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# Qualitative Phytochemistry Of Siddha Formulation Vatharoga Chooranam

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***Abstract:*****Introduction**: The Siddha system, one of the oldest documented medical traditions originating from the Indian subcontinent, has a rich history rooted in ancient times. Its founders, the Siddhars, have recorded numerous remedies throughout the ages. Vatharoga Chooranam (VC), a herbo-mineral formulation, is traditionally recommended for treating various vatha diseases, according to classical Siddha texts. Among the 80 vatha diseases, Cervical Spondylosis is one condition that may potentially benefit from VC. In today's globalized world, ensuring the authenticity of such formulations through drug standardization and proper dissemination is crucial. VC was standardized following PLIM guidelines, marking an important step toward its acceptance in Western practices.**Materials and Methods:** VC was produced in strict compliance with Good Manufacturing Practices (GMP). The standardization process involved phytochemical analysis, testing for pesticide residues, sterility testing using the pour plate method, an aflatoxin assay, and heavy metal analysis. This study was conducted at Noble Research Solution's facility, following the rigorous PLIM standards. The phytochemical analysis took place at Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai.**Results:** The research revealed the presence of carbohydrates, saponins, phenols, phytosterols, diterpenes, flavonoids, tannins, quinones, glycosides, gum, and mucilage in the phytochemical analysis.No microbial colonies or growth were observed.**Conclusion:** The published findings can serve as a valuable foundation for future clinical research and further standardization efforts. **Key words**: Vatharoga Chooranam (VC),Cervical Spondylosis,Degenerative disorders, phytochemistry, Dr.M.G.R Medical university, Chennai, heavy metal analysis, pesticide residue, sterility test**.**

**Index Terms –** Vatharoga Chooranam (VC),Cervical Spondylosis, Heavy metal analysis, Aflotoxins, PLIM.

## 1.INTRODUCTION

Vatharoga Chooranam (VC), a traditional medicine rooted in the Siddha system, has been historically prescribed for the treatment of various "vatha" disorders, including cervical spondylosis. As traditional remedies gain increasing interest in modern healthcare, there is a growing need to ensure the safety, quality, and efficacy of such formulations. This study aims to evaluate VC for its phytochemical composition, the presence of potential contaminants, and microbial burden to ensure its safety for broader use. The safety profile of VC was assessed through rigorous testing for heavy metals, pesticides, and microbial contamination. Advanced techniques like Atomic Absorption Spectroscopy (AAS) and sterility testing were employed to determine the levels of these substances. The results were compared against established AYUSH and WHO guidelines, ensuring that VC meets the safety standards required for its application in modern healthcare. This assessment contributes significantly to the standardization of VC, paving the way for its wider acceptance and integration into contemporary medicinal practices.

## 2.MATERIAL AND METHODS

The polyherbal formulation was identified in the Siddha Classical Literature "SARABENDIRAR VAITHIYA MURAIGAL SIROROGA SIGITCHAI , PAGE NO:[119][1]".The ingredients for this formulation are included in Table-1[1-6].

**TABLE-1 INGREDIENTS OF VATHAROGA CHOORANAM**

| S.NO | INGREDIENTS | BOTANICAL NAME | QUANTITY |
|---|---|---|---|
| 1 | [2]Kadukurogini | *Picrorhiza Scrophulariflora* | 3 Palam |
| 2 | [3]Karkadagasingi | *Rhus succedanea* | 3 Palam |
| 3 | [4]Kandankathiri ver | *Solanam Burattense* | 1 Palam |
| 4 | [5]Jatamanjil | *Nardostachys grandiflora* | 1 Palam |
| 5 | [6]Siruthekku | *Clerodendrum serratum* | 1 Palam |
| 6 | Indhuppu | *Sodium Chloride impura* | 1 Palam |

## 3.COLLECTION,IDENTIFICATION AND AUTHENTICATION OF THE DRUG

All necessary plant materials were procured from a raw drug shop located at Parry's Corner in Chennai, Tamil Nadu. These materials were subsequently verified and confirmed by botanical and pharmacological experts at the Government Siddha Medical College Hospital in Arumbakkam, Chennai – 106.

### 3.1PURIFICATION OF THE DRUGS

All the drugs were purified according to Siddha Literature.

### 3.2PREPARATION OF THE DRUG

**PROCEDURE:**

All the purified ingredients listed in Table 1 (1-6) were taken in above mentioned quantity, mildly roasted, pounded and finely powdered.The obtained powder was sieved well using sieving cloth.Then Vatharoga Chooranam was stored in an air tight container.

