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ICP-OES and FTIR Analysis of Siddha Herbo-mineral formulation of *Vedi Annabedhi Chenchooram*

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ABSTRACT

The *Vedi Annabedhi Chendhooram* (VABC) is a vital herbo-mineral formulation in Siddha medicine, traditionally employed for treating various conditions such as dysentery, dropsy, jaundice, anemia, fever and ascites. This study aimed to assess the presence of heavy metals and other elements in VABC, ensuring adherence to WHO guidelines. Ingredients were meticulously collected, purified, and prepared as per Siddha literature "*Siddha Vaithya Pathartha Guna Villakam*" (*Thathu – Jeeva Vilakam*), authored by Dr. C. Kannusamipillai. Here, the drug was subjected to standardization by simultaneous ICP-OES analysis equipment (PERKIN ELMER OPTIMA 5300 DV). The FTIR spectra sample was analyzed by using Bruker Alpha FTIR Spectrophotometer. The results, obtained through ICP-OES analysis, affirm the absence of toxic levels of heavy metals in *Vedi Annabedhi Chendhooram*, establishing its safety for use in Siddha medicine. FTIR spectroscopy complementarily scrutinized the presence of organic substances. From the ICP-OES analysis reveals that *Vedi Annabedhi Chendhooram* were free from toxicity there by proving the safety of its utilization in Siddha system. FTIR spectroscopy has been used to study the presence of organic substances in the sample. The findings form a crucial foundation for subsequent pharmaceutical analyses and efficacy studies. This study underscores the importance of Vedi *Annabedhi Chendhooram* in Siddha medicine, paving the way for its safe and effective utilization.

Keywords: *Vedi Annabedhi Chendhooram*(VABC), Siddha medicine, ICP-OES, FTIR.

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INTRODUCTION

The Siddha system, rooted in South India, stands as one of India's oldest traditional medicine systems, integrating ancient medicinal practices, spiritual disciplines, mysticism and alchemy. It offers a holistic approach, addressing preventive, promotive, rehabilitative, curative and rejuvenative health needs. In the Siddha tradition, substances like Ferrous Sulphate (*Annabedhi* in Tamil), Potassium Nitrate (known as*Vediuppu, Potiluppu, Enangan, Padairasn, Boomikoormai, Navacharamithru* in Tamil) and others undergo purification processes[(1)] before being used in medicinal preparations. These purified substances are integral components of numerous Siddha formulations, totaling 64 varieties 32 for internal use and 32 for external applications. Notabley, "Chendhooram", a red-colored powder with a remarkable shelf life of 75 years, is a significant internal medicine within this system.

The *Vedi Annabedhi Chendhooram* (VABC)[(2)] holds a crucial position in Siddha medicine, serving as a significant herbo-mineral formulation traditionally utilized for the treatment of diverse conditions, including dysentery, dropsy, jaundice, anemia, fever and ascites. Beyond its established indications, this formulation is also employed by traditional medical practitioners to address gynecological disorders such as polycystic ovarian syndrome, fibroid uterus and excessive vaginal bleeding.

For the development of a new drug, standardizing traditional Siddha formulations is paramount importance. While many Siddha medicines exhibit effectiveness, their lack of standardization possesses a significant challenge. Additionally, herbo-mineral formulations may contain toxic elements, necessitating thorough standardization to ensure the safety profile and therapeutic utility of the drug. In this context, the drug underwent standardization through simultaneous ICP-OES analysis using the PERKIN ELMER OPTIMA 5300 DV equipment to detect heavy metals and confirming the absence of toxic levels of heavy metals. Furthermore, FTIR spectra samples were analyzed using the Bruker Alpha FTIR Spectrophotometer, aids in minimizing adulteration, undoubtedly contributing to a comprehensive understanding of the characterization of the chosen ingredients in *Vedi Annabedhi Chendhooram*, ensuring both safety and quality.

MATERIALS AND METHOD

The herbo-mineral formulation of *Vedi Annabedhi Chendhooram* (VABC) selected from the book *Siddha Vaithiya Pathartha Guna Villakam (Thathu-Jeeva Varkam),* authored by Dr. C. Kannusamipillai.

