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Sophisticated Instrumental Approach towards Standardization and Analytical Evaluation of Novel Siddha Formulation *Keelvayu Nivarana Chooranam*

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Abstract

In recent days the trend on standardization of siddha formulations is even become advanced and more precise with available modern sophisticated analytical instruments. In which Scanning Electron Microscope (SEM), Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD) plays very vital role as the siddha medicines treated as pharmaceutical preparations has to comply with the standard expected by the regulatory authorities. Further to achieve the global standard it become mandate that the standard and quality of the drug has to be ascertained through proper standardization methods. According to recent research it was evident that the people throughout the world have started exploring the drugs from siddha origin as it's devoid of side effects. Siddha system of traditional medicine prominently depends on herbs as a major ingredient further there is a constant need of exploring the nature of such preparation's through modern analytical methodologies which will become documentary evidence for the future researcher to work on correlation between structural activity relationships with respect to biological activity. Hence the main aim of the present investigation is to identify the functional group of formulation *Keelvayu Nivarana Chooranam*(KVNC) by FT-IR and also characterize the same by using sophisticated techniques like SEM and XRD. The data's obtained from the present investigation offers valuable information with respect to surface morphological features of the formulation KVNC with the size range of 500nm - 1µm.FT-IR analysis of the sample KVNC reveals the presence of 15 prominent absorption peaks. Further it evident the presence of peaks corresponds to presence of phenolic functional group. The major diffraction peaks are identified in XRD analysis of KVNC shows that the particles are in the average size range of 48-75nm. The structure, size and shape of the particle plays significant role in bio absorption of the drug. Further from the results obtained from the present investigation it was concluded that the formulation KVNC has functional bioactive components such as phenol and hydroxyl group which gains paramount importance in pharmacological activity of the drug and also being a micro formulation with the nano sized particle range it offers better bioavailability towards the treatment of several disease.

Keywords: Siddha,*Keelvayu Nivarana Chooranam*, Standardization, SEM,FT-IR, XRD.

1. Introduction

Ancient Indian literature comprises a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances [1].However, a key obstacle which has hindered the acceptance of the alternative medicines in the developed countries is the lack of documentation and rigorous quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort toward standardization of the plant-based medicines.

The World Health Organization (WHO) has estimated that around 65%–80% of the world population, especially in developing countries, depends essentially on plants for their primary healthcare [2]. THM use has been steadily rising with almost 70%–95% of citizens in major developing countries using THM for their primary health care needs [3]. Use of THM is quite convincing, since it is affordable for all people regardless of their income [4]. The WHO has defined herbal medicine as "herbs, herbal materials, herbal preparations and finished herbal products that contain active ingredients obtained from parts of plants, or plant materials, or combinations thereof" used to treat ailments [5-7] throughout the world [8].

Nanotechnology provides the tools and technology platform for the investigation and transformation of biological systems, and biology offers inspiration models and bio-assembled components to nanotechnology. Nanobiotechnology is defined as a field that applies the nanoscale principle and techniques to understand and transform bio systems (living and non-living) and which uses biological principles and materials to create new devices and systems integrated from the nanoscale [9]. It is widely believed that formulation contains nano particle will effective bind with the bacterial membrane and aids in penetrating the cellular component of the organism thereby preventing its replication.

Siddha also have standardized protocols for purification and detoxification of certain phytocomponents used in specific formulations. As this will greatly reduce the toxicity and also enhance the therapeutic efficacy of the formulation. The chances of occurrence of adverse event is very minimal in siddha when compare to any other therapies in the world this is mainly because the 90 % of ingredients used in the preparing formulations are compatible with the biological system of the humans and animals. Hence event of adverse events are less. Anti-dotes and counter therapy modifications are even available in siddha system in case of rare occurrence of some unexpected interactions.

The main objective of the present investigation is to identify the functional group of formulation *Keelvayu Nivarana Chooranam*(KVNC) by FT-IR and also characterize the same by using sophisticated techniques like SEM and XRD.

