Studies on Contributions of FTIR Spectroscopic analysis in the Purification process of Veeram (Hg$_2$Cl$_2$) used in Siddha formulations

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**ABSTRACT**

In Siddha system Arsenics are called as Paasaanam (toxins). Veeram is one of the p aasaanam, its chemical formula is Hg$_2$Cl$_2$ (Calomel). Internally arsenic based medicines are used for rheumatoid arthritis, generalized body pain, syphilis, epilepsy and cancer. Various organic agents are used to purify the veeram such as milk, tender coconut, bitter guard and lemon juice. In this study raw veeram and products obtained after purification were analyzed using FTIR Spectroscopy with a view to understand the need and mechanism of this purification processes. FTIR analysis was carried out before and after purification. Efforts were made to study various chemical changes veeram undergoes during this process. FTIR of the raw Veeram and its processed samples were recorded between 4000-400 cm$^{-1}$. In the study raw drug showed only nine functional groups in the region between 3789.36 cm$^{-1}$ and 468.75 cm$^{-1}$. Bitter gourd treated veeram peaks were observed in the region between 3526.99 and 476.62 cm$^{-1}$ and a total of 13-peaks were obtained. Milk treated veeram showed peaks in the region between 3851.10 and 470.39 cm$^{-1}$ and total of 16-peaks were obtained. This method indicated presence of large number of functional groups. Lemon juice treated veeram showed peaks in between 3839.33 and 471.99 cm$^{-1}$ total of 15-peaks were obtained. Tender coconut veeram peaks were observed in between 3877.84 and 477.33 cm$^{-1}$ and total of 15-peaks were obtained. From this data number of functional groups increased in purified samples which indicated that the toxic veeram is not only detoxified but also interacted with functional groups of purifying agents there by therapeutic potency is enhanced.

**INTRODUCTION**

Fourier Transform Infrared Spectroscopy (FTIR). The widespread use of IR spectroscopy is for the identification of drugs, polymorphic modifications, excipients and raw materials used in pharmaceutical manufacturing. This is mainly due to the sensitivity and the ease with which spectra can be obtained on any sample including insoluble solids, polymers, solutions, gases. Identical IR spectra of two samples (superimposable spectra) are excellent evidence that the two samples have the same
chemical analysis. IR spectrometric analysis is a handy tool in the detection of functional groups of biomolecules, thus aiding in their structural elucidation, thereby confirming the presence of active molecules responsible for the therapeutic activity of Ayurveda & Siddha drugs. It can also be used in the understanding of the complex formulation of different metals with phytoconstituents (Ouhaddouch et al., 2019).

In Siddha system of medicine metals, minerals, plants and animal products are used to prepare the medicines. The metals and minerals are further classified into four categories, namely Metals -12, Mineral-24, Arsenic-64 and Hydro chemicals -120 (Thyagarajan, 2004). These are unique and specific to the traditional medical system. A small dosage is sufficient for the metallic formulation to control the illness completely, it acts very fastly, single medicine has so many therapeutic values, it has significantly longer shelf life, older prepared medicines possess rich medicinal values, and geological variations will not occur in metallic drugs (Thyagarajan, 2004; Devi et al., 2019). In this system, arsenic is called as Paasaanam. In Tamil language Paasaanam means Toxins. According to the Siddha system, pasanam is either native or synthetic – they are further subdivided into thirty-two kinds.

Veeram is one of the arsenic (Paasaanam), it is commonly known as corrosive material, chemical name is mercuric chloride, and the chemical formula is Hg₂Cl₂ (Mariyammal, 2016). In our traditional system, it has various names sawveeram, poovinthu, sarakku chunnam and parangi pasanam (Thyagarajan, 2004; Mariyammal, 2016). In Sanskrit, it is called sawveera. In other regional languages such as Telugu, Kannada, Malayalam its name is sawveera pasanam (Narayanaswami and Uthamarayan, 1993) Veeram is found as the main ingredient in many Siddha formulations like Thirithoda mathirai, Amirtha vennai, sawveera centuram, ayaveera centuram, Chanda marutha centuram, ashta Bairava kuligai, emathanda kuligai, maha veera mezhugu, pancha sutha mezhugu and ghanthaga sudar thailam (Uthamarayan et al., 2008; Kuppusamymudaliyar and Uthamarayan, 2009).

