Phytochemical and Physicochemical Analysis of Nilavagaai Chooranam A Siddha Herbo-Mineral Preparation

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Abstract
Background: Standardization of Siddha medicine is necessary one in this scientific word ‘Nilavagaai chooranam’ is a herbo-mineral preparation indicated for various diseases including skin diseases.
Aim and Objective: To do the phytochemical and physicochemical analysis for the drug ‘Nilavagaai chooranam’
Material and Methods: The drug prepared as per the method mentioned in the classical Siddha literature. The drug is subjected to physicochemical analysis such as total ash, loss on drying, microbial load, heavy metal analysis, pesticide residues, aflatoxins, thin layer chromatography and high performance thin layer chromatography as per the pharmacopoeical laboratory standards of Indian medicine.
Results and Conclusion: The drug had no microbial contamination and shows positive for the presence of alkaloids, saponins, phenols, tannins, diterpenes, gum & mucilage, quinones etc. The ash value is 9.13%. The heavy metals such as lead and mercury are below detectable limit.
Keywords: Herbo-mineral, Siddha, Physicochemical analysis, High performance thin layer chromatography.

I. INTRODUCTION:
Siddha system of medicine is one of ancient system of medicine which is practiced among the Tamil speaking people. Sage Siddhars are the founder of this system. The medicine in this system is prepared from raw material of herbs, metals, minerals and animal products. ‘Nilavagaai chooranam’ is one of the Siddha drug chosen from the text Aathma Ratchamirdhamenum Vaidhya Saara Sangirakam1. It is indicated for various diseases like venereal itching, scabies, pitha diseases, ptyalism, some poisons and accumulation of gas in some part of the intestines. The use of scientific tools is essential to validate the traditional claim. Though Siddha drugs are considered to be safe and effective, it is the utmost duty of the physicians to standardizing the Siddha prepared medicine before trying out in human being. The drug is a herbo-mineral drug which contains only one mineral Indhuppu and the others are herbal ingredients.

II. AIM AND OBJECTIVE:
The aim of this study is to do phytochemical analysis, physicochemical analysis and HPTLC finger printing for the drug ‘Nilavagaai chooranam’.

III. MATERIALS AND METHODS:

Ingredients of Nilavagaai chooranam1:
Nilavagaai samoolam (Cassia senna Linn.) -10 palam (350 grams)
Milagu (Piper nigrum Linn.)
Kadukkai (Terminaliachebula Retz.)
Thandrikka (Terminaliabellerica Roxb.)
Seeragam (Cuminumcyminum Linn.)
Vaaluvai (Celastruspaniculatus Willd.)
Sirunaagapoo (Mesuaferrea Kosterm.)
Elam (Elettariacardamomum Linn.)
Ilavangapattai (Cinnamomverum Presl.)
Kadugurogini (Picrorhizakurroa Pennell.)
Sivadhai (Oerculinaturpetillum Linn.)
Thalisapathiri (Taxusbaccato Mirb.)
Jadhikkai (Myristicafragrans Houtt.)

- each ¼ palam (8.75 grams)
Kirambu(Syzygium aromaticum Linn.)
Thippili(Piper longum Linn.)
Chevviyam(Root of Piper nigrum Linn.)
Indhuppu(Sodium chloride)
Koogaineer(Maranta arundinacea Linn.)
Chukku(Zingiber officinale Roscoe.)
Senisarkarai(Sugar) — Equal amount for Chooranam

Purification of raw drugs:
The raw drugs are purified as per the method mentioned in the Siddha literature.

Method of preparation:
The purified raw drugs were dried and powdered separately, then mixed well together and then added with equal amount of white sugar and stored in clean dry air tight container.

Dosage: 1 Gram (Thirikadi), twice a day
Adjuvant: Ghee
Duration: 48 days

Analytical specification of chooranam:
1. Description: microscopic and macroscopic,
2. Loss on drying at 105°C,
3. Total ash,
4. Acid-insoluble ash,
5. Water soluble ash,
6. Water soluble extractive,
7. Alcohol soluble extractive,
8. Detection of alkaloids,
9. Detection of carbohydrates,
10. Detection of glycosides,
11. Detection of saponins,
12. Detection of phenols,
13. Detection of tannins,
14. Detection of Flavonoids,
15. Detection of proteins,
16. Detection of diterpenes,
17. Gum and Mucilage,
18. Test for Quinones,
19. Test for aflatoxins: B1, B2, G1, G2,
20. TLC/HPTLC with marker,
21. Test for heavy / toxic metals: lead, cadmium, mercury, arsenic,
22. Pesticide residue: organochlorine pesticides, organophosphorus pesticide, pyrethroids,
23. Sterility test by Pour plate method,
24. Test for Specific pathogen.