Collection and Authentication of the Drugs:

The required raw drugs were purchased from raw drug store at Tambaram and authenticated

by Geo Scientist, Faculty of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur, Chennai – 603203.

Ingredients of VABC:

- Purified *Annabedhi* (Ferrous Sulphate) – 75 gms
- Purified *Vediuppu* (Pottasium Nitrate) – 5 gms
- Lemon (*Citrus Limon*) – Sufficient quantity

Purification of ingredients:

Annabedhi:

A solution was prepared by dissolving one kilogram of *Annanedhi* in one liter of water, followed by filtration using muslin cloth to remove dust and impurities. To the filtered solution, ten drops of *Ganthaga Thiravagam* (Sulfuric acid) were added. The resulting mixture was boiled and taken at the stage of distilled as ripeness of salt.[1]

Vediuppu:

Combine four liters of water with 1kg of *Vediuppu* and simmer over low heat. Add four eggs to the boiling mixture, and skim off any bubbles containing impurities using a wooden spoon. Transfer the mixture to a different container, seal it with a mud cloth, filter it, and store it in areas without aeration. The next day, filter the water, add allow the salt to dry in the shade. Repeat this process seven times for purification.[1]

Preparation process:

The detoxification of the drug followed the methods outlined in an authorised Siddha textbook. (1) Fresh lemon juice was utilized for the process. *Annabedhi* and *Vediuppu* underwent separate purification processes, and they were combined with a generous amount of lemon juice. The mixture was meticulously ground until it attained a waxy consistency. And then it was shaped into small circular cakes and left to undergo the drying process. Subsequently, the desiccated cakes were arranged within a mud vessel, covered by another mud vessel, and enveloped in seven layers of cloth (S*eelaiman*). This arrangement underwent calcination using 70 cow dung cakes. Four pudams of the resultant product encountered further processing, culminating in the production of a powdered substance.[2]

Instrumental methods:

The elemental composition of the samples was determined using ICP-OES and FT-IR. The ICP-OES used in the study is Perkin Elmer Optima 5300 DV (Simultaneous) based on Argon plasma. The FTIR spectra samples were recorded between 4000 and 400 cm^{-1} using Bruker Alpha FTIR Spectrophotometer with ZnSe ATR technique at 2 cm^{-1} resolution. ICP-OES and FTIR analysis were conducted at the Sophisticated Analytical Instrument Facility, IITM, located in Chennai-36.

ICP-OES Study of *Vedi Annabedhi Chendhooram* (VABC)

Principle of ICP Optical Emission Spectrometry (ICP-OES) [3]

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry. When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the rays' intensity. To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

EQUIPMENT

Equipment for ICP optical emission spectrometry consists of a light source unit, a spectrophotometer, a detector and a data processing unit. There are several types of equipment based on differences in the Spectrophotometer and the detector. The most common type is shown in Figure 1.

Sequential type

A spectrophotometer with a Czerny-Turner Monochromator, and a detector with a photomultiplier is most common for this type. With this equipment, programmed wavelength of the spectrophotometer is consecutively varied to measure multiple elements. This causes rather long measuring time, however, with its high resolution spectrophotometers, it is favorable for measurement of high-matrix samples. Figure 1

Simultaneous Type

This type typically uses an echelle cross disperser in spectrophotometers and semi-conductor detector such as CCD for the detector. Echelle cross disperser disperses light of measurable wavelength range two-dimensionally by combining prism and echelle diffraction grating. Combination of echelle cross disperser and a CCD detector enables multi-element measurement at any wavelength. The most notable feature of this equipment is the high-speed measurement, providing information on all 72 measurable elements in measurements of 1 to 2 minutes normally. (Figure 2)

