

## Physicochemical Standardization of Siddha Syrup Preparation Madhulai Manappagu

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### Abstract

#### Context:

Siddha system of medicine is one of the pioneer systems of medicine practiced in southern part of India particularly in Tamilnadu. The system of medicine also deals with Paediatric diseases in a separate chapter "Balar Vakatum". Among the diseases of the children, nutritional disorders play a major role. "Pandu Noi" (Anaemia) is one among the nutritional disorders which hinders the growth and development of the children. It is caused by the deficiency of Iron in children. This Iron deficiency of anaemia is the most common nutritional disorder affecting not only the children but also the adult population particularly the pregnant and menstruating women in developing countries like India. In order to treat this disorder in children, safe and easily administrable syrup "Madhulai Manappagu" (A syrup formulation with *Punica granatum* - Pomegranate juice as a main ingredient) which is in common Siddha practices and explained in the text "Siddha Vaithya Thirattu", is selected to screen for its physico chemical characteristics as a step to validate it scientifically.

#### Aim:

To standardize the herbal syrup preparation Madhulai Manappagu on the basis of qualitative and quantitative methods as per the analytical specifications of syrup/manappagu prescribed by the Protocol testing of ASU drugs by Pharmacopoeial laboratory for Indian Medicines.

#### Materials and Methods:

The syrup is prepared as described in the text "Siddha Vaithya Thirattu". The medicine is subjected to physicochemical and phytochemical analysis, microbial load, aflatoxin assay, sterility test, pesticide residue analysis, heavy metal analysis, Gas chromatography – Mass spectrometry analysis, High Performance Thin Layer Chromatography and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as per the Pharmacopoeial laboratory standards of Indian medicine.

#### Results and Conclusion:

The syrup is in liquid form which is reddish brown in colour. It is highly viscous and opalescent with a pleasant odour. The specific gravity of the syrup is 1.2670. The aflatoxin assay revealed that the syrup is free of aflatoxins. The results of analysis for pesticide residue show no traces of pesticides residues in the syrup. The formulation is free of microbial contamination and shows positive for the presence of steroids, alkaloids, flavonoids, tannins, phenols, carbohydrates, glycosides etc. The quantitative analysis of the syrup estimates about  $2.80 \pm 0.11$  mg/gm of flavonoids,  $0.632 \pm 0.04$  mg/gm of alkaloids,  $0.37 \pm 0.03$ mg/gm of tannins and  $0.97 \pm 0.05$  mg/gm of phenol. The total ash value is  $5.66 \pm 1.69\%$ . The heavy metals such as arsenic, lead, mercury, cadmium are below detectable limit. The result Analysis of GC-MS Study of the syrup reveals the presence of 12 prominent peak in which the 11<sup>th</sup> prominent peak reveals the presence of ascorbic acid. This ascorbic acid is essential for the absorption of iron from the intestines and aids in Erythropoiesis.

The results of the present study ensures the safety profile of the Madhulai Manappagu – Siddha herbal syrup intended for paediatric usage and indicative of presence of active phytoconstituents that are responsible for its efficacy in treating the Pandu Noi (Iron Deficiency Anaemia) in children.

**Key Words :** Madhulai Manappagu, Pandu, Iron deficiency Anaemia, Physicochemical analysis, Phytochemical analysis

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### I. Introduction

India is a land of treasures of various tradition, culture, language, religion etc. wherein medicine is one among them. Indian medicine has got its own antiquity, tradition, practice and classification. Siddha system of Medicine – the medical knowledge of Tamil people that has developed its roots from southern most part of

peninsular India is one among them. Though the period of origin of this system is time immemorial, it is believed to be blessed and graced by Lord Siva who is the founder of this system. The term "Siddha" itself gives the meaning of completeness and hence the system of medicine is a complete medical system that not only deals with Medicine but also describes the way of life in this materialistic world<sup>1</sup>.

Siddha system of Medicine deals the diseases of the children separately in a section "Balavakatam". Pandu Noi (Anaemia) which is called as Veluppu Noi<sup>2</sup> in Siddha Medicine is a blood disorder commonly affecting children, adolescent girls, pregnant and menstruating women. Though the aetiological factors are many, nutritional deficiency, particularly Iron deficiency is the prime cause for anaemia. Iron deficiency anemia (IDA) is one of the commonest nutritional disorders in children worldwide. Various studies in India have reported the prevalence of anemia in infants and children varying from 60% to 80%<sup>3-7</sup>. Iron deficiency in school age children results in growth retardation, poor immunity, abnormal behavioral approach, and poor cognitive and memory development<sup>8</sup>. Though Anaemia is serious and even life-threatening, it is largely preventable and treatable by increasing dietary intake (such as iron supplements), healthier hygiene and sanitation practices, regular deworming etc. Anaemia can be overcome through the various traditional systems such as Ayurveda, Yoga & Naturopathy, Unani, Siddha, Homoeopathy (AYUSH). India is the only country where AYUSH traditional systems have been codified<sup>9</sup>.