### 4. STANDARDIZATION OF THE DRUG

**I. PRELIMINARY PHYTOCHEMICAL SCREENING OF VATHAROGA CHOORANAM** The preliminary phytochemical screening test was carried out for each aqueous extract of VATHAROGA CHOORANAM as per the standard procedure mentioned here under

**1. Detection of alkaloids:**

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

**Mayer's Test**: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow colour precipitate indicates the presence of alkaloids.

a) **Wagner's Test**: Filtrates were treated with Wagner's reagent (Iodine in PotassiumIodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**2. Detection of carbohydrates:**

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**Molisch's Test**: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acidis added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

a) **Benedict's Test**: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

### 3. Detection of saponins

**Foam Test**: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of Saponins.

### 4.Detection of phytosterols:

**Salkowski's test**

Extracts was treated with chloroform and filtered; the filtrates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand for few minutes. Golden yellow color indicates the presence of Triterpenes

### 5. Detection of phenols Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colorindicates the presence of phenols.

### 6. Detection of tannins Gelatin Test:

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

### 7. Detection of Flavonoids

**Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation ofyellow color precipitate indicates the presence of flavonoids.

### 8.Detection of diterpenes:

**Copper Acetate Test:**

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

### 9.Gum and Mucilage:

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

### 10.Test for glycosides

**Liebermann's test**

2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, violet color change into blue and green indicates the presence of glycosides.

**11.Test for Quinones:**

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

The Preliminary phytochemical studies of aqueous extract of **VATHAROGA CHOORANAM** were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of **VATHAROGA CHOORANAM.**

| S.No. | Phytochemicals | Test Name | $H_2O$ Extract |
|---|---|---|---|
| 1 | Alkaloids | Mayer's Test | -ve |
| | | Wagner Test | -ve |
| 2 | Carbohydrates | Molisch's Test | +ve |
| | | Benedict Test | +ve |
| 3 | Saponin | Foam Test | -ve |
| 4 | Phytosterols | Salkowski's | +ve |
| 5 | Phenols | Ferric Chloride Test | +ve |
| 6 | Tannins | Gelatin Test | +ve |
| 7 | Flavonoids | Lead acetate | +ve |
| 8 | Diterpenes | Copper Acetate Test | +ve |
| 9 | Gum & Mucilage | Test for Gum & Mucilage | +ve |
| 10 | Glycosides | Liebermann's test | -ve |
| 11 | Quinones | Test for Quinones | +ve |

**+ve/-ve present or absent if component tested**

Certified that the above stated are the phytochemical properties for the given sample.


**II.HEAVY METAL ANALYSIS BY AAS**

Standard: Hg, As, Pb and Cd - Sigma

**a.Methodology**

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

**b.Sample digestion**

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3. **c.Standard reparation**

As & Hg- 100 ppm sample in 1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO3.

**BDL- Below Detection Limit**

**Report and Inference**

Results of the present investigation have clearly shows that the sample has no traces of heavy metal such as Arsenic and Cadmium were as the sample evident the presence of Lead and Mercury at 7.174 and 0.358 ppm.

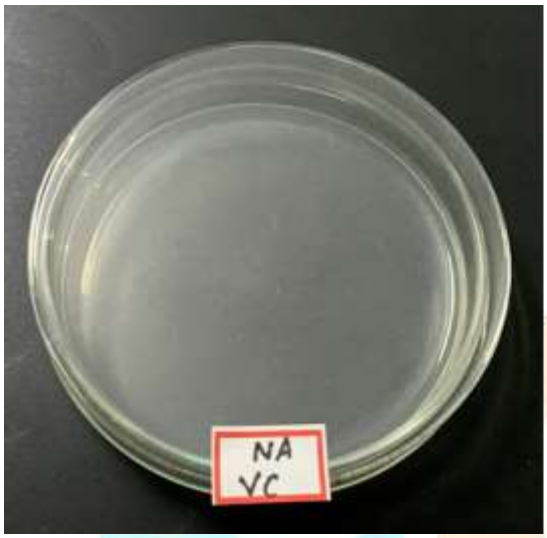
**III.STERILITY TEST BY POUR PLATE METOD**

**Objective**

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

**Methodology**

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45ºC were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37º C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.