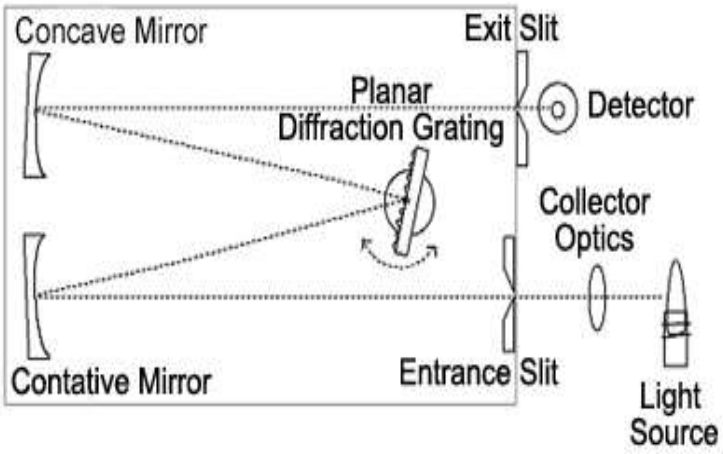

Figure 1: Sequential Type ICP-OES

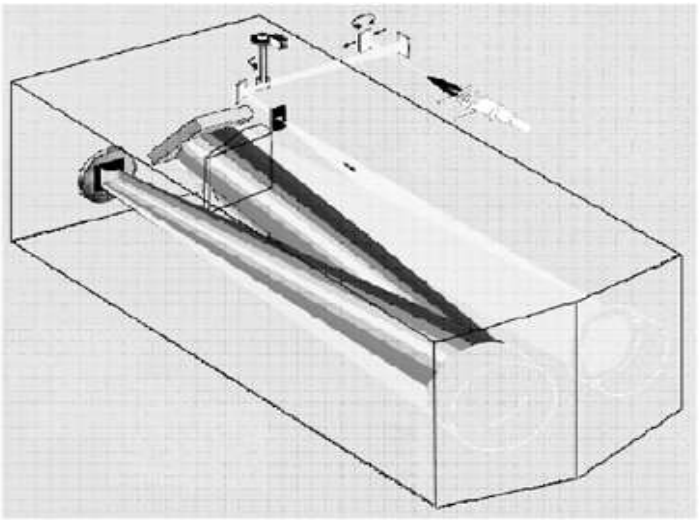

Figure 2: Simultaneous ICP-OES

VABC analysis:

VABC has attracted attention because it is thought to contain a person's health history on some level and is thought to act as an excretory organ for heavy metal in the body. However, there are problems because there are few usable samples and knowledge about multiple elements is required. With simultaneous analysis equipment, we can collect useful information with a small amount of sample.

Equipment: Simultaneous ICP-OES, PERKIN ELMER OPTIMA 5300 DV

Sample: 0.40340g of VABC is measured, and then dissolved in a decomposition vessel with nitric acid into 10ml solution. Partial spectral profile and analysis results shown in table 1. .

FTIR

FTIR is an important and more advanced technique to identify the functional group. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there is no two unique molecular

structures producing the same infrared spectrum. It is recorded as the wavenumber and the peaks seen in the spectrum indicates the amount of material present.

The Perkine Elmer Spectrum in Fourier Transform Infrared (FTIR)[4] Spectrometer was used to derive the FTIR Spectrum of UP placed in Potassium Bromide (KBr) discs with scan rate of 5 scan per minute at the resolution 4cm^{-1} in the wave number range 4000-400 were recorded the FTIR Spectrum under Standard condition. FTIR Spectra were used to determine the presence of the functional groups and bands in the drug (VABC). The recorded spectrum shows in figure 3. The standard table of FTIR is given in table 2.