2. Materials and Methods

2.1.Ingredients

The formulation *Keelvayu Nivarana Chooranam* comprises of the following ingredients

Nannariverpattai (Hemidesmus indicus) - 116 g
Parangipattai (*Smilax chinensis*) - 116 g
Seemai Amukara (Withania somnifera) - 116 g
Chittaraththai (Alpinia officinarum) - 58 g

2.2. Source and authentication of raw drug:

All the raw drugs werebought from Indigenous authentic country drug shop at Parry's corner, Chennai, Tamil Nadu, India. All the raw drugs were identified and authenticated by the *Gunapadam* experts in Government Siddha Medical College, Arumbakkam, Chennai – 106. The specimen sample of all the herbs have been preserved in PG *Gunapadam* department individually for future reference. **Ref No: GSMC/PGGM/014-017/2014-2017**

2.3. Purification of Raw Materials

All the raw drugs required for the formulation of KVNC were purified as per the Siddha literature. *Nannariverpattai*were washed in the running tap water to remove the soil and impurities.*Parangipattai* was dried and powdered and then it was purified by *Pittaviyal* method (steam cooking in milk). A mud pot was taken and it was half filled by milk and half filled by pure water. The mouth of the pot was sealed by a cloth. This *chooranam* then placed over the cloth and the pot was heated. The same drug was later dried and powdered then sieved again.*Amukara* was dried and powdered and then it was purified by *Pittaviyal* method (steam cooking in milk). A mud pot was taken and it was half filled by milk and half filled by pure water. The mouth of the pot was sealed by a cloth.

This *chooranam* then placed over the cloth and the pot was heated. The same drug was later dried and powdered then sieved again. *Chittaraththai* were washed in the running tap water to remove the soil and impurities.

Formulation of *Keelvayu Nivarana Chooranam* [10]

All the above purified ingredients were powdered in an iron mortar separately and it was sieved by a cotton cloth. Then these powders were mixed together and bottled up. It was labeled as *Keelvayu Nivarana Chooranam* (KVNC).

2.4. Drug Storage

The prepared test drug was stored in a clean, air tight glass container. The contents were inspected frequently to avoid moisture and insects.

Dose : 1g twice a day

Adjuvant : Honey

2.7. Fourier Transform – Infra Red Spectroscopy Study [11-12]

Fourier Transform – Infra Red Spectroscopy Study (FTIR) IR data acquired with Spectrum one: FT-IR Spectrometer with scan range of MIR 450-4000 cm-1. About 20 mg of the test sample SPK was taken on a micro spatula and grounded well with required quantity of KBr salt. Sample admixed with KBr with trituration aided by mortar and pestle until to get a uniform fine powder of sample- KBr mixture. Further mixture was loaded in pellet die and subjected to 5000-10,000 psi in pelletizer. Resulting pellet was placed in FTIR sample holder and expose to IR radiation to get the spectra.

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX) [13-14]

The study was conducted in a very fine powder of the drug and the sample was quick frozen in liquid nitrogen. The sample was mounted rigidly on a specimen holder called specimen stub. The mounted sample was placed inside the microscope's vacuum column evaporator through an air tight door. On expelling air from the air pump, a beam of electrons passed from an electron gun. This beam travelled through a series of magnetic lenses designed to focus the electrons. The focused beam moved back across the mounted sample row by row by a set of scanning coils. As the electron beam hit each spot on the sample, secondary electrons are backscattered from its surface. A detector counts these electrons and sends the signals to an amplifier. The final image was built up from the no of electrons emitted from each spot on the sample. The micrographs obtained give sufficient data about the topography of the subjected sample. Model- SEM-Hitachi with the scan range of 3400n and resolution of 1.2 nm gold particle separation on a carbon substrate .Magnification from a min of 12 x to greater than 1, 00,000 X

XRD spectral Study

The XRD spectrum of test drug was Bruker discover D8 X ray diffractometer. Cu K Alpha radiation was used for recording the spectra. The range of diffraction angle 10-70^{o} operating at 30kV and 20 mA. The pattern was recorder from the angle 5 to 80 degree at a scanning rate of 3 degree/second [15].

3.Results

3.1. Result Analysis of FT-IR spectrum of the formulation KVNC

The results obtained from the FTIR instrumental analysis of the sample KVNC reveals the presence of 15 peaks intense absorption peaks corresponds to the presence of hydroxyl and phenol functional. As shown in figure 1.

