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Scientific validation of standardization of Narayana chendrooram (Kannusamy Paramparai Vaithiyam) through the Siddha and modern techniques

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Abstract

Siddha system is one of the ancient system of medicine. In this system the knowledge of drugs is not only limited to herbs, but also expands to metals and minerals. *Narayana chendhooram* (Nc) (Reference : Kannusamy Paramparai Vaithiyam, Author : Vaiththiya Viththuvan Mani S.Kannuchamipillai, Page No : 327, Published B.Rathnanayakar and Sons, Chennai – 600 079) is one of the minero metalic formulation which is used to treat more than three hundred diseases.
Standardization of Siddha drug is a burning topic in herbal drug industry today. Standardization is difficult because the Siddha formularies are usually mixtures of many constituents and the active principle in most cases is unknown, however it is possible to generate a physico - chemical finger print for the standardization.

Keywords: *Narayana chendhuram* (Nc), XRD, EDAX, ICP – OES, SEM, FTIR.

Introduction

Siddha system of medicine is a peculiar science and is a wonderful system of medicine and it cannot be compared with any other system of medicines.

Medicines in Siddha system are prepared from herbs, minerals, metals and animal products also. Mineral substances which are 220 in numbers, having salt as its basis and *Rasa, Ghandhaka and Pashanas* (poisons) as ingredients for the mineral and metallurgical medicines. So the treatment followed in this system is by transforming the said minerals into *parpam, chenduram and chunnam.* This process of transforming is called as that of binding and it is only after binding these materials that they can be turned into that of *parpam*, *chenduram*, etc to be administered as medicines.

Chenduram (Red oxide) is prepared in which metallic or arsenic compounds are made into red coloured powders by the process of burning, frying or insulating or keeping them in specialized *pudams* by grinding them with decoctions, *ceyaneers*, juices, etc.

My trial drug *Narayana Chenduram* (NC) is a minerometalic formulation which is traditionally used in the treatment of various diseases. Recommended dosage is 32mg twice daily with different adjuvant according to the diseases.

In the present paper, a Siddha minerometalic preparation *Narayana Chenduram* was prepared and subjected to physico-chemical analysis and instrumental studies such as SEM, XRD, FT-IR and ICP-OES.

Materials and Methods

Narayana Chenduram :

The Siddha medicine *Narayana Chenduram* was prepared in the Gunapadam laboratory, Government Siddha Medical College, Palayamkottai, Tamilnadu. The drug was prepared by the methods as told in the book Kannusamy Paramparai Vaithiyam, Author : Vaiththiya Viththuvan Mani S.Kannuchamipillai, Page No : 327, Published B.Rathnanayakar and Sons, Chennai – 600 079)

Ingredients:

Navalavana thiravagam (Pala Upputhiravagam)

Common Name in Tamil	English Name	Chemical Name	Chemical Formula	Amount
Vediyuppu	Saltpeter	Potassium Nitrate	KNO_3	525 gram (15 palam)
Padikaram	Alam Stone	Potassium Aluminium Sulfate	$KAl(SO_4)_2 – 12H_2O$	350 gram (10 palam)
Venkaram	Borex	Disodium tetra borate	$Na_2B_4O_7.H_2O$	35 gram (1 palam)
Navacharam	Ammonic Chloridum	Ammonic Chloridum	NH_4Cl	35 gram (1 palam)
Pooneeru	Fullers Earth	Hydrous Aluminum Silicate	Pooneeru	35 gram (1 palam)
Annabedi	Green vitriol	Ferrous sulphate	$FeSO_4.H_2O$	35 gram (1 palam)
Thurusu	Blue vitrol	Copper Sulphate	$CuSO_4.H_2O$	17.5 gram (1/2 palam)
Indhuppu	Rock Salt	Impure Sodium Chloride	Nacl	17.5 gram (1/2 palam)
Kaluppu	Common Salt	Sodium Chloride	Nacl	17.5 gram (1/2 palam)

Preparation method of Thiravagam

520gm of 7th Saturated solution of Vediuppu, 35gm Purified Annabedi, 350 gm Padikaram and 17.5g Indhuppu are taken

Along with that, 17.5gm Thurusu, 17.5gm Kaluppu, 35gm Venkaram and 35gm Navacharam are taken and then mix it with 35 gm of Pooneeru.

Above ingredients are grind into fine powder undergoes distillation process.