In Siddha system, it acts as alterative, antiseptic and possesses caustic activities. Internally these arsenic-based medicines are used for rheumatoid arthritis, generalised body pain, syphilis or gonorrhoea, epilepsy, ophthalmia, gastric ulcer and cancer (Thyagarajan, 2004). It may safely be prescribed in hydrocephalus, dropsical affections, glandular enlargements and hepatic problems (Pillai, 1992). Veeram based medicine Ayaveera chenthuram is used for sciatica; its mention has also been made in the clinical research papers of (Seethalakshmi et al., 2017; Shibi et al., 2012).

Its therapeutic dose is 2 mg to 4 mg; in large doses, it is highly poisonous (Murugesamudalier, 1988). Large doses symptoms are ptyalism, adhesion of cheeks and gums. If there is the appearance of toxic symptoms, specific herbal antidotes are prescribed in Siddha textbooks. Few of the antidotes mentioned are Tribulus terrestris plant juice or Indigofera tinction plant paste or Vernonia cinerea plant juice or coconut toddy (Gurusironmani, 1999; Murugesamudalier, 1988). Advance toxicology research work was carried out on veeram based Siddha formulation Arumuga chenthuram by (Murugan et al., 2016).

The purification processes of these metals in the Siddha system of medicine removes toxicity and enhances efficacy. FTIR analysis of veeram and purified veeram samples were performed to detect the presence of functional groups or organic ligands in the treated samples. This purification process of veeram study is a small step towards the right direction to prove the safety of Siddha drugs and in determining the changes from raw drug to purified drug.

An earlier report on similar spectroscopy research work of veeram and processed veeram was existing using mega sanjeevi pills in the year 2012. (Sathish et al., 2012)

**METHODOLOGY**

Veeram was obtained from Raw drug shop of Chennai and authenticated using the database (7487-94-7 CAS DataBase). Detoxified materials bitter gourd, lemon and tender coconut were procured from the vegetable market, Thanjavur; Tamil Nadu and milk were obtained from SASTRA University gosala, SASTRA University, Thanjavur.

**VEERAM purification process**

In this study veeram (Figure 1) was purified using four processes as per the methods mentioned in Siddha textbook (Aananthan and Saganthala, 2008). The methods are briefly explained below. Method one is Bitter gourd treated veeram (BTV), second is Milk treated veeram (MTV), Third is Lemon juice treated veeram (LTV), and last fourth method is Tender coconut treated veeram (TTV).

**BTV process** - bitter guard is cut horizontally, and 25 gm of sample kept in the centre portion and tied the bitter guard tightly together using a thread and placed in an earthen vessel which is filled with lemon juice (625 ml) using thula yanthra murai.
Thula yanthra murai - In this procedure, the material is boiled in a fluid containing vessel in which sample is tied like a bundle and suspended with the help of a thread into the liquid or boiled in the steam. The other end of the thread is tied to the rod. The pot is then kept on the stove and heated (Pillai, 1992). The sample is boiled for one hour with the juice/steam (Figure 3).

MTV process – In the milk treatment process, the sample was soaked in cow’s milk in an earthen pot. Kept under sunlight till the milk is thoroughly dried, after drying, is obtained purified sample (Figure 4).

LTV process – In this method also the thula yanthram is used. In this process, lemon juice is used instead of tender coconut (Figure 5).

TTV process – In this method also, the thula yanthram method is followed. In this method, the sample is subjected directly to tender coconut steam. The sample was tied in a kada cloth with the use of thread and heated using tender coconut steam (Figure 6).

