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# Systemic validation of Herbo- mineral formulation *Udara Noi Nivarana Thiravagam* by instrumental analysis

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## Abstract

Siddha medicine is a complementary system of medicine, drawing global attention in recent times since it contains many formulations, able to treat and cure many diseases even degenerative disorders with no adverse or side effects. It comprises thirty-two types of internal formulations with distinct preparation techniques mentioned in various classical Siddha texts. These traditional medicines require newer standardization techniques to prove their safety and efficacy. *Udara Noi Nivarana Thiravagam* is a Siddha herbo-mineral formulation used for the treatment of peptic ulcer disease (PUD). This work was proposed to formulate and standardize *Udara Noi Nivarana Thiravagam* as per the guidelines set forth by PLIM. *Udara Noi Nivarana Thiravagam* was standardized by various parameters like FT-IR, ICP-OES and GC-MS. All parameters showed desirable results concerning the standard limits. The set parameters were sufficient to evaluate *Udara Noi Nivarana Thiravagam* and this could be used as reference standards for the quality control and quality assurance of the drug.

**Keywords:** Siddha; *Udara Noi Nivarana Thiravagam*; FT-IR; ICP-OES; GC-MS

## 1. Introduction

*Thiravagam* is known by various names like *Pugai Neer, Dravaga Neer* denotes the collection of distillates accompanied by fumes primarily from distillation of salts with or without adding herbs. The term *Pugai Neer* is attributed to the fumes emanating by the heating of non-herbal sources that may include salts or higher minerals. As considering the composition of *Pugai neer* it is divided into *Kadum kara dravagam* and natured *Kadum sara dravagam*. Since they are highly concentrated they are used in minimal dosage forms, with high potency and extended shelf life. It is also used in *Siddhar rasavatham* (alchemical practices) which is an integral part of the Siddha system. The distillates are mainly used in alchemical practices and work as a catalytic agent for higher-order medicine manufacture, synthetic elemental preparation or as a potent medicine for specific diseases. These medications also had their role in the field of critical care management and chronic ailments or contagious diseases[1]. In that context, the traditional Siddha herbo-mineral drug *Udara Noi Nivarana Thiravagam* is considered as *pugai neer* or *Thiravagam* since it was prepared by mixing alkali, salts and herbs. It has an alkaline pH, so it is also considered as *Kadum sara Thiravagam.* It is a safe and effective drug for the treatment of peptic ulcer disease. But still, no scientific studies have been carried out to prove its traditional claim. Hence an attempt had been made to discover a new drug with high efficacy with lesser or no side effects and cost effective to treat PUD. This work mainly deals with the study of systemic validation of *Udara Noi Nivarana Thiravagam* to prove the safety, efficacy and purity by instrumental methods such as FT-IR, ICP-OES and GC-MS.

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## 2. Material and methods

### 2.1. Collection & Authentication of raw drugs

The raw drugs *kariyuppu, karchunnam, uzhamun* and root bark of *mutsangan* are brought from a local drug shop in Tirunelveli, Tamilnadu. Fresh leaves of *Pedalium murex* were brought from Thuckalay, Kanyakumari district, Tamil Nadu.

The raw materials were identified and authenticated by the experts of PG Gunapadam Dept Government Siddha Medical College, Tirunelveli. The identified raw materials were conserved in the laboratory of PG Gunapadam Government Siddha Medical College, Tirunelveli for further reference.

### 2.2. Purification of the drugs [2,3]

The raw drugs were purified by the methods mentioned in Siddha classical literature.

#### *2.2.1. Kariyuppu - Sodium chloride*

Kariyuppu was dissolved in seawater or rainwater and filtered. The filtrate is boiled into a semisolid state. Then it is placed under daylight. It was allowed to dry and scrapped from the vessel.

#### *2.2.2. Karchunnam - Limestone*

*Karchunnam* was heated in water and it was dried under daylight.

#### *2.2.3. Uzhamun - Sodium bicarbonate*

*Uzhamun* was mixed with water and the filtrate was boiled until to a semisolid state then it was placed under daylight and allowed to dry. The dried salt bars were scrapped from the vessel.

