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Physiochemical and sophisticated instrumental analysis-ftir and sem of herbomineral formulation-naaga chendhooram through plim guidelines

S.Janani^{*1}, K.Megala², M.D.Saravana Devi³, V.Velpandian⁴

^{*1}PG Scholar, PG, Gunapadam Department (Pharmacology), Government Siddha Medical College, Chennai, TN, India

²PG Scholar, PG, Gunapadam Department (Pharmacology), Government Siddha Medical College, Chennai, TN, India

³ Professor, HOD, PG PG, Gunapadam Department (Pharmacology), Government Siddha Medical College, Chennai, TN, India

⁴ Professor, PG, Gunapadam Department (Pharmacology), Government Siddha Medical College, Chennai, TN, India

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Abstract

Background: The current study is pointed at the characterization of the physicochemical analysis of the traditional Indian Siddha medicine, Naaga Chendooram (NAC). It is a metal-based medicine which cures especially breast cancer. A literature survey revealed that a scientific study was lacking on this drug. Materials and Methods: Naaga chendhooram was made as per the Veeramamunivar siddhar method. The ingredients were Zinc, Potassium nitrate, Zingiber officinale, Curcuma longa and Carum copticum and Aspera bidentate. The prepared drug was analysed in Fourier Transform Infra-Red (FTIR) for its chemical properties which help in the identification and localisation of chemical species, scanning electron microscopy (SEM). FT-IR spectroscopy has been used to study the presence of organic substances, physiochemical, organoleptic and biochemical were analysed. Results: Loss on Drying at 105 °C $1.053 \pm 0.1747\%$, Total Ash $91.6 \pm 3.7360\%$, Acid insoluble Ash $0 \pm 0\%$, Water soluble Extractive $6.367 \pm 2.779\%$, Alcohol Soluble Extractive $1.46 \pm 0.73\%$, pH 7.5. It has the acid radical-carbonate and sulfates and basic radical- mercury and in the instrumental analysis, it has arsenic group. Conclusion: Findings divulge that samples need more studies to standardize the drug and to determine the importance of the Siddha drug preparation technique, which may disclose the scope for chemical modulation by traditional methods in succeeding years.

Keywords: Naaga chendhooram, SEM, FTIR, Standardization, PLIM guidelines.

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*Corresponding Author

S.Janani

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Introduction

In the Siddha system of medicine, Theraiyar 'Denotes the ineffable efficacy of Naagam in treating great maladies'. In Siddha's books, it shows that 'Zinc bring back the dead. These are the words used by Yugi muni an ancient siddhar in Siddha in his work Yugi muni

vaithiya chindhaamani to describe the efficacy of Naaga chendh ooram. Must utilise only the purified zinc for the further preparations like parpam and chendhooram which will helps in the therapeutics of infertility and also acts in molecular level diseases like cancer especially in the drug therapy of breast cancer which is highlighten in the book of 'VEERAMAMUNIVAR VAAGADA TIRATU". Whatever is in the macrocosm, is in the microcosm' [1]. It is safe to say based on the above words of Siddhar Sattaimuni that the elemental composition of human body almost mirrors the elemental composition of our Earth, even recent researches support this statement. Zinc, a critical nutrient, controls cell proliferation, differentiation, and

viability, as well as programmed cell death, in a variety of physiological and pathological processes. These features are especially focused on the structural role of zinc ions in numerous proteins as well as in the transcription factors. In modern system of medicine nanoparticles of zinc oxide plays a notable role. Furthermore, zinc oxide nanoparticles have UV blocking properties. Compared with other metal oxide Nanoparticles, ZnO NPs(nanoparticles) with the comparatively economical friendly and relatively innocuous property reveals excellent biomedical applications, such as anticancer, targeted delivery and gradual release of therapeutic agents, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bioimaging and they are mainly used in the personal care products like cosmetic products and products which used for sunscreen because of their strong UV absorption properties [2]. Although the mechanisms of cytotoxicity caused by ZnO-NPs are not fully understood, the formation of hydroxyl radicals (OH•), superoxide anion, and per hydroxyl radicals from the surface of ZnO is thought to be important. When nanoparticles come into contact with cells, cellular defence mechanisms kick in to keep the damage to a bare minimum [3, 4]. However, if the creation of extremely active free radicals boosts the cells anti-oxidative defences, it results in biomolecule oxidation, which can lead to cell death. Recent research has shown that bioactive chemicals derived from macroalgae can be used to develop new pharmacological and flexible innovative agents with more potential in cancer research, diagnosis, and treatment [5].