Fourier Transform Infrared Spectroscopy

FTIR is the acronym for Fourier Transform Infrared Spectroscopy. FTIR is a spectroscopic technique that utilises lower energy radiation to induce vibrational and rotational excitation of atoms and groups of atoms within molecules. Because of the variety of symmetry of atomic groups and their differences in atomic masses and electronic structure, the absorption patterns for a specific species will be unique, which allows for their identification. The infrared spectroscopic technique is the most popular vibrational spectroscopic technique used to identify the functional groups in organic and inorganic compounds. IR spectroscopic technique utilises lower energy IR radiation (10000-100 cm⁻¹).

Infrared spectroscopy is the study of the reflected, absorbed or transmitted radiant energy in the region of the electromagnetic spectrum with ranging wavelength from 0.8 to 500nm. A more commonly used measurement is the frequency and is expressed in wavenumber. The IR spectrum is usually divided into three regions, namely – near IR (12500 to 4000 cm⁻¹), mid-IR (4000 to 400 cm⁻¹) and far IR (400-20 cm⁻¹). Only the mid-IR region is usually referred to simply as infrared and is widely used in the analysis of drugs and pharmaceuticals. Fourier transform spectrophotometer is the recent advancement in the field of Infra-Red spectroscopy, which has many advantages over dispersive instruments. They can be scanned.
Table 1: FTIR Peaks obtained in veeram and various purification process products of veeram

| S.No | Sample | No. of Peaks | Obtained Peaks                                      |
|------|--------|--------------|-----------------------------------------------------|
| 1    | Veeram | 9            | 3789.36, 3516.31, 3572.26, 2419.80, 1939.94, 1613.17, |
|      |        |              | 1366.75, 1232.62, 468.75                            |
| 2    | BTV    | 13           | 3526.99, 3585.73, 3437.68, 2924.09, 2854.65, 1830.44, |
|      |        |              | 1613.86, 1560.02, 1459.08, 1169.88, 1115.01, 501.02, |
|      |        |              | 476.62                                              |
| 3    | MTV    | 16           | 3851.10, 3525.23, 3584.86, 2853.70, 2924.47, 1743.02, |
|      |        |              | 1613.17, 1557.80, 1460.34, 1378.68, 1243.10, 1167.98, |
|      |        |              | 1114.16, 1019.23, 721.53, 470.39                     |
| 4    | LTV    | 15           | 3839.33, 3625.97, 3585.30, 3568.40, 3526.18, 3437.31, |
|      |        |              | 2855.77, 2925.42, 1944.14, 1613.94, 1560.03, 1172.22, |
|      |        |              | 1116.80, 690.43, 471.99                              |
| 5    | TTV    | 15           | 3877.84, 3646.01, 3526.35, 3585.90, 2854.46, 2924.96, |
|      |        |              | 2342.66, 1922.26, 1738.28, 1613.76, 1559.97, 1459.18, |
|      |        |              | 1260.06, 1168.13, 477.33                              |

RESULTS AND DISCUSSION

Raw and four processed samples were analysed for their functional groups by using FTIR at Central Instrumental Facility, CARISM, SASTRA University, Thanjavur. FTIR of the raw Veeram and its intermediates obtained during various purification processes were recorded between 4000-400 cm⁻¹ in an FTIR spectrometer (Spectrum 100, Perkin Elmer, USA). The samples were mixed with KBr and pelletised for analysis. A comparison of FTIR Spectra of Raw Veeram and products obtained during various purification processes indicated the changes occurred in the fingerprint regions. Some peaks are disappearing, and many new peaks appear. Table 1 presents the peaks obtained in veeram and products obtained during the various purification process of veeram. Table 2 explained the FTIR Peaks of veeram and various purified products of veeram obtained in
Table 2: Effect of Imperata cylindrica Linn rhizomes extracts on biochemical parameters on last day

