction of cardiac and skeletal muscles. It is essential for the activity of nerves. Calcium present in body in great abundance. It is necessary for muscle contraction and normal transmission of nerve impulses. It is also played a vital role in the maintenance and regulation of acid-base balance. (16) Iron mainly exerts its functions through the compounds in which it is present. Haemoglobin and myoglobin are required for the transport of O₂ and CO₂. Iron is associated with effective immune competence of the body. (17)

APC was scanned under FESEM to know the shape, size, arrangement and texture of particles. Chendhuram was well agglomerated. The FESEM report and particle size analysis data indicated that the test drug obtained was nanometre in size. EDAX report revealed the presence of high calcium ions. Calcium is rich in the APC due to the presence of kodipavalam which contains 85% calcium carbonate in raw drug. (17) For the identification of the compounds present in the APC, XRD was carried out in which prominent peaks of ferric oxide were seen which confirms that final product is oxide form of ferrous. XRD pattern explored the formation of crystalline compound which is again due to the impact of incineration processes. (18) In the FTIR spectra, peaks range from 400 to 4000cm⁻¹. The probable functional groups which can be correlated to some of the peaks present in the region are given Table 6. The region between the 400 to 1500cm⁻¹ looks complex and many peaks (Fe-O) were exhibited in this range.

Conclusion

The data obtained in this study suggests that quality specifications for APC can be developed using tests described in Siddha along with analytical tools available today. The main limitation of the study, there

is no proper control specimen(s). Hence, this is a major challenge for herbo - mineral mixtures, as they vary from batch-to-batch. Analysis of more samples from different batches with variable composition and/or raw materials needs to be performed as a step forward to characterization and standardization of APC.

Acknowledgement

The author wishes to acknowledge Dr. M. Jeyanthinath, M.S, Ph.D, Associate Professor, Madurai Kamarajar University, Madurai for his help in analysis of the drug and extended support in conducting this research work.

Financial support: Nil

Conflict of Interest: The authors have declared no conflicts of interest in this study.

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