In Siddha Medicine, many numbers of formulations are mentioned in various literatures to treat anaemia. Most of the formulations are using metals and minerals as the main ingredient of the medicine. But there are formulations that are based on only with herbal raw drugs as their ingredients. One of such herbal formulation is "Madhulai Manappagu" mentioned in the text "Siddha Vaidya Thirattu"<sup>10</sup>. This formulation is supposed to have promising results in the disorder of anaemia by improving the Haemoglobin level in the blood. This results in better cognitive improvement, mental development, and immune boosting mechanism of the child to have a better citizen of the country.

## **II. Materials And Methods**

As per the reference literature<sup>10</sup> the syrup "Madhulai Manappagu" is prepared with the following ingredients,

- |    |                                                               |          |
|----|---------------------------------------------------------------|----------|
| 1. | Madhulam Pazham Saru (Pomegranate Juice)<br>(Punica granatum) | - 500 ml |
| 2. | Kalkandu (Sugar Candy)(Sugar crystals)                        | - 500 gm |
| 3. | Panneer ( Rose Water)                                         | - 500 ml |
| 4. | Thaen ( Honey)                                                | - 500 ml |

### **Process:**

Mix all the four and prepare syrup by carefully boiling. The syrup may be prepared by heating 1, 2 & 3 and then honey may be mixed with it after it has cooled. Then it is preserved in an airtight glass container.

### **Dose:**

8 – 15 ml twice or thrice a day with twice water.

### **Indications:**

Velluppu Noi. (Pandu).

### **Physicochemical analysis**

#### **Organoleptic characters**

##### **Color**

About 10 ml of Manappagu was taken in a clean glass beaker and tested for its color by viewing against a white opaque background under direct sunlight<sup>11</sup>.

##### **Odour**

About 10 ml of Madhulai Manappagu was placed in 100 ml of beaker and tested for its odour by wafting the air above the beaker. Further organoleptic characteristic features of the finished product Madhulai Manappagu was done by visual observation such as touch, smell and tasting the test drug and respective observations were detailed.

#### **Determination of specific gravity**

Fill the dry sp. gravity bottle with prepared samples in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 50°C and hold for 30 min. Carefully wipe off any substance that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh. Calculate the weight difference between the sample and reference standard<sup>12, 13, 14</sup>.

#### **Determination of Viscosity**

Viscosity determination were been carried out using Ostwald viscometers. Measurement of viscosity involves the determination of the time required for a given volume of liquid to flow through a capillary. The liquid is added to the viscometer, pulled into the upper reservoir by suction, and then allowed to drain by gravity back

into the lower reservoir. The time that it takes for the liquid to pass between two etched marks, one above and one below the upper reservoir, is measured<sup>12, 13, 14</sup>.

#### **Percentage Loss on Drying**

10gm of test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed<sup>13</sup>.

*Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100*

#### **Determination of Total Ash**

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 °C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug<sup>13</sup>.

*Total Ash = Weight of Ash/Wt of the Crude drug taken X 100*

#### **Determination of Acid Insoluble Ash**

About 0.5gm of the ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash<sup>13</sup>.

*Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100*

#### **Determination of Water Soluble Ash**

About 0.5gm of the ash obtained by total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter is collected in crucible and will be washed with hot water, and ignite for 15 minutes at a temperature not exceeding 450°C. Weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug<sup>13</sup>.

*Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100*

#### **Determination of Alcohol Soluble Extractive**

About 5 g of test sample will be macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug<sup>13</sup>.

*Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100*

#### **Determination of Water Soluble Extractive**

About 5 g of the test sample will be macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug<sup>13</sup>.

*Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100*

#### **Determination of p<sup>H</sup>**

About 5 g of test sample will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to PH evaluation using PH meter<sup>13</sup>.

#### **Preliminary Phytochemical Analysis**

##### **Test for Alkaloid**

Test drug was extracted with 2ml of HCl was added. To this acidic medium 1ml of dragendroffs reagent was added on, orange or red precipitate produced immediately indicate the presence of alkaloids<sup>15</sup>

##### **Test for flavonoid**

0.1ml of the sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Shows the presence of yellow color indicates the presence of Flavonoids<sup>15</sup>.