| Sample | Hydrogen stretching region (4000 to 2700 cm⁻¹) | Triple bond (2700 to 1950 cm⁻¹) | Double bond (1950 to 1550 cm⁻¹) | Fingerprint region (1500 to 700 cm⁻¹) | Unknown Region |
|--------|---------------------------------------------|-------------------------------|---------------------------------|-----------------------------------|----------------|
| Veeram | 3789.36, 3516.31, 3572.26 | 2419.80 | 1939.91 & 1613.17 | 1366.75 & 1232.62 | 468.75 |
| BTV    | 3526.99, 3585.73, 3437.68, 2924, 2854    | -    | 1830, 1613.86, 1560.02 | 1459, 1169.88, 1115.02 | 501.02, 476.62 |
| MTV    | 3851, 3584.86, 3525.23, 2924.47, 2853.7 | -    | 1743, 1614.2, 1557.8 | 1460, 1379, 1243, 1168, 1114.2, 721.53 | 470.39 |
| LTV    | 3839, 3626, 3585, 3568, 3526, 3437, 2856, 2925 | -    | 1944, 1613.9, 1560.03 | 1172.22, 1116.80, 721.53 | 690.43, 471.99 |
| TTV    | 3877.84, 3646.01, 3585.9, 3526.4, 2925, 2854.5 | 2342.66 | 1922.3, 1738, 1613.8, 1560 | 1459.2, 1260, 1168.13 | 477.33 |

Different regions.

**RAW VEERAM**

FTIR spectra of *Veeram* revealed peaks in the region of 3789.36 cm⁻¹ to 468.75 cm⁻¹. As shown in Figure 7. Total of 9- peaks were obtained which includes three phenols groups, one carboxylic acid stretching, one hydroxyl peak, one benzene ring (aromatic), one alkyl region and two amide bands (*Charde et al., 2016*).

Total of nine peaks was obtained which includes three peaks in hydrogen stretching region, one peak in the triple bond region, two peaks in the double bond region, two peaks in fingerprint region and one peak in unknown region (*Balan et al., 2019*). Three phenol functional groups of 3789.36 cm⁻¹, 3516.31 cm⁻¹ and 3572.26 cm⁻¹ are present in hydrogen stretching region and denotes the presence of phenol group. Only one carboxylic group 2419.80 cm⁻¹ is present in the triple bond region. 1939.91 cm⁻¹ & 1613 cm⁻¹ is C-C aromatics functional groups present in double bond region. 1366.75 cm⁻¹ is alkyl peak, and amide ring is present in the fingerprint region.

Unknown region peak 468.75 cm⁻¹ indicates the presence of halogen bonds. Already spectroscopic studies on Ayurvedic products have identified halogen bond, which is the agreement with the present work (*Charde et al., 2016*).

**BTV**

In the FTIR spectra of BTV peaks were observed in the region of 3526.99 to 476.62 cm⁻¹. The graph is given in Figure 8. Total of 13- peaks were obtained...
which includes two phenols groups, two alcohol peaks, one benzene ring (aromatic), one ketone functional group, two sulphur bands, one benzene ring, two alkyl stretches, one ammonia and one amide peak (Charde et al., 2016). Total of thirteen peaks was obtained which includes five peaks in hydrogen stretching region, three peaks in the double bond region, three peaks in fingerprint region and two peaks in an unknown region (Balan et al., 2019). 3526.99 cm\(^{-1}\), 3585.7 cm\(^{-1}\), 3437.68 cm\(^{-1}\), 2924 cm\(^{-1}\) and 2854 cm\(^{-1}\) are present in hydrogen
strecthing region. In this region the first two peaks are phenols, next two peaks are alcohol, and the last one is a benzene ring. 3437.68 denotes the presence of a significant quantity of hydroxyl groups (OH) in the structure, the same peak was identified in the mercury-based Siddha medicine *Arumuga chenthumaram* (Shibi et al., 2012). The 2924 cm⁻¹ peak is attributed to vibration of axial deformation of C-H of the CH₂ group (Balan et al., 2019). 3- groups are in the double bond region, 1830 cm⁻¹ is ketone functional group, 1613.86 cm⁻¹ is a broad and sharp peak denotes the occurrence of the amine group. And 1560 cm⁻¹ signified the amide peak. Three peaks are presented in the fingerprint region, in that 1459 cm⁻¹ are 1169.88 cm⁻¹ sulphur bands and 1115.02 cm⁻¹ is peptide group.