End product (Thiravagam) (pH : 0.0) is taken and kept in a sealed China Glay Bottle bottle.

Narayana Chenduram

Common Name in Tamil	English Name	Chemical Name	Chemical Formula	Amount
Rasam	Mercury	Hydrargyrum	Hg	300gm
Saathilingam	Natrural Cinnabar	Mercury sulfide	HgS	150gm
Aritharam	Orpiment	Arsenic Sulfide	As_2S_3	100gm
Ganthakam	Native Sulph	Sulpher	S	120gm
Manosila	Red orpimen	Tetra Arsenic tetra Sulphide	As_4S_4	40gm

Preparation method of Naraya Chenduram

300gm Purified Rasam obtained from Jathilingam, 150gm Purified Jathilingam Purified Aritharam 100 gm, Purified Gandhagam 120 gm, Purified Manoselai 40 gm are grind, along Sankaththiravagam for 12 hours (4 saamam) and dried.

Dried powder form is kept in a Glass ware which is sealed with 7 layers mud past plaster & Close with the Marble stem cork.

An earthen vessels in taken & its fill with sand upto two inchers (4 Finger bread) & glass ware in pleace of with in it and again fill with sand up to the Neck of the bottle. This vessels is covered with in earth vessels and sealed with glay.

The mud pot is insureted by 4 Saamam (12 hours) Dheepakkini and Kamalakkini then for cooling whole night, next day pot is insureted by 4 Saamam Kamalakkini and Kaadakkini and then cooling, after cooling "*Chendhooram*" will get ready.

Standardization methods of *Narayana Chenduram :*

As per Siddha aspect:

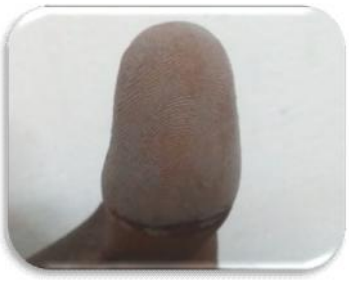
Colour:

Mostly *Chenduram* is in Black red colour. *Narayana Chenduram* was in dark red colour. It shows the perfect colour of *Chenduram*.

Taste:

Properly prepared *Chenduram* should be completely tasteless. If any taste present in *Chenduram*, it indicates that the preparation was not prepared well. A small amount of *Chenduram* was kept in the tip of the tongue, which is tasteless and felt mild irritation, due to its alkaline nature. The final product *Narayana Chenduram* was analyzed as per Siddha classical standardization methods. Based on the results it was suitable for further analysis.

Finger print test:



A pinch of *Narayana Chenduram* was taken and rubbed in between the thumb and the index finger. It entered into the lines of the fingers. It confirmed the fineness of *Chenduram*.

Floating on water:

A pinch of *Narayana Chenduram* was sprinkled over the water in a glass container. The *Chenduram* particles did not sink into the water but floated on the surface of water. It indicates the lightness of *Narayana Chenduram*

Lustre:

If any glowing particles are seen in the *Chenduram*, it shows that the drug is not prepared properly. The *Narayana Chenduram* was taken in a petri dish and observed for any luster in daylight via magnifying glass. No luster was observed in the *Chenduram*.

S.No	Parameter	Results of VABC	Interpretation
1.	Colour	Black Red colour, no shining	Indicates incineration process.
2.	Taste	Tasteless	Indicates complete incineration process.
3.	Finger print test	Impinged in the furrow of fingers	Indicates the fine particles of powder.
4.	Floating on water	Floats on the surface of water	Lightness of the Drug.
5.	Lustre	Lustre less	No glowing particles seen. It indicates complete incineration process.

As per Modern aspects

S.No	Parameter	Result
1.	Colour in day light	Black Red
2.	Odour	Pleasant odour
3.	Sense of touch	Nice
4.	Appearance	Powder
5.	Taste	Tasteless
6.	Solubility	Sparingly soluble in water and well soluble in acids (Conc.HCl and Conc.H_2SO_4)
7.	Action on heat	No change
8.	Flame test	No change
9.	Nice	Fine powder
10.	Water floated test	Completely floated

Interpretation:

Action on heat:

No strong white fumes evolved indicating the absence of carbonate.

Flame test:

No bluish green flame appeared indicating absence of copper.

Physico-chemical parameters:

Physico-chemical analysis of the sample of NC (values are mean of three determinations ± SEM).