**Observation**

No growth was observed after incubation period reveals the absence of specific pathogen

**Result**

No growth / colonies was observed in any of the plates inoculates with the test sample.

| Test | Result | Specification | As per AYUSH/WHO |
|---|---|---|---|
| *Total Bacterial Count* | Absent | NMT $10^5$CFU/g | As per AYUSH specification |
| *Total Fungal Count* | Absent | NMT $10^3$CFU/g | |

**III.AFLATOXINS**

**Standard**

Aflatoxin B1

Aflatoxin B2

Aflatoxin G1

Aflatoxin G2

**Solvent**

Standard samples was dissolved in a mixture of chloroform and acetonitrile (9.8 : 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2

**Procedure**

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly, the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

| Aflatoxin | Sample VC | AYUSH Specification Limit |
|---|---|---|
| B1 | Not Detected - Absent | 0.5 ppm (0.5mg/kg) |
| B2 | Not Detected - Absent | 0.1 ppm (0.1mg/kg) |
| G1 | Not Detected - Absent | 0.5 ppm (0.5mg/kg) |
| G2 | Not Detected - Absent | 0.1 ppm (0.1mg/kg) |

**Result:** The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2.

**IV.PESTICIDE RESIDUE**

**a.Extraction**

Test sample were extracted with acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample wasperformed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone iscompletely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

**Test Result Analysis of the Sample VC**

| **Pesticide Residue** / **I.Organo Chlorine Pesticides** | **Sample VC** | **AYUSH Limit (mg/kg)** |
|---|---|---|
| Alpha BHC | BQL | 0.1mg/kg |
| Beta BHC | BQL | 0.1mg/kg |
| Gamma BHC | BQL | 0.1mg/kg |
| Delta BHC | BQL | 0.1mg/kg |
| DDT | BQL | 1mg/kg |
| Endosulphan | BQL | 3mg/kg |
| **II.Organo Phosphorus Pesticides** | | |
| Malathion | BQL | 1mg/kg |
| Chlorpyriphos | BQL | 0.2 mg/kg |
| Dichlorovos | BQL | 1mg/kg |
| **III. Organo carbamates** | | |
| Carbofuran | BQL | 0.1mg/kg |
| **III.Pyrethroid** | | |
| Cypermethrin | BQL | 1mg/kg |

BQL- Below Quantification Limit

**Result:** The results showed that there were no traces of pesticides residues such as Organo chlorine,Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

## 5. DISCUSSION

The present study investigated the physicochemical properties, heavy metal content, sterility, aflatoxin content, and pesticide residue of Vatharoga Chooranam (VC), a polyherbal formulation.

Phytochemical Screening: The aqueous extract of VC revealed the presence of various bioactive compounds, including carbohydrates, saponins, phytosterols, phenols,tannins, flavonoids, diterpenes, and quinones. The absence of alkaloids and glycosides (as indicated by Liebermann's test) suggests specific characteristics of the formulation.

Heavy Metals: The analysis showed the presence of Lead and Mercury at 7.174 and 0.358 ppm in the

sample. While these levels were reported, their safety within the context of Ayurvedic or WHO standards is not mentioned. Further investigation is required to determine if these levels pose any health concerns.

Sterility and Aflatoxin Content: The sterility test indicated no bacterial or fungal growth, signifying that the product meets sterility standards (as per AYUSH/WHO) for safe consumption. Additionally, no aflatoxins (B1, B2, G1, or G2) were detected, ensuring the absence of these harmful toxins.

Pesticide Residue : The results indicated that no pesticide residues, including organochlorines, organophosphates, carbamates, or pyrethroids, were detected in the sample analyzed.

This exploratory study delves into the fundamental phytochemical characteristics of VatharogaChooranam (VC). The findings presented here in serve as a preliminary framework for future investigations into this Herbo-Mineral formulation. By building upon the insights gleaned from this study, subsequent research can delve deeper into the intricate properties and potential therapeutic applications of VC.

**Conclusion:**

This study offers initial insights into the physicochemical properties and quality of Vatharoga Chooranam. The presence of various bioactive compounds indicates its potential for therapeutic use. However, the detection of heavy metals (lead and mercury) requires further investigation to ensure safety. The absence of bacterial and fungal contamination, aflatoxins, and most pesticide residues reflects good quality standards, with no pesticide residues detected in the sample. Overall, this research provides a basis for further studies to assess the efficacy and safety of Vatharoga Chooranam.

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