##### **Test for Steroids**

When the sample reacted with chloroform, acetic acid and conc.H<sub>2</sub>SO<sub>4</sub> and formed a blue and green colour. Which indicates confirmed the presence of steroid<sup>15</sup>.

##### **Test for Glycosides**

The test drug were mixed with a little anthrone reagent on a watch glass. One drop of concentrated sulphuric acid was added and made into a paste, warmed gently over water bath. The presence of glycosides was identified by dark green coloration<sup>15</sup>.

##### **Test for Tannins**

The test drug was mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins<sup>15</sup>.

***Test for carbohydrate***

Fehling's Test: Fehling's A and Fehling's B solutions, each 1ml were mixed and boiled, for 1ml and 2 ml of test drug were added heated in boiling water bath for 10 min, appearance of yellow and then brick red precipitate indicates the presence of reducing sugars.

***Test for Saponins***

The test drug was shaken with water and copious lather formation indicates the presence of saponins<sup>15</sup>.

***Test for Proteins (Biuret Test)***

To test drug 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins<sup>15</sup>.

***Test for Phenol***

To test drug a few drops of alcohol and ferric chloride solution was added. Bluish green or red indicates the presence of phenol<sup>15</sup>.

***Qualitative Analysis***

***Total Flavonoid***

Total flavonoid content in the drug Madhulai Manappagu was determined using aluminum chloride method. In this method Quercetin was used as standard and flavonoid contents were measured as quercetin equivalent. For this purpose, the calibration curve of quercetin was drawn. 1ml of standard or extract solution was taken into 10ml volumetric flask, containing 4ml of distilled water. 0.3ml of 5% NaNO<sub>2</sub> added to the flask. After 5min, 0.3ml 10% AlCl<sub>3</sub> was added to the mixture. At the 6th min add 2ml of 1M NaOH was added and volume made up to 10ml with distilled water. The absorbance was noted at 510nm using UV-Visible spectrophotometer<sup>16</sup>.

***Estimation of Alkaloid***

2g of the sample manappagu was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hr. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed<sup>17</sup>.

***Estimation of Tannin***

The tannin content was determined using FolinCiocalteu assay. Aliquot extract of test sample of 100 µL was added to 750 µL of distilled water, 500 µL Folin-Ciocateureagent and 1000 µL of 35 % sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). The mixture was shaken vigorously after diluting to 10 mL of distilled water. The mixture was incubated for 30 min at room temperature and read at 725 nm. Distilled water was used as blank. Tannic acid standard solutions were prepared and standard calibration curve was plotted with varying concentration. The total tannins content were expressed as Tannic acid mg/gm, as calculated from the prepared standard curve<sup>18</sup>.

***Determination of total Phenol content***

1 ml of test was added to deionized water (10 ml) and Folin–Ciocalteu phenol reagents (1ml). After 5 minutes, 20% sodium carbonate (2 ml) was added to the mixture. After being kept in total darkness for 1 hr, the absorbance was measured at 750 nm using a spectrophotometer. Amounts of total Phenol was calculated using Gallic acid calibration curve. The results were expressed as Gallic acid equivalents (GAE) mg/g of dry plant matter<sup>19</sup>.

***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 micro 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 300- 600nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of components present in each sample and R<sub>f</sub> values were tabulated<sup>20</sup>.

**Gas Chromatography–Mass Spectrometry (GCMS) Analysis**

GCMS (Clarus 500 Perkin – elmer (Auto system XL)), NIST Ver.2.1 MS data library

Specification :

Start Time(min)	End Time(min)	Start m/z	End m/z Scan	Speed
2.50	18.00	50.00	650.00	2000

Sample Inlet Unit :GC

GC-MS Plays a key role in the analysis of unknown components of plant origin. GC-MS ionizes compound and measures their mass numbers. Ionization method includes EI (Electron Ionization). The EI method produces ions by colliding thermal electrons emitted from a filament with sample gas molecules. This method provides high stability in ionization and obtained mass spectra show good reproducibility. The EI method provides good result for quantitative analysis as well. Quantitative analysis with GC-MS, in which only ions specific to the compounds are measured, is highly selective method without interfering components. Gas chromatography Technique involves the separation of volatile components in a test sample using suitable capillary column coated with polar or non-polar or intermediate polar chemicals. Elite-1 column (100% Dimethyl polysiloxane) is a non-polar column used for analysis of phyto-components. Elite -5 column (5% phenyl and 95% methyl polysiloxane) is an intermediate column and also used for the estimation of Phytochemical. An inert gas such as hydrogen or nitrogen or helium is used as a carrier gas .The compounds of test sample is evaporated in the injection port of the GC equipment and segregated in the column by absorption and adsorption technique with suitable GC programme.