Parameters	Total Ash	Values
Ash value	1. Water soluble ash 2. Acid insoluble ash	2.80 ± 0.011 2.85 ± 0.010
Extractive value	1. Water soluble extractive value	7.10 ± 0.410
Loss on drying	Loss on drying at 70^0C	11.33 ± 0.340
pH analysis		8.210

Bio-Chemical analysis of "Narayana chenduram"

Preparation of the extract:

100 mgs of the chenduram is weighed accurately and placed in to a clean beaker added a few drops of concentrated hydrochloric acid and evaporated it well. After evaporation cooled the content and added a few drops off concentrated nitric acid and evaporated it well. After cooling the content add 20 ml of distilled water and dissolved it well. Then it is transformed it to 100 ml volumetric flask and made upto 100 ml with distilled water. Mix well. Filtered it. Then it is taken for analysis.

Qualitative analysis

S.No	Experiment	Observation	Inference
01	**Test for calcium** 2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution	A white precipitate is formed	Indicates the presence of Calcium
02	**Test for sulphate** 2ml of the extract is added to 5% Barium chloride solution.	A white precipitate is formed	Indicates the presence of Sulphate
03	**Test for chloride** The extract is treated with silver nitrate solution	No white precipitate is formed	Absence of Chloride
04	**Test for carbonate** The substance is treated with concentrated Hcl.	No brisk effervescence is formed	Absence of Carbanate
05	**Test for starch** The extract is added with weak iodine solution	No Blue colour is formed	Absence of Starch
06	**Test for ferric iron** The extract is acidified with Glacial acetic acid and potassium ferro cyanide.	No blue colour is formed	Absence of ferric Iron
07	**Test for ferrous iron** The extract is treated with concentrated Nitric acid and Ammonium thiocyanate solution	Blood red colour is formed	Indicates the presence of ferrous Iron
08	**Test for phosphate** The extract is treated with Ammonium Molybdate and concentrated nitric acid	yellow colour is formed	Indicates the presence of Phosphate
09	**Test for albumin** The extract is treated with Esbach's reagent	No yellow precipitate is formed	Absence of Albumin
10	**Test for tannic acid** The extract is treated with ferric chloride.	No blue black precipitate is formed	Absence of Tannic acid
11	**Test for unsaturation** Potassium permanganate solution is added to the extract	It gets decolorized	Indicates the presence of unsaturated compound
12	**Test for the reducing sugar** 5ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8-10 drops of the extract and again boil it for 2 minutes.	No colour change occurs	Absence of Reducing Sugar
13	**Test for amino acid** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% Ninhydrin is sprayed over the same and dried it well.	No Violet colour is formed	Absence of Amino acid
14	**Test for zinc** The extract is treated with Potassium Ferro cyanide.	No white precipitate is formed	Absence of Zinc

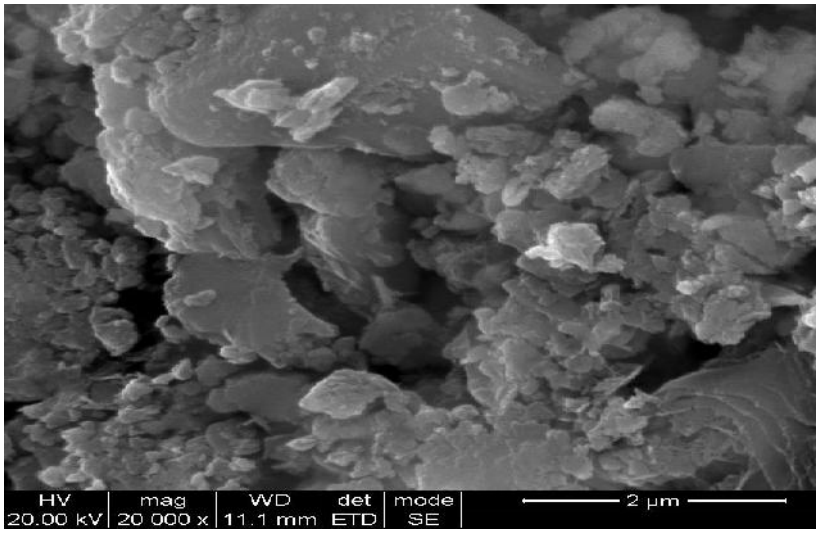
Instrumental analysis:

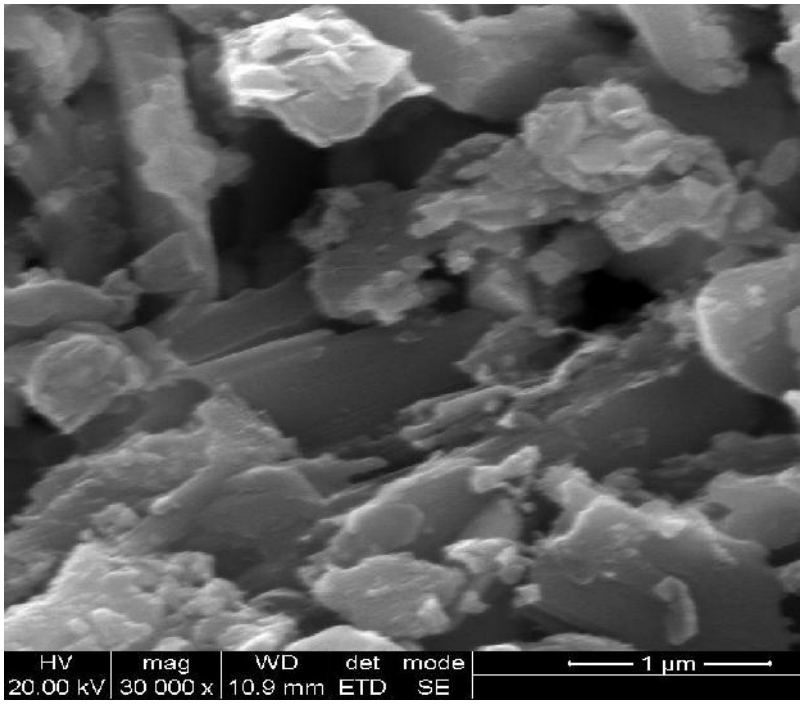
SEM (scanning Electron Microscopy):

The SEM analysis gives the surface morphology, grain size, particle size, distributions, material homogeneity and inner metallic distributions.

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with particles in the sample, producing various signals that contain information about the sample's surface topography and composition.

SEM images of NC as below:





SEM analysis of the drug NC shows the particle size varies from below 1µm to 2µm.

The micro particles shows,

Sustain release of the drug during the transportation through absorption.

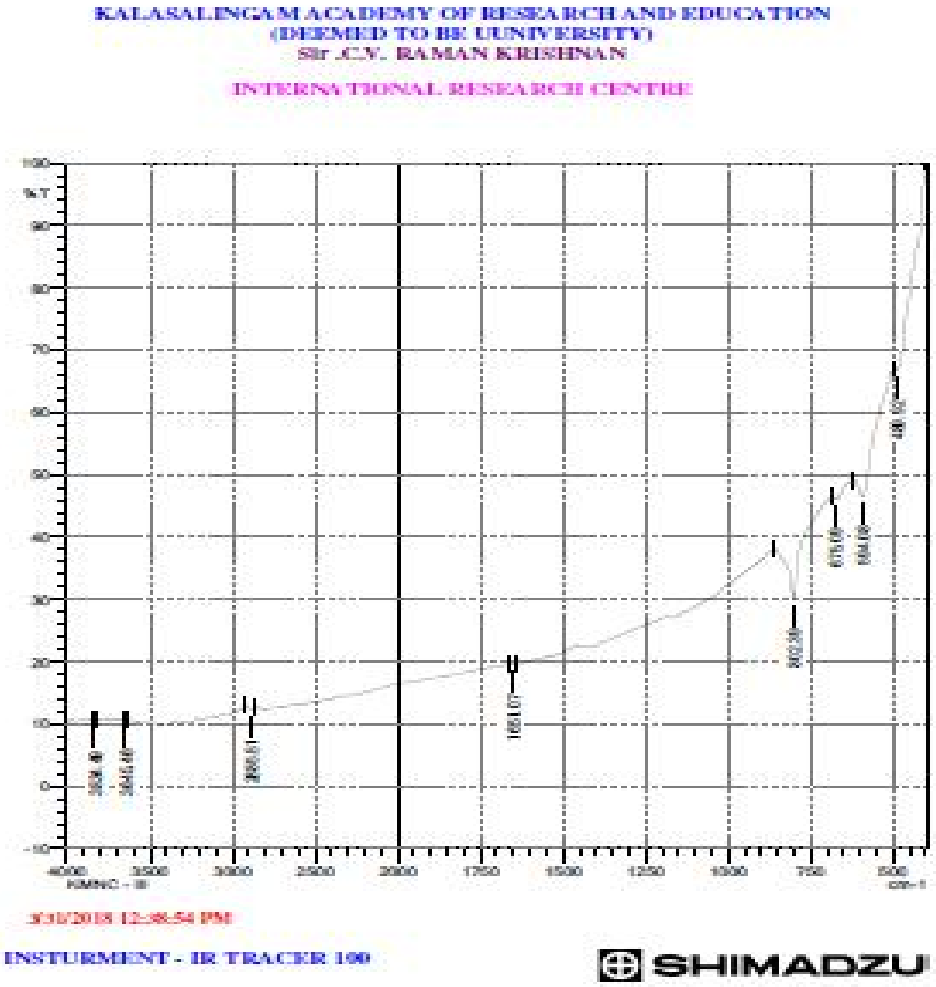
Alters distribution of the drug
Subsequently clears the drug
Increase the drug therapeutic efficacy
Increases the bio-availability
Reduces the side effects.