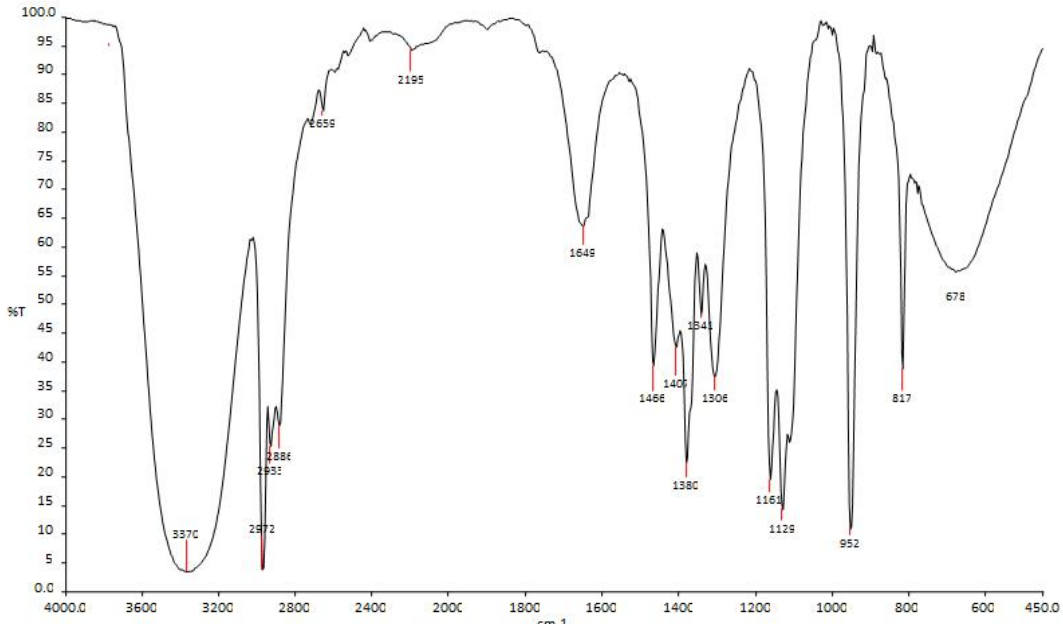

Figure 1: FTIR Spectrum of the formulation KVNC

3.2. Result Analysis of SEM images of the formulation KVNC

The particle size and chemical elements were assessed by SEM is one of the most widely used instruments in research areas. Results of the present study debits that the surface of the sample grains is uniformly arranged in agglomerates. They are micro particles ranging from 500nm - 1µm size. As shown in figure 2.

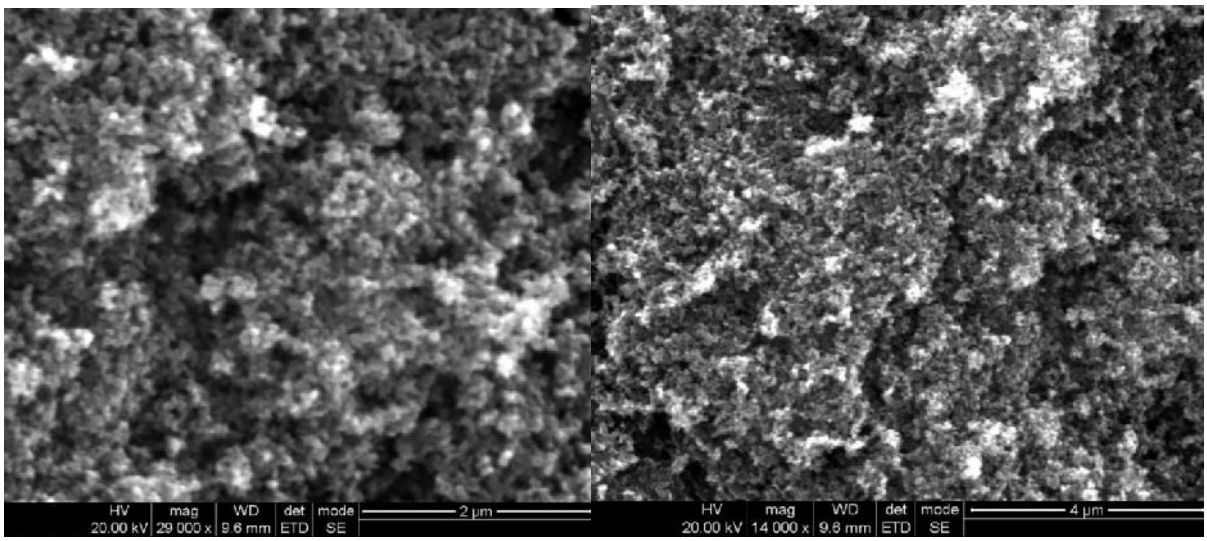

Figure 2: SEM analysis of the formulation KVNC

3.2. Result of XRD analysis of the formulation KVNC

The structure, size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio absorption of the drug. From the major diffraction peaks obtained from XRD analysis KVNC it was concluded that average particle size range 48-75nm is association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in KVNC act as additional supplement and possibly helps in increase the efficacy of the formulation. As shown in figure 3.