In Indian alchemy, chendhooram were the formulations of metals or their own salts such as gold, silver, tin, stannum, lead, zinc, mercury, and in the same way organic macromolecules produced from the juices of herbals by alchemical operations making them biologically equivalent. These herbo mineral formulations are made by incinerating metals or their salts with medicinally beneficial herbs or extracts on a regular basis to eliminate their toxicological effects, and they're eaten with butter, honey, milk, or ghee [6]. The majority of medicines are combinations of substances, and because of their synergistic action, negative effects are reduced, boosting bioavailability via body cells. Treating minerals with medicinal herb juices can help reduce particle size to nano levels (less than 100 nm), which increases effectiveness [7]. In siddha, Naaga chendhooram (formulation prepared from zinc)- from

the book of VEERAMAMUNIVAR VAAGADA THIRATU Breast cancer, acid peptic disease, and vaadha diseases can all be cured with this remedy. Despite this, the current investigation work was undertaken to standardise the sastric siddha formulation Naaga chendhooram (NAC), which has been used for the regimens of various ailments, using physiochemical, organoleptic characters, and instrumental evaluations in accordance with AYUSH regulations.

2. Materials and Procedures

Drug Selection

Naaga chendhooram is taken as a trail drug for breast cancer from the siddha literature VEERAMAMUNIVAR VAAGADA THIRATU.

Collection of the Drugs

Sennayuruvi (*Aspera bidentata*) were freshly collected from kolli hills.

Naagam (Zinc), vediuppu (Potassium nitrate), omam (*Carum copticum*). Manjal (*Curcuma longa*), sukku (*Zingiber officinale*) were brought from authenticated drug store Ramasamy chetty country shop at Parys, Chennai.

Identification and Authentication of Drugs:

All plant and raw materials were point out and authenticated by Botanist and experts of Gunapadam, (Pharmacology) Department in Government siddha medical college, Arumbakkam Chennai. A specimen sample of each raw material has been labelled as 1015/PGG/321912105/GSMC-CH/2019-2022,1016/PGG/321912105/GSMC-CH/2019-2022,1017/PGG/321912105/GSMC-CH/2019-2022,1018/PGG/321912105/GSMC-CH/2019-2022,1019/PGG/321912105/GSMC-CH/2019-2022, 1020/PGG/321912105/GSMC-CH/2019-2022 were kept in the department of Gunapadam.

Dosag: 65-130mg

Form of Medicine: Chendooram

Time of Administration: Two times a day in an empty stomach.

Indications : Breast Cancer, Vatha Diseases, Acid

Purification of Naaga Chendhooram

The medications were refined using the SARAKAKUGALIN SUTHI SEI MURAI Siddha literary literature.

Purification of Naagam:

Materials Required:

Eluppai Ennai (Madhuca Oil): As Required

Butter: As Required

Goat's Milk: As Required

Procedure

A big iron spoon's inner side will be coated with Madhuca oil and butter in equal ratio and 140 grams of zinc be taken it should be heated with the help of a blacksmith furnace until it melts and pour the melted Zinc into goat's milk. Zinc should be completely immersed in goat's milk and let cool. The process is repeated 20 times.

Purification of VEDIUPPU:

- Potassium nitrate -700gm
- Seawater -1 lit

Procedure

Potassium nitrate is dissolved in seawater and filtered. The filtrate is heated in an iron pan till it is in the drained state then transferred into the copper pan and let it cool, turned into salt. Mixed the salt with two times its weightage in water, and repeated the above procedure 5-7 times.

Purification of OMAM (*Carum copticum*) Omam was detoxified by soaking it for three hours in limestone water and then drying it.

Purification of SUKKU (*Zingiber officinale*)

Chukku was detoxified by removing the outer covering and immersing it for three hours in limestone water.

Purification of MANJAL (*Curcuma longa*)

Manjal was purified by removing the outer layer of skin.

Purification of SENNAAYURUVI (*Achyranthus bidentata*)

The entire plant of *Achyranthus bidentata* was thoroughly cleaned of dust and contaminants, and the root was solely purified with running water.