So the minimal quantity of the medicine is enough to treat the diseased conditions.

FTIR (Fourier Transform Infra Red) spectral analysis:

FT-IR study was selected to identify the inorganic materials of the sample (NC). The FT-IR spectra of the sample were recorded between 400cm^{-1} and 4000cm^{-1}. FTIR analysis results in absorption spectra provide information about the functional group and molecular structure of a material.

FTIR results of NC :



FTIR spectrum - functional groups:

S.No	Frequency	Bond	Functional groups
1.	3834	C-H Stretch	Aliphatic
2.	3645	C-H Stretch	Alcohol strong bond
3.	2885	C = C	Alkanes
4.	1651	C = C	Alkanes
5.	802	N - H	Amides
6.	675	C - Cl bond	Alkyl halides
7.	594	C - Cl bond	Alkyl halides
8.	489	C - Cl bond	Alkyl halides

FTIR results confirming the presence of various active molecules like Aliphatic, Alcohol, Alkanes, Amides and Alkyl halides are responsible for the therapeutic activities of the Siddha drug (NC).

Aliphatic :

Aliphatic R group are nonpolar and hydrophobic. Hydrophobicity increases with increasing of C atoms in the hydrocarbon chain. Although these amino acids prefer to remain inside protein molecules.

Amines groups:

Amines groups act as neurotransmitters.
It is involved in protein synthesis.
This group of substances has antihistaminic and analgesic activity.

Alcoholic groups:

Alcoholic group of substances act as antimicrobial and antiseptic agents.

Phenolic groups:

Phenolic group act as neurotransmitters.

This group of substance has antimicrobial, antiseptic and antioxidant activities.

Alkanes groups:

Alkanes have little biological activity.

They protect against bacteria and fungi.

Alkyl halides:

Alkyl halides are also known as haloalkanes. It is required for the synthesis of glutathione and anti-oxidant activity.

ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry):

ICP-OES is an analytical technique used for the detection of trace metals. The intensity of this emission is indicative of the concentration of the elements within the drug KMNC.

ICP-OES Results of NC :

S.No	Elements	Wavelength (nm)	Concentration
1.	Al	396.152	BDL
2.	As	188.979	08.059 mg/L
3.	Ca	315.807	42.100 mg/L
4.	Cd	228.802	BDL
5.	Cu	327.393	BDL
6.	Fe	238.204	08.761 mg/L
7.	Hg	253.652	01.300 mg/L
8.	K	766.491	53.801 mg/L
9.	Mg	285.213	01.984 mg/L
10.	Na	589.592	24.320 mg/L
11.	Ni	231.604	BDL
12.	Pb	220.353	BDL
13.	P	213.617	106.341 mg/L
14.	S	180.731	701.204 mg/L

Specification of Siddha, Ayurveda and Unani products intended for exports:

BDL- Below Detecting Limit:

1% = 10000 ppm, (1 ppm = 1/1000000 (or) 1 ppm = 0.0001%)

The toxic metals and the permissible limits:

Heavy metals	**WHO & FDA limits**
Arsenic (As)	10ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.3ppm

ICP-OES results of NC shows that the heavy toxic metals like As, Hg, Pb and Cd are BDL. The minerals like sodium, potassium, calcium, phosphorus, Iron, magnesium and sulphur are present.