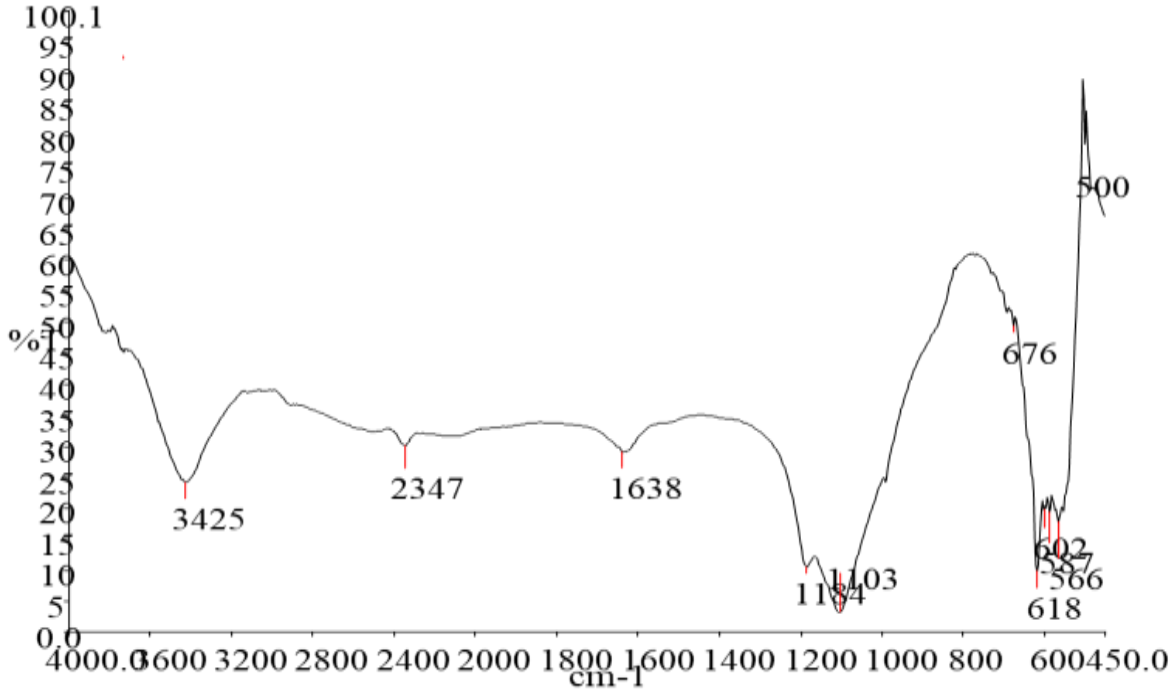

Figure 3: FTIR Spectrum of VABC

RESULTS AND DISCUSSION

Table 1: Elements of *Vedi Annabedhi Chendhooram* (VABC) - (Wt: 0.40340g)

S. No	Elements	Wavelength(nm)	Detected Levels (in VABC)
1.	Al	167.020	BDL
2.	As	188.979	BDL
3.	Fe	238.204	650.256 mg/L
4.	Hg	253.652	BDL
5.	K	766.491	494.821 mg/L
6.	Mg	285.213	00.342 mg/L
7.	Mn	257.610	BDL
8.	Na	589.592	01.320 mg/L
9.	Pb	220.353	BDL
10.	P	213.617	76.341 mg/L
11.	S	180.731	211.254 mg/L

* BDL – Below Detectable Limit

Table 2: Functional group and Wavelength of VABC

Frequency, cm^{-1}	Bond	Functional group
3425	O–H stretch, H–bonded	Alcohols, phenols
2347	O=C=O stretching	Carbon dioxide
1638	–C=C– stretch	Alkenes
1184	C–O stretch	Alcohols, Carboxylic acids, Esters, Ethers
1103	C–N stretch	Aliphatic amines
676	–C≡C–H: C–H bend	Alkynes
618	–C≡C–H: C–H bend	Alkynes
602	C–Br stretch	Alkyl halides
587	C–Br stretch	Alkyl halides
566	C–Br stretch	Alkyl halides
500	C-I stretching	Alkyl halides

DISCUSSION

Heavy metal Viz. arsenic (As), mercury (Hg), lead (Pb), Aluminum (Al) , Manganese (Mn) and other elements such as iron (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), sulfur (S), of VABC on table 3 was found to be within the permissible limits as per WHO guidelines. The FTIR results shows the observed water O-H stretch, O-H stretch, H-C-H stretch, C=O stretch, N-H stretch, –C=C– symmetric stretch, H—H bend, C-O stretch, C-H bend, C-C stretch which indicates that the presence of functional groups Amide, Phenols and alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Ether, Alkyne.

CONCLUSION

The ICP-OES analysis results affirm *Vedi Annabedhi Chendhooram* (VABC) is devoid of toxicity, substantiating its safety within the Siddha system. Additionally, FTIR studies indicate the absence of harmful chemicals and minerals. Therefore, VABC demonstrates its safety for prolonged use. These analysis serve as the foundation for the pharmaceutical assessment of VABC, paving the way for subsequent investigations into its safety and efficacy. This study marks a significant advance towards the scientific validation of VABC in the context of Siddha medicine.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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