Figure 1: Sample of Nilavagaai Chooranam (NVC)

Table 1: Organoleptic characters of Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>State</th>
<th>Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Brownish green</td>
</tr>
<tr>
<td>Nature</td>
<td>Hard coarse powder</td>
</tr>
<tr>
<td>Odor</td>
<td>Strong Characteristic</td>
</tr>
<tr>
<td>Flow Property</td>
<td>Free flowing</td>
</tr>
</tbody>
</table>
Particle Size$^{2,3}$

Figure 2: Electron microscopic observation of particle size for the test sample- Nilavagaai Chooranam (NVC)

![Electron microscopic observation of particle size for the test sample- Nilavagaai Chooranam (NVC)](image)

Table 2: Mean, Std. deviation and Std.error of Particle Size for the test sample- Nilavagaai Chooranam (NVC)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>253.8</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>147.5</td>
</tr>
<tr>
<td>Std. Error</td>
<td>60.23</td>
</tr>
</tbody>
</table>

Report

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be $253.8 \pm 147.5 \mu m$

IV. RESULTS AND OBSERVATION:

PHYSICOCHEMICAL ANALYSIS OF NILAVAGAAI CHOORANAM

1. Loss on Drying:

An accurately weighed 2g of Nilavagaai chooranam formulation was taken in a tarred glass bottle. The crude drug was heated at $105^\circ C$ for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

2. Determination of total ash:

Weighed accurately 2g of Nilavagaai chooranam formulation was added in crucible at a temperature 6000C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

3. Determination of acid insoluble ash:

Ash obtained from the given sample, was boiled for 5min with 25ml of 1hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was
Phytochemical and Physicochemical Analysis of Nilavagaai Chooranam A Siddha Herbo-

Mineral

burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

4. Determination of water soluble ash:
Total ash 2g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

5. Determination of water soluble Extractive:
5gm of air dried drug, coarsely powered Nilavagaai chooranam was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 10 ml of filtrated solution was evaporated in a tarred flat bottom shallow dish, further dried at 100°C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

6. Determination of alcohol soluble extractive:
1 gm of air dried drugs, coarsely powdered Nilavagaai chooranam was macerated with 20 ml. alcohol in closed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrated solution was then evaporated in a tarred flat bottom shallow dish, dried at 100°C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Table 3: Results of Physicochemical Parameters for the test sample- Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on drying</td>
<td>4.56%</td>
</tr>
<tr>
<td>2</td>
<td>Total ash value</td>
<td>9.13%</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>1.11%</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>5.09 %</td>
</tr>
<tr>
<td>5</td>
<td>Water soluble extraction</td>
<td>18%</td>
</tr>
<tr>
<td>6</td>
<td>Alcohol soluble extraction</td>
<td>16%</td>
</tr>
</tbody>
</table>

The above stated physicochemical properties of the given sample certified to be present.

PRELIMINARY PHYTOCHEMICAL SCREENING OF NILAVAGAAI CHOORANAM

The Preliminary Phytochemical screening test was carried out for each extracts of Nilavagaai chooranam as per the standard procedure.

1. Detection of alkaloids:
Extracts were dissolved individually in dilute Hydrochloric acid and filtered.
a) Mayer’s Test: Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow colored 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.
a) 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 acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

3. Detection of glycosides:
Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.
Cardiac glycoside (Keller-Killiani test): Extract was shaken with distilledWater (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed byH2SO4 (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

4. Detection of saponins
a) 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.

5. Detection of phenols Ferric Chloride Test:
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates 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 containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.
7. Detection of Flavonoids
   a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

8. Detection of proteins
   a) Xanthoprotein Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

9. 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.

10. 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.

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 Nilavagaai 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 Nilavagaai chooranam.

Table 4: Results of Phytochemical analysis for the test sample- Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Test Name</th>
<th>H2O Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>Molisch’s Test</td>
<td>-ve</td>
</tr>
<tr>
<td>3</td>
<td>Glycoside</td>
<td>Cardiac glycoside’s Test</td>
<td>-ve</td>
</tr>
<tr>
<td>4</td>
<td>Saponin</td>
<td>Foam Test</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>Ferric Chloride Test</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>Gelatin Test</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>flavonoids</td>
<td>Lead acetate test</td>
<td>-ve</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>Xanthoprotein Test</td>
<td>-ve</td>
</tr>
<tr>
<td>9</td>
<td>Diterpenes</td>
<td>Copper Acetate Test</td>
<td>+ve</td>
</tr>
<tr>
<td>10</td>
<td>Gum &amp; Mucilage</td>
<td>Extract + Alcohol</td>
<td>+ve</td>
</tr>
<tr>
<td>11</td>
<td>Quinones</td>
<td>NAOH + Extract</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve/-ve present or absent if component tested

The above stated phytochemical properties for the given sample certified to be present.