#### *2.2.4. Mutsangan - Azima tetracantha*

Mutsangan (*Azima tetracantha*) root bark was taken and the outer covering of the roots was removed with a knife.

#### *2.2.5. Yanai Nerunjil - Pedalium murex*

The leaf of the Yanai Nerunjil (*Pedalium murex*) was cleaned with a cloth and the dried and infected leaves were removed.

### 2.3. Preparation of the drug - Udara Noi Nivarana Thiravagam [2,3]

*Udara Noi Nivarana Thiravagam* was prepared as per classical Siddha text.

The ingredients of the drug are as follows

Kariyuppu (Sodium chloride): - 1400 gms

Karchunnam (Limestone): - 1400 gms

Uzhamun (Sodium bicarbonate): - 1400 gms

Root barks of Mutsangan (*Azima tetracantha*): - 700 gms

Root barks of Yanai Nerunjil (Pedalium murex): - 700 gms

Well water: - 5.2 litres

The mineral drugs and the root of *Azima tetracantha* and leaves of *Pedalium murex* are ground well and transferred to the distillation apparatus (*Valaiyanthiram*) and intensely heated. During the process of heating the drugs were completely decomposed and expel the fumes. The fumes were condensed at the condenser submerged in cold water and the drug was collected in a vessel. It was stored in an airtight glass container.

### 2.4. Instrumental analysis[4]

*2.4.1. Fourier Transform - Infra Red Spectroscopy (FT-IR)*

Vibrational spectroscopy is an extremely useful tool in the elucidation of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy.

In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for the characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible.

*2.4.2. Inductively coupled plasma optical emission spectrometry (ICP-OES)*

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

The intensity of each line is then compared to previously measured intensities of known concentrations of the elements, and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix. Examples of the application of ICP-OES include the determination of metals, arsenic present in traditional medicines, and trace elements bound to proteins.

*2.4.3. Gas chromatography Mass spectroscopy (GC-MS)*

It is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance or separating the different components of a mixture (the relative amounts of such components can also be determined).

In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture. In gas chromatography, the *mobile phase* (or "moving phase") is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen. The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation).

The instrument used to perform gas chromatography is called a *gas chromatograph* (or "aerograph", "gas separator"). The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness. In most modern GC-MS systems, computer software is used to draw and integrate peaks and match MS spectra to library spectra.

## 3. Results and discussion

### 3.1. Fourier Transform - Infra-Red spectroscopy (FT-IR) results Udara Noi Nivarana Thiravagam

Results from the Figure.1 and table.1 represents the drug contains a phenolic group; which exerts anti-oxidant activity. They are responsible for chemo-preventive properties like anti-carcinogenic, anti-mutagenic and anti-inflammatory activities. The anti-oxidant potential of phenols scavenges the free radicals produced by oxidative stress regenerates the cells and prevents diseases. The alcoholic group present in the drug has mild antimicrobial activity. The Nitro groups are responsible for anxiety reduction activity.