**MTV**

FTIR spectra of MTV showed peaks in the region of 3851.10 to 470.39 cm⁻¹. The graph is shown in Figure 9. A general overview of MTV indicates the presence of a large number of functional groups. Total of 16- peaks were obtained which includes one hydrocarbon bond, two ammonia structures, four alkyl group, one carbonyl, three amide linkage, three phosphate stretches, one steroid organic compound and one Halogen ring (Charde et al., 2016). Total of sixteen peaks was obtained which includes five peaks in hydrogen stretching region, three peaks in the double bond region, seven peaks in fingerprint region and one peak in unknown region (Balan et al., 2019). 3851 cm⁻¹, 3584.86 cm⁻¹, 3525.23 cm⁻¹, 2924.47 cm⁻¹, 2853.7 cm⁻¹ are hydrogen stretching region. It is a milk-based method the sharp peak 3851.10 cm⁻¹ may be due to the presence of lipid functional group (lipid molecules contain hydrocarbons). 3584.86 cm⁻¹ and 3525.23 cm⁻¹ peaks are due to the presence of the amine group—IR absorbance peak at 2923.80 cm⁻¹ due to alcohol overlapping. The sharp absorption peak at 2853 cm⁻¹ is due to the presence of alkane stretching. The two amide linkage one is long and sharp peak 1613.17 cm⁻¹, and another one is 1557.80 cm⁻¹ are located in the double bond region (Sureka et al., 2019). The absorption peak at 1460.18 cm⁻¹ due to the presence of sulfonyl chloride (S=O) functional group stretching (Sureka et al., 2019). 1378.68 cm⁻¹, 1243.10 cm⁻¹, 1167.98 cm⁻¹, 1114.16 cm⁻¹, 1019.23 cm⁻¹, 721.53 cm⁻¹ peaks are presented in fingerprint region. 721.53cm⁻¹ is an organic steroid compound (Materiais 2019). It is a halogen Bromide compound (Ragavendran et al., 2011). 470.39 cm⁻¹ is a halogen bond. Halogen is the electronegative elements such as fluorine, chlorine, iodine and bromine (Nandiyanto et al., 2019).

**LTV**

In the FTIR spectra of LTV peaks were observed in the region of 3839.33 to 471.99 cm⁻¹. A graph is presented in Figure 10. Total of 15- peaks were obtained which includes one hydrocarbon peak, one hydroxyl compound, three ammonia structures, four alcohol groups, one benzene ring, one amide functional linkage, two phosphate stretches, one steroid organic compound and one halogen ring (Charde et al., 2016). Total of fifteen peaks was obtained which includes eight peaks in hydrogen stretching region, three peaks in the double bond region, two peaks in fingerprint region and two peaks in unknown region. 3839.33 cm⁻¹, 3625.97 cm⁻¹, 3585.30 cm⁻¹, 3568.40 cm⁻¹, 3526.18 cm⁻¹, 3437.31 cm⁻¹, 2855.77 cm⁻¹ and 2925.42 cm⁻¹, are present in hydrogen stretching region. 3839.33 cm⁻¹ peak is only one hydrocarbon peak, and 3625.97 cm⁻¹ is the hydroxyl compound. Three functional group of 3585.30 cm⁻¹, 3568.40 cm⁻¹, 3526.18 cm⁻¹ are amine linkage. 3437.31 cm⁻¹ and 2925.42 cm⁻¹ peaks denote the presence of a significant quantity of hydroxyl groups (OH) in the structure (Balan et al., 2019). This alcoholic peak was also identified in the mercury-based Siddha medicine *Arumuga chenthumaram* (Shibi et al.). 1944.14 cm⁻¹, 1613.94 cm⁻¹ and 1560.03 cm⁻¹ are located in double bond region. Peak 1944.14 cm⁻¹ signified the presence of an alcoholic group. 1613.94 cm⁻¹ indicates the existence of aromatic rings and 1560.03 cm⁻¹ means the presence of the amide functional group. 1172.22 cm⁻¹ and 1116.8022 cm⁻¹ groups are situated in fingerprint region. These two peaks symbolised the occurrence of phosphate groups. Unknown region peaks of 690.43 cm⁻¹ and 471.99 cm⁻¹ referred to steroid and halogens, respectively (Ragavendran et al., 2011).