Aflatoxin Assay:

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.

Test solution: Concentration 1 µg per ml

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.
Table 5: Test results of Aflatoxin Assay for the test sample- Nilavagai Chooranam (NVC)

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>Sample NVC</th>
<th>AYUSH Specification Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Not Detected - Absent</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>B2</td>
<td>Not Detected - Absent</td>
<td>0.1 ppm</td>
</tr>
<tr>
<td>G1</td>
<td>Not Detected - Absent</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>G2</td>
<td>Not Detected - Absent</td>
<td>0.1 ppm</td>
</tr>
</tbody>
</table>

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

**TLC Analysis**

- **Instrument:** CAMAG TLC SCANNER III
- **Sample Applicator:** Camag Linomat - IV applicator with N2 gas flow.
- **Photo documentation System:** Digi store - 2 documentation system with Win Cats & Video scan software.
- **Scanner:** Camag HPTLC scanner - 3 (030618), Win Cats - IV.
- **Development Chamber:** Camag HPTLC 10X10, 10 X 20 twin trough linear development chamber.
- **Quantity applied:** 5, 10 µl for extracts and 5 µl for standards
- **Stationary phase:** Aluminium Coated Silica Gel – Merck
- **Plate thickness:** 0.2 mm.
- **Mobile Phase:** Chloroform: n-Butanol: Methanol: Water: Acetic Acid (4:1:1:0.5:0.5)
- **Scanning wavelength:** 254 nm
- **Laboratory condition:** 26 ± 5°C and 53 % relative humidity

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Chloroform: n-Butanol: Methanol: Water: Acetic Acid (4:1:1:0.5:0.5). After the run plates are dried and were observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

**High Performance Thin Layer Chromatography Analysis**

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantitation of nano grams level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials.

**Chromatogram Development**

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

**Scanning**

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.
Figure 3: TLC Visualization for the test sample Nilavagaai Chooranam (NVC) - TLC plate visualization at 366 nm

Figure 4: 3D View of Chromatogram
Figure 5: HPTLC finger printing for the test sample- Nilavagaai Chooranam (NVC)

Table 6: Rf value of HPTLC finger print for the test sample- Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>Start Height</th>
<th>Max Rf</th>
<th>Max Height</th>
<th>Max %</th>
<th>End Rf</th>
<th>End Height</th>
<th>Area</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.17</td>
<td>1.0</td>
<td>0.25</td>
<td>81.9</td>
<td>29.87</td>
<td>0.29</td>
<td>0.2</td>
<td>2745.5</td>
<td>28.69</td>
</tr>
<tr>
<td>2</td>
<td>0.52</td>
<td>3.3</td>
<td>0.55</td>
<td>22.8</td>
<td>8.33</td>
<td>0.59</td>
<td>10.2</td>
<td>577.2</td>
<td>6.03</td>
</tr>
<tr>
<td>3</td>
<td>0.61</td>
<td>4.6</td>
<td>0.65</td>
<td>14.8</td>
<td>5.40</td>
<td>0.68</td>
<td>5.8</td>
<td>406.6</td>
<td>4.25</td>
</tr>
<tr>
<td>4</td>
<td>0.77</td>
<td>5.1</td>
<td>0.86</td>
<td>154.6</td>
<td>56.40</td>
<td>0.92</td>
<td>0.2</td>
<td>5839.5</td>
<td>61.03</td>
</tr>
</tbody>
</table>

Report

HPTLC finger printing analysis of the sample reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present within it. Rf value of the peaks ranges from 0.16 to 0.64. Further the peak 4 and 1 occupies the major percentage of area of 56.40 and 29.87 % which denotes the abundant existence of such compounds.

HEAVY METAL ANALYSIS BY AAS

Standard: Hg, As, Pb and Cd – Sigma

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.