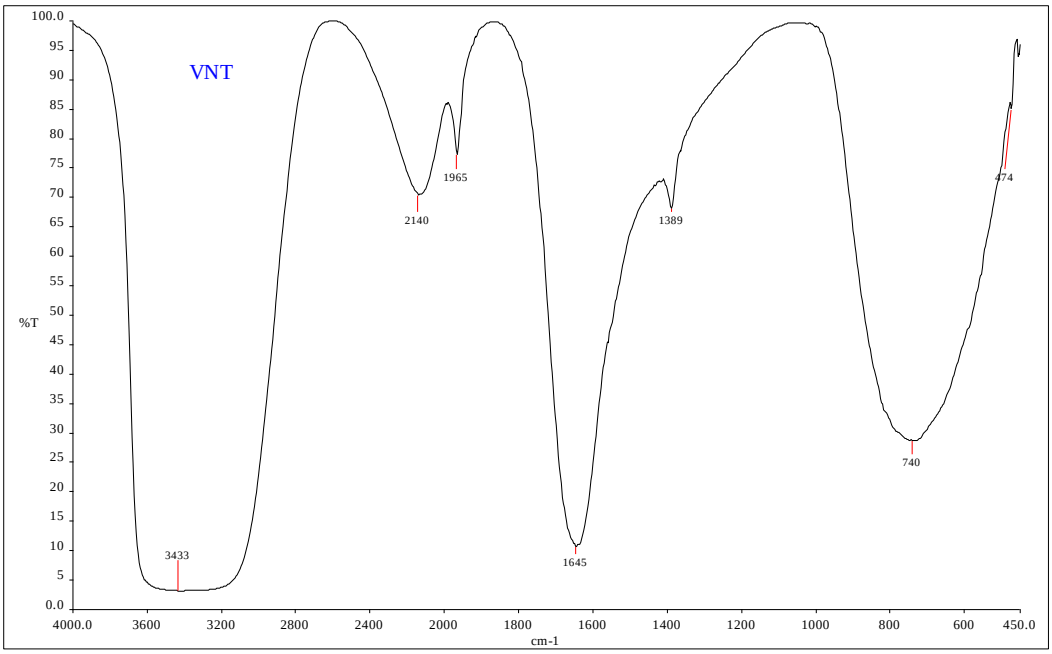


**Figure 1** Fourier Transform - Infra-Red spectroscopy (FT-IR) of *Udara Noi Nivarana Thiravagam*

**Table 1** FT-IR Interpretation of *Udara Noi Nivarana Thiravagam*

| Characteristic absorption(s) in cm-1 | Functional groups | Stretch |
|---|---|---|
| 3433 | Alcohol, phenols | (oH Stretch bonded ) |
| 2140 | Alkynes | (-C ≡ C-stretch) |
| 1965 | Carbonyl | (C ≡ C) |
| 1945 | Alkenes | (-C=C- stretch) |
| 1389 | Nitro groups | (N ≡ O bend) |
| 740 | alkenes | (= C-H bend) |
| 474 | Poly sulfides | S-S stretch |

### 3.2. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) results of Udara Noi Nivarana Thiravagam

The result from the table.2 reveals the below detection limit (BDL) of heavy metals like Aluminum (AL), Arsenic (As), Cadmium (Cd), Copper (Cu), Mercury (Hg), Nickel (Ni), and Lead (Pb). The result indicates the presence of calcium, Iron, Potassium, Magnesium, Sodium, Phosphorous, Zinc and Sulfur was found to be within the permissible limits as per WHO guidelines.

**Table 2** ICP-OES Interpretation of *Udara Noi Nivarana Thiravagam*

| SL.NO | ELEMENTS | FREQUENCY | MEAN CONCENTRATION (MG/L) |
|---|---|---|---|
| 1. | Al | 396.152 | BDL |
| 2. | As | 188.979 | BDL |
| 3. | Ca | 315.807 | 223.510 mg/L |
| 4. | cd | 228.802 | BDL |

| | | | |
|---|---|---|---|
| 5. | Cu | 327.393 | BDL |
| 6. | Fe | 238.204 | 0.240mg/l |
| 7. | Hg | 253.652 | BDL |
| 8. | K. | 766.491 | 25.251mg/l |
| 9. | Mg | 285.213 | 05.180mg/l |
| 10. | Na | 589.592 | 217.350mg/l |
| 11. | Ni | 231.604 | BDL |
| 12. | Pb | 220.353 | BDL |
| 13. | P | 213.617 | 15.181mg/L |
| 14. | S | 180.731 | 04.153mg/l |
| 15. | Zn | 206.200 | 0.545mg/l |