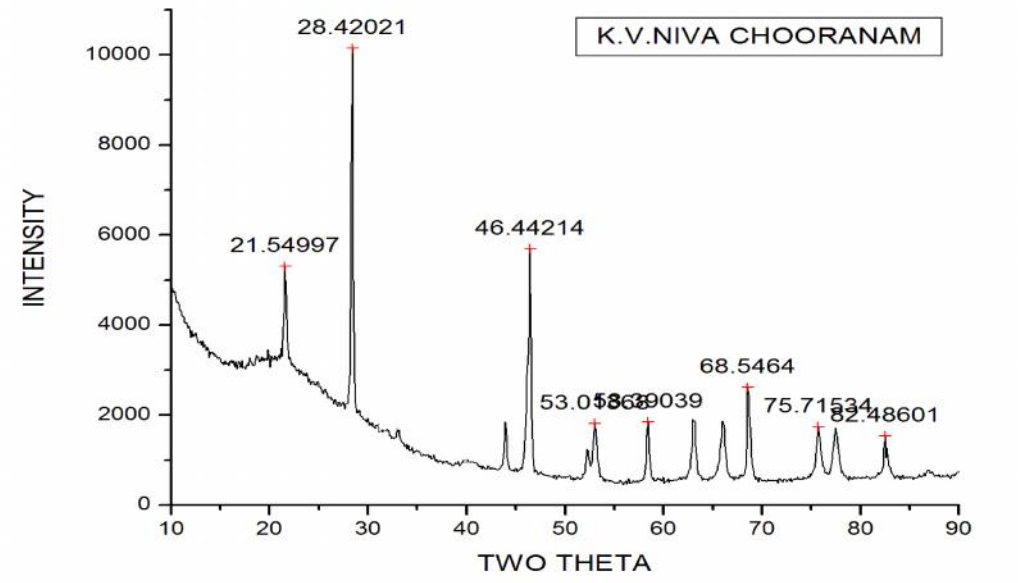

Figure 3: XRD analysis of the formulation KVNC

4. Discussion

Indian traditional medicine or medicinal plants are also considered as a vital source of new drug. Mainstreaming of such medicine is important for the people. Several steps have been taken in India to promote such medicine and to integrate them into clinical practice. Evidence based incorporation of Indian traditional medicine in clinical practice will help to provide quality healthcare to all. FT-IR analysis of the sample KVNC reveals the presence of 15 prominent absorption peaks. Further it evident the presence of peaks corresponds to presence of phenolic and hydroxyl functional group. Researchers suggested that presence of phenolic functional groups contributes to significant higher level of biological activity such as anti-oxidant property of the formulation [16].

In 2005, WHO published a report of a global survey about National Policy on Traditional Medicine and Regulation of Herbal Medicines. This report indicated that about 50 countries already have their national policy and laws or regulations on traditional medicines. Through systematic standardization a formulator can profile the preparation with respect to the following (i) Physiochemical parameters (ii) Category of phytocomponents (iii) Nature of individual chemical component (iv) Structural and functional group analysis (v) Correlation of mechanism with respect to the functional group present in bioactive phytocomponents (vi) Drug stability (vii) Pharmacokinetic profiling (viii) Receptors on which the drug acts (ix) Chances of possible interaction. Because of the emerging knowledge in the field of drug standardization. Now siddha preparation which satisfy the quality and standard are being exported and it is in practice by other countries like Sri Lanka, USA and Indonesia.

Present investigation offers valuable information with respect to surface morphological features of the formulation KVNC with the size range of 500nm - 1µm.The major diffraction peaks are identified in XRD analysis of KVNC shows that the particles are in the average size range of 48-75nm. The structure, size and shape of the particle plays significant role in bio absorption of the drug. Nanoparticles have important properties that can be used to progress the drug delivery. Where larger particles would have been unequipped from the body, cells adopt these nanoparticles because of their size. Complex drug delivery mechanisms are being developed, together with the capability to get drugs through cell membranes and into cell cytoplasm. Efficacy is important because various diseases depend upon processes within the cell and can only be impeded by the drugs that make their way into the cell [17].

5. Conclusion

From the results of the present study it was observed that the siddha formulation KVNC contains biologically significant functional groups like hydroxyl and phenolic moieties and further SEM and XRD data reveals the presence of particle with size range of micro to nano scale range which again evident the improved bioavailability and penetrability of the drug in to the biological system.

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