Standard Operative Procedure of Drug-Naaga Chendhooram

- ❖ Powdered the purified Potassium nitrate (Vediuppu) 700 gram, *Zingiber officinale* (Sukku) 35 grams, *Carum copticum* (Omam) 350 grams, *Curcuma longa* (Manjal) 35 grams separately and mixed together.
- ❖ Place Purified zinc in a new earthen vessel and heat it until melt, Added the powdered drugs in small quantity for the process of kirasam and fried it. Repeated this process until the powdered drugs got over. At the final stage of

this process, zinc turned into powder. Made the *Achyranthus bidentata* into small pieces and added it into prepared zinc powder and repeated the Kirasam process again until it turned into pomegranate flower colour.

- ❖ In kalvam, added the above prepared zinc powder and tirturated it with lemon juice for 12 hours, then place it in an agal and cover it with 5 layers of clay smeared cloth, dry it. Then it should be subjected to incineration process (Varatti equal to 7 times the weight of kavasam). Allowed it to cool. Then the chendhooram was collected and stored in an airtight glass container and the prepared drug is labelled as NAC.

Organoleptic Characters

The organoleptic characteristics of the samples were evaluated using Siddiqui et al approach. 'S Organoleptic evaluation is the examination of a formulation based on colour, smell, taste, texture, and other factors [8, 9].

Qualitative Analysis Investigation

Qualitative analysis of this trial drug NAC has been done according to PLIM guidelines, Physiochemical analysis, biochemical analysis, heavy metal analysis, sterility method and sophisticated instrumental analysis like FTIR and SEM were done at, NOBLE RESEARCH INSTITUTE, PERAMBUR, CHENNAI.

PHYSIOCHEMICAL ANALYSIS [10, 11]

- Finding whole ash was done
- Resolution of acid-insoluble ash was done
- Resolution for the alcohol-soluble extractive
- Water-soluble determination extractive was done
- pH determination is also done
- solubility test was done

Biochemical analysis was done for the following test [12]

- Carbonates, Chlorides, Sulfates, Sulphides, phosphates, Fluoride and Oxalate, Borates, Nitrates, Lead, Arsenic, Mercury, Copper, Ferric, Ferrous, Zinc, Silver, and Magnesium.

Test for Sterility Was Done By Pour Plate Method [13]

Heavy Metal Analysis by AAS was done [14]

- Standard reparation
- As & Hg- 100 ppm sample in 1mol/L
- HCl Cd & Pb- 100 ppm sample in 1mol/L HNO₃.

Instrumental Analysis

Sem-Edx Analisis was done [15]

FT-IR Spectrum Analysis was done [16]

Results and Discussion

Organoleptic Characters



Fig1: organoleptic characters

Table 1: Organoleptic characters

State	Solid
Nature	Very Fine
Odour	Characteristic
Touch	Soft
Flow Property	Free Flowing
Appearance	Pale Brownish

Table 2: Solubility profile

S.No	Solvent Used	Solubility / Dispensability
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

Table 3: Confirmatory Specification for Chendhooram

S.No	Parameter	Observation for NAC
1.	Fineness	Confirms the fineness standard based on particle size analysis and sample flow property.
2.	Float on Water	Confirms the test
3.	Smokeless	Confirms the test
4.	Taste less	Confirms the property
5.	Lustreless	Confirms the property



Fig 2: Fineness – Finger ridge deposit Analysis



Fig 3: Float on Water

Physiochemical Analysis

Table 4: Physiochemical Analysis of NAC

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	1.053 ± 0.1747
2.	Total Ash (%)	91.6 ± 3.7360
3.	Acid insoluble Ash (%)	0±0
4.	Water soluble Extractive (%)	6.367 ± 2.779
5.	Alcohol Soluble Extractive (%)	1.46± 0.73.
6.	pH	7.5

Biochemical Analysis:

Test for Acid Radicals Specific Radical Test Report

- Test for carbonates- Positive - Indicates Presence
- Test for sulfates- Positive - Indicates Presence

Test for Basic Radicals Specific Radical Test Report

- Test for Mercury- Positive- Indicates Presence

Sterility Test by Pour Plate Method

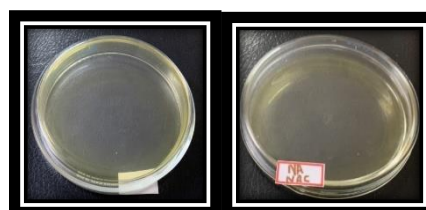


Fig 5: Sterility test for NAC

In any of the plates inoculated with the test material, no growth or colonies were seen.