### 3.3. Gas Chromatography Mass Spectroscopy (GC-MS) results of Udara Noi Nivarana Thiravagam:

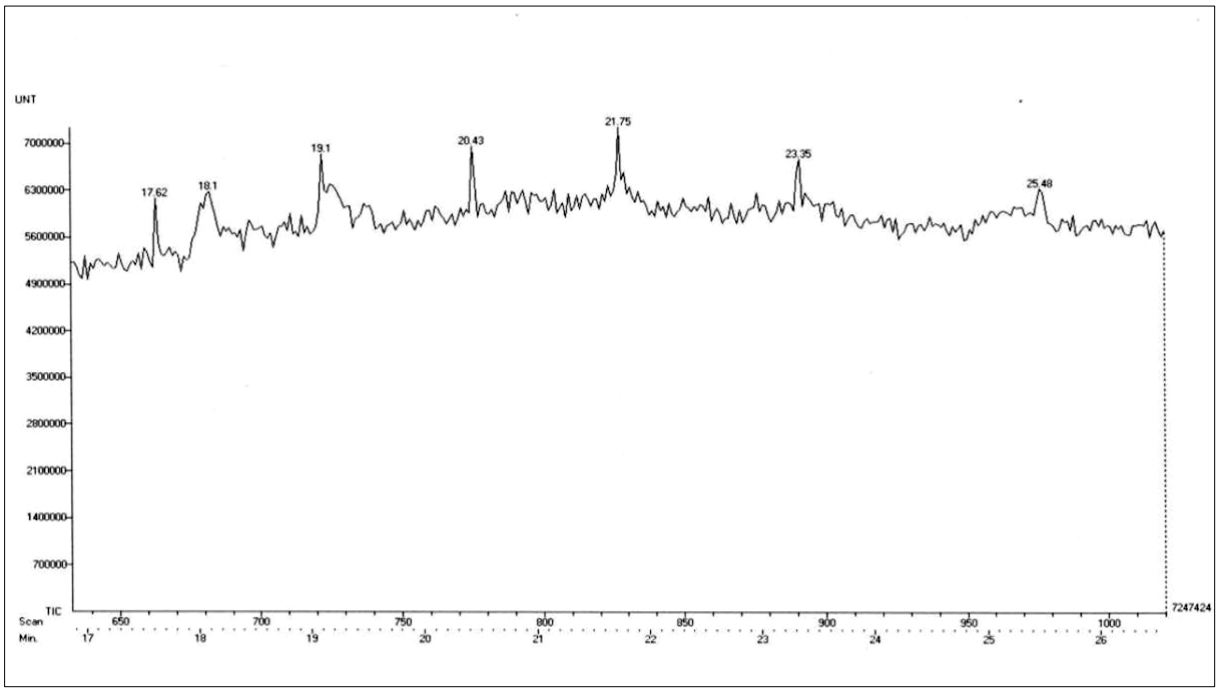


**Figure 2** GC-MS analysis of Udara Noi Nivarana Thiravagam

The Gas chromatogram results from Figure.2 and table.3 of *Udara Noi Nivarana Thiravagam* revealed 7 prominent spikes with retention time ranging from 17.62 to 25.48 the seven compounds belong to ester and steroid groups. Steroids have anti-inflammatory activity and Esters and their groups are effective in reducing pain.

**Table 3** Gas Chromatography - Mass Spectroscopy (GC-MS) Interpretation of *Udara Noi Nivarana Thiravagam*

| PEAK NO | RENENTION TIME | NATURE OF THE COMPOUND |
|---|---|---|
| 1. | 17.62 | Methyl ester |
| 2. | 18.1 | Methyl ester |
| 3. | 19.1 | Steroid |
| 4. | 20.43 | Methyl ester |

| | | |
|---|---|---|
| 5. | 21.75 | Ester |
| 6. | 23.36 | Steroid |
| 7. | 25.48 | Ester |

## 4. Conclusion

The *Udara Noi Nivarana Thiravagam* samples were evaluated organoleptically and with classical parameters and confirmed its perfect method of purification and preparation. Sophisticated analytical instruments like FT-IR, ICP-OES and GC-MS analysis were found to be informative. As there is no standard instrumental analysis profile available for *Udara Noi Nivarana Thiravagam.* The results obtained by various parameters of *Udara Noi Nivarana Thiravagam* may be taken as standard parameters for future reference.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

The author hereby declares that she has no conflicts of interest to disclose.

### *Statement of ethical approval*

The present research work does not contain any studies performed on animal/human subjects by any of the authors.

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