**TTV**

In the FTIR spectra of TTV peaks were observed in the region of 3877.84 to 477.33 cm⁻¹. The graph presented Figure 11 reveals all the peaks observed. 15- peaks were obtained which includes one hydrocarbon peak, one hydroxyl compound, two ammonia structures, two alcohol groups, one phenol stretch, two carbonyl bonds, three benzene rings, one peptide linkage, one phosphate compound and one halogen ring (Charde et al., 2016). Total of fifteen peaks was obtained which includes six peaks in hydrogen stretching region, one peak in the triple bond region, four peaks in the double bond region, three peaks in fingerprint region and one peak in the unknown region. In this process following peaks are existing in hydrogen stretching region 3877.84 cm⁻¹, 3646.01 cm⁻¹, 3526.35...
Siddha based An earlier report on spectroscopy analysis of an unknown region halogen group. Its phosphate group. Last peak 477.33 is situated in the region between 3361 and 500 cm⁻¹ records some similar peaks. In that study also similar in all organic agent treated samples except raw veeram where it has not interacted with any organic agents/groups. In one more veeram based Siddha medicine veeramezhugu standardisation research work, spectroscopy analysis revealed peaks in the region between 3960.75 cm⁻¹ and 428.47 cm⁻¹ (Rajalakshmi et al., 2014). Similar stretching at 3572.26 cm⁻¹ is present in raw veeram as observed in the present study. Other FTIR peaks at 1459.08 cm⁻¹ and 476.62 cm⁻¹ in BTV and peaks at 3584.86 cm⁻¹, 2924.47 cm⁻¹, 1460 cm⁻¹ and 1019.23 cm⁻¹ in MTV, peaks at 3568.40 cm⁻¹, 2925.42 cm⁻¹ and 471.99 cm⁻¹ in LTV and peaks observed at 1459.18 cm⁻¹ and 477.33 cm⁻¹ in TTV sample observed in the present study were all in agreement the earlier report (Rajalakshmi et al., 2014; Sudeer et al., 2015).

CONCLUSIONS

In this study, veeram was purified as per the procedure given in Siddha textbooks such as Gunapadam, Sarakku Suthi sei muraikal and Siddha Formulary of India. Raw veeram and product obtained after various purification process of veeram were subjected to FTIR analysis. According to this data, some peaks disappear, and many new peaks appear. All the purified samples of veeram showed a peak at around 3500 cm⁻¹ indicating the presence of an aromatic amine group or N-H stretch vibrations. A week intensity peak at 2920 cm⁻¹ observed indicated C-H stretch and presence of secondary amine or N-H bending vibrations at 1613 cm⁻¹. A peak is present at 1613 cm⁻¹ similarly in all samples, indicating the existence of a carbonyl group. These groups must have come from the secondary metabolites present in the purifying agents and must have interacted with Veeram and contributed to the purifying process in making the product less toxic with enhanced therapeutic potentials.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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