Table 5: Sterility Test of NAC

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10 ⁵ CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10 ³ CFU/g	

Table 6: Heavy metal analysis of NAC:

Name of the Heavy Metal	Absorption Max λ max	Result Analysis	Maximum Limit
LEAD	217.0 nm	BDL	10 ppm
ARSENIC	193.7 nm	BDL	3 ppm
CADMIUM	228.8 nm	BDL	0.3 ppm
MERCURY	253.7 nm	0.23 ppm	1 ppm

BDL- Below Detection Limit

Instrumental Analysis

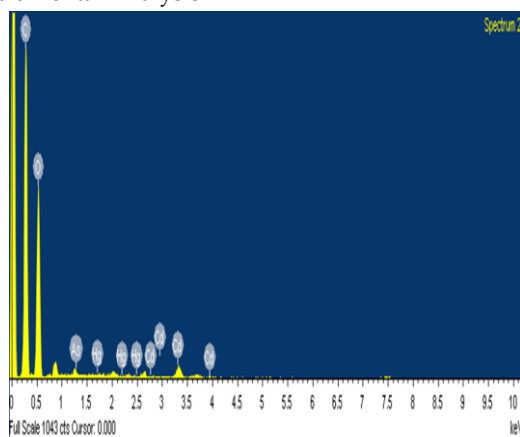


Fig 5: SEM-EDX analysis of the sample NAC

Tab 7: Elemental Peak Table

Element	App Conc.	Intensity	Weight %	Weight %	Atomic %
	Conc.	Corrn.		Sigma	
C K	89.84	1.3336	49.61	1.03	56.79
O K	51.21	0.7510	50.24	1.04	43.18
As L	0.02	1.0634	0.01	0.21	0.00
Cd L	0.28	0.7350	0.28	0.27	0.03
Hg M	-0.16	0.8047	-0.14	0.41	-0.01

Totals			100.00	

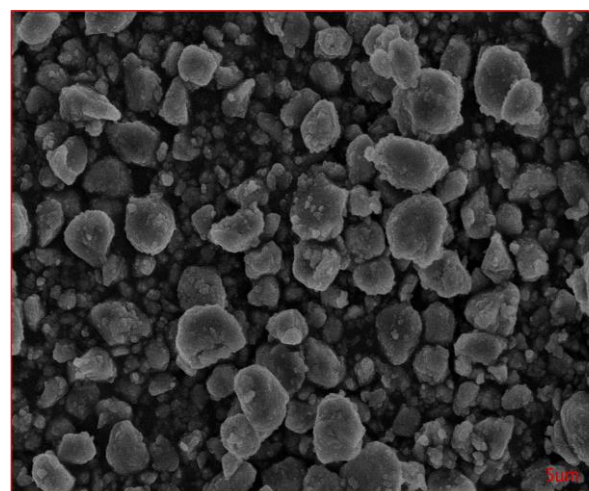


Fig 6:SEM image of NAC – Cluster View

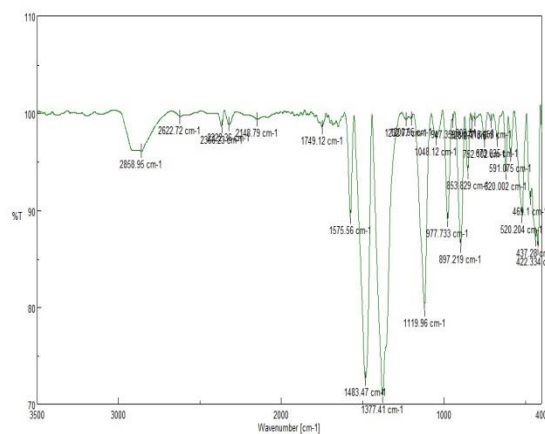


Fig 7:FT-IR SPECTRUM OF Naaga Chendhooram – NAC

Ft-IR Peak Table

- Peak at 2858 cm⁻¹ due O-H stretching vibration
- Peak at 1575 cm⁻¹ due to C=N stretching vibration
- Peak at 1483 cm⁻¹ due to C-H sym. deformation
- Peak at 1377 cm⁻¹ due to N-O stretching vibration
- Peak at 1119 cm⁻¹ due to C-O stretching vibration
- Peak at 977 cm⁻¹ due to C-H vibration
- Peak at 897 cm⁻¹ due to N-H out-of-plane bending vibrations
- Peak at 591 cm⁻¹ due to N=N=N bending vibration
- Peak at 520 cm⁻¹ due to C-C skeleton vibration
- Peak at 422 cm⁻¹ due to aromatic C-CN in-plane bending vibration