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

Standard preparation

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

Table 7: Results of Heavy Metal analysis for the test sample- Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>Name of the Heavy Metal</th>
<th>Absorption Max A max</th>
<th>Result Analysis</th>
<th>Maximum Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>253.7 nm</td>
<td>BDL</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Lead</td>
<td>217.0 nm</td>
<td>0.36</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Arsenic</td>
<td>193.7 nm</td>
<td>0.14</td>
<td>3 ppm</td>
</tr>
<tr>
<td>Cadmium</td>
<td>228.8 nm</td>
<td>BDL</td>
<td>0.3 ppm</td>
</tr>
</tbody>
</table>
BDL - Below Detection Limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metals such as Mercury and Cadmium. Further the results show the presence of arsenic and lead at 0.36 and 0.14 ppm whose level is below the prescribed limit.

Pesticide Residue

Extraction

Test sample were extracted with 100 ml of 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 was performed 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 R and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Table 8: Test Result of Pesticide Residue for the test sample- Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>Pesticide Residue</th>
<th>Sample NVC</th>
<th>AYUSH Limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Organo Chlorine Pesticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha BHC</td>
<td>BQL</td>
<td>0.1mg/kg</td>
</tr>
<tr>
<td>Beta BHC</td>
<td>BQL</td>
<td>0.1mg/kg</td>
</tr>
<tr>
<td>Gamma BHC</td>
<td>BQL</td>
<td>0.1mg/kg</td>
</tr>
<tr>
<td>Delta BHC</td>
<td>BQL</td>
<td>0.1mg/kg</td>
</tr>
<tr>
<td>DDT</td>
<td>BQL</td>
<td>1mg/kg</td>
</tr>
<tr>
<td>Endosulphan</td>
<td>BQL</td>
<td>3mg/kg</td>
</tr>
<tr>
<td>II. Organo Phosphorus Pesticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>BQL</td>
<td>1mg/kg</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>BQL</td>
<td>0.2 mg/kg</td>
</tr>
<tr>
<td>Dichlorovos</td>
<td>BQL</td>
<td>1mg/kg</td>
</tr>
<tr>
<td>III. Pyrethroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>BQL</td>
<td>1mg/kg</td>
</tr>
</tbody>
</table>

BQL - Below quantification Limit

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

STERILITY TEST BY POUR PLATE METHOD

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 admixed with sterile distilled water and the mixture were been used for the sterility evaluation. About 1ml of the 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. Grown colonies of organism was then counted and calculated for CFU.
Figure 6: Sterility Test by Pour Plate Method

Observation
No growth was observed after incubation period. Reveals the absence of specific pathogen

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

Table 9: Results of Microbial Load for the test sample- Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Specification</th>
<th>As per AYUSH/WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bacterial Count</td>
<td>Absent</td>
<td>NMT 10^5 CFU/g</td>
<td>As per AYUSH specification</td>
</tr>
<tr>
<td>Total Fungal Count</td>
<td>Absent</td>
<td>NMT 10^3 CFU/g</td>
<td></td>
</tr>
</tbody>
</table>

Test for Specific Pathogen

Methodology
One part of the test sample was dissolved in 9 mL of sterile distilled water and the test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°C for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation in each differential media.

Table 10: Detail of Specific Medium and their abbreviation

<table>
<thead>
<tr>
<th>Organism</th>
<th>Abbreviation</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-coli</td>
<td>EC</td>
<td>EMB Agar</td>
</tr>
<tr>
<td>Salmonella</td>
<td>SA</td>
<td>Deoxycholate agar</td>
</tr>
<tr>
<td>Staphylococcus Aureus</td>
<td>ST</td>
<td>Mannitol salt agar</td>
</tr>
<tr>
<td>Pseudomonas Aeruginosa</td>
<td>PS</td>
<td>Cetrimide Agar</td>
</tr>
</tbody>
</table>

Observation
No growth was observed after incubation period. Reveals the absence of specific pathogen

Result
No growth / colonies were observed in any of the plates inoculated with the test sample.

Table 11: Test results for Specific Pathogen for the test sample- Nilavagaai Chooranam (NVC)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Specification</th>
<th>Result</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-coli</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus Aureus</td>
<td>Absent</td>
<td>Absent</td>
<td>As per AYUSH specification</td>
</tr>
<tr>
<td>Pseudomonas Aeruginosa</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
</tbody>
</table>
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Figure 7: Culture plate with E-coli (EC) specific medium

Figure 8: Culture plate with Salmonella (SA) specific medium

Figure 9: Culture plate with Staphylococcus Aureus (ST) specific medium
V. CONCLUSION:
Based on the above results, it can be assumed that the drug ‘Nilavagaai chooranam’ has validated the traditional claim.

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