Sodium:

Sodium regulates the acid-base balance of the body fluids.
Sodium is required for the maintenance of osmotic pressure of the body fluids.
Sodium is involved in the intestinal absorption of glucose and amino acids.
Sodium is necessary for the normal muscle irritability and permeability of the cells.
Sodium maintains the extracellular osmotic pressure.

Potassium:

Potassium is required for the regulation of acid-base balance and water balance of the body fluids.
Potassium maintains the intracellular osmotic pressure.

Calcium:

Calcium ions are necessary for the maintenance and regulation of acid-base balance and water balance in the body.
Calcium influences the membrane structure and transport of water and several ions across it.

Phosphorus:

Phosphorus plays an important role for the formation and utilization of high energy production.
Phosphorus involves the function of repair of body cells especially hepato cells and tissues.
Phosphate buffer system is important for the maintenance of pH in the blood (7.4) as well as the cells.
Phosphorus contributes the formation of ATP, ADP and Creatine Phosphate, Phospholipids, Coenzymes and Enzymes of intermediatory metabolism.

Sulphur:

Sulphur is minor constituent of fat, body fluid, skeletal minerals. It is component in most proteins since it is contained in the amino acid.

Iron:

Iron compound have many uses. It is important in forming complex with molecular oxygen in Hemoglobin and myoglobin.

Magnesium:

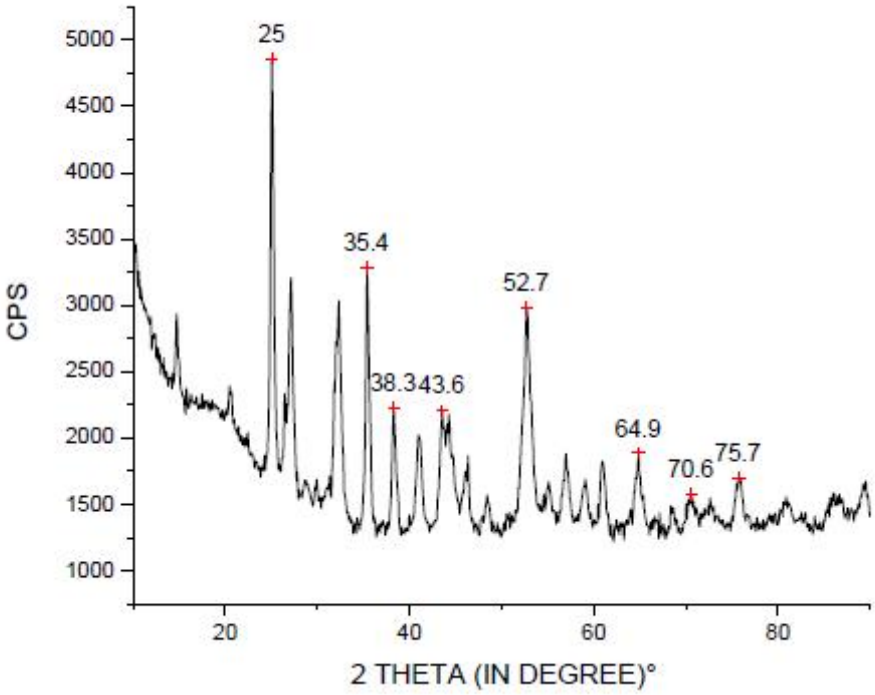
Magnesium is an essential element in biological system. It is an essential mineral nutrient for life. Adenosine triphosphate, main source of energy in cells, must be bound to the magnesium ion in order to be biologically active.

XRD (X-Ray Diffraction):

X-Ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized and average bulk composition is determined.

This XRD finger print shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of Herbo-mineral formulations. Modern techniques are necessary to standardize and bring out the high quality mineral products owing to their complex nature. The different peaks show the presence of minerals in the samples.

XRD –Results of NC



Conclusion

Biochemical analysis showed the presence of Calcium, Sulphate and Ferrous iron. From these results to know effectiveness of the presence of these constituents.

SEM analysis shows that the micro particles present in the test drug. So very minimal quantity of the medicine is enough to treat the diseases.

FTIR spectroscopy shows that different functional groups are present in the test drug.

In XRD different peaks shows that the presence of various minerals in the test drug.

In ICP-OES study shows that the heavy toxic metals As, Pb, Cd and Hg were found to be BDL his ensured that the safety of this formulation (NC).

The biological active principles showed its efficacy. The electrolytes and elements present in this formulation prove its neutraceutical efficacy. Microparticles of the trial formulation indicate the high bio-availability of NC.

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