Interpretation

Tab 1: that NAC shows the physical parameters like colour, odour touch, and appearance revealed that is a Pale Brownish, soft in nature due to the particle size i.e bioavailability, it has the free-flowing property having the 7.5 slightly alkaline Ph. The chendhooram answered the following tests showing that it was properly processed. When held between the index and thumb and fanned out, it had no metallic shine and was thin enough to fit easily between finger lines. A modest amount of chendhooram was spread on the water's surface. And the chendhooram floated on the surface. This denoted that, the particle are less in weight and well nonpresence of organic materials etc. The results of Tab 4 obtained from the physicochemical analysis clearly reveal that the Loss on Drying value was 1.053 ± 0.174 , and the percentage of loss on drying was within the allowable range thus suggesting that the formulation can be stored for a long period and would not easily be attacked by microbes.

The total ash value was 91.6 ± 3.736 it helpful in determining the quality as well as purity of a crude drug, the percentage value of total ash indicates that the inorganic contents of the formulation are below the limits [17].

The acid-insoluble Ash value was 0 ± 0 , Acid insoluble ash value indicates siliceous impurities indicating that the chendhooram contains a negligible amount of impurities.

Water-soluble Extractive (%) 6.367 ± 2.779 and Alcohol Soluble Extractive (%) was 1.46 ± 0.73 which proves that the secondary metabolites are extractable with the above solvents and it shows higher polar secondary metabolites such as tannins, flavonoids, phenol etc., Now a days purpose of biochemical analysis chemically constituents may be therapeutically active or inactive.

This drug was positive for acid radical- carbonates, sulfates and basic radical mercury.

1. The carbonates present in the drug which is highly recommended for their Calcium carbonates with antiageing and antioxidant property [18].
2. The sulfates present in the drug which indicates
 - sulfated fucoidans with greater anticancer activities [19].
 - Sulfated and benzaldehyde chitosans appear to be promising candidates for anticancer medication development, particularly in the MCF-7 cell line. 20 sulfated chitosans has Antioxidant activity, Chelating effect, and

Radical scavenging effect [21].

3. Mercuric radical was present in the drug which indicated that
 - oxidative stress and cell death by cytotoxicity and induction of apoptosis.
 - mercurial species are able to induce T-cell death by activating specific apoptotic(programmed cell death) cascades [22].
 - More bioavailability of mercuric chloride is because the alkalinity of bile promotes the absorption of $HgCl_2$ [23].

The above table 5 indicates that there is no growth was observed after the incubation period. transmission of germs.

The results of the current analysis, as shown in Table 6, clearly show that the sample has no evidence of heavy metals such as arsenic, lead, or cadmium, but does include mercury at 0.23 ppm.

SEM-EDX Analysis

The presence of carbon, oxygen, and a minor quantity of cadmium and arsenic in the formulation is confirmed by SEM-EDX analysis. The particle size, surface, and uniform distribution of particles are shown by NAC; the particle size is 5m and the surface is smooth. It is unrestricted in its movement. As a result, the medications will have a better chance of moving smoothly through the gastrointestinal tract without causing irritation, resulting in increased bioavailability.

FT-IR Analysis

The major sharp peaks of NAC correspond to the approximate frequency 2858 cm^{-1} to 422 cm^{-1} . These peaks are epitomized by the functional group like alcohol, amines, alkyl, secondary amines, nitro and aliphatic groups.

Conclusion

In this study, NAAGA CHENDHOORAM, a more potent therapeutic drug was prepared and analyzed based on the standard procedures. The powder property of the samples was good for assimilation and flowability. The organoleptic characteristics of the drug reveal the detoxification process and preparation processes were done in a sterile condition and samples were alkaline in nature so that the drug has more bioavailability. Findings divulge that samples need more studies to standardize the drug and to determine the importance of the Siddha drug preparation

technique, which may disclose the scope for chemical modulation by traditional methods in succeeding years.

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Conflict of interest

There are no conflicts of interest declared by the authors.

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Author Contribution

All authors contributed equally

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