**ICP-MS- Heavy Metal Analysis Report**

Inductively Coupled Plasma Mass Spectrometry (ICP-MS): ICP-MS is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals and several non-metals at concentration below one part in 10<sup>12</sup> (parts per trillion). Samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios. It is an automated, simple and unique quantitative and qualitative analysis. It measures elemental isotopes ratio.

**Procedure**

Digestion of sample is carried out by transforming 0.5 gm of the sample into a closed beaker and 5 ml of concentrated HNO<sub>3</sub> was added and digested to near dryness. 16 M nitric acid was further added each time to the sample and digested until the clear solution was obtained. 5ml of 12 M Hydrochloric acid was added to ensure complete digestion .The digested solution was cooled to room temperature and made to the final volume of 100 ml with deionized water. Sample solutions were then filtered through membrane (0.45micron) filter. Finally, the digested samples were used for metal analysis using inductively coupled plasma Mass Spectrometry (Perkin Elmer DRC-e Model) .Each sample was digested in triplicate. A blank solution was also prepared in a similar manner<sup>21, 22</sup>.

Machine Model: **Agilent 7700 ICPMS**

**Aflatoxin Assay By TLC (B1,B2,G1,G2)**

**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 front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm<sup>23</sup>.

**Test for Specific Pathogen**

**Methodology**

About 5ml of test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by spread 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.

**Abbreviation**

Organism	Abbreviation
<i>E-coli</i>	EC
<i>Salmonella</i>	SA
<i>Staphylococcus Aureus</i>	ST
<i>Pseudomonas Aeruginosa</i>	PS

**Sterility Test by Pour Plate Method**

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / unsterile 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**

About 1ml of the test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°C 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 37° C for 24-48 hours. Grown colonies of organism was then counted and calculated for CFU.

**Pesticide Residue Analysis**

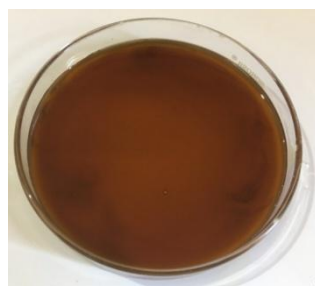
**Extraction**

About 10 g weight equivalent of test substance 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 40°C 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<sup>24, 25</sup>.

**III. Results And Discussion**

Out of 32 types of Internal Medicines described in Siddha Literatures, Manappagu is a type of Internal Medicine that can be correlated with the syrup preparation<sup>26</sup>. Usually, as manappagu preparations are having herbal ingredients and honey in its formulation, they are palatable and are easy to administer even to the children. But nowadays, in the commercial world, there is a possibility for adulteration of the ingredients and hence there is necessity for standardization of the formulation. Though, Madhulai Manappagu is in general practice for many more years across the country, the scientific standardization details are not available. Standardization gives information on chemical, biological, physico-chemical profile of madhulai manappagu. In the present study, the organoleptic characters, physico-chemical characters as per AYUSH guidelines are carried out and are compared with AYUSH standards. Phytochemical quantification of the syrup is also studied using HPTLC technique.

**Organoleptic characters**



Madhulai Manappagu

State	Liquid
Appearance	Reddish Brown
Nature	Highly viscous
Odor	Pleasant odour
Clarity	Opalescence
Taste	Sweet
Specific Gravity	1.2670
Viscosity at 50°C (Pa s)	60.85

The sweet taste and liquid state consistency is due to the addition of juice of pomegranate, sugar candy and honey with rose water. The pleasant odour is attributed to juice and rose water. Viscous nature is due to the presence of honey in the preparation.

**Results of Physicochemical Analysis**

The values obtained in respect of physicochemical analysis of Madhulai Manappagu are tabulated as below.

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	39.43± 1.98
2.	Total Ash (%)	5.66 ± 1.69
3.	Acid insoluble Ash (%)	0.83 ± 0.24
4.	Water Soluble Ash (%)	0.93 ± 0.20
5.	Alcohol Soluble Extractive (%)	38.8 ± 2.2
6.	Water soluble Extractive (%)	50.47 ± 4.1
7.	PH (%)	7.2 ± 0.6

Loss on drying indicates the total volatile content and moisture content of drug. The loss on drying at 105 °C of madhulai manappagu was 39.43± 1.98. This is due to the presence of polyphenolic content in the pomegranate juice<sup>27</sup>. High values of Loss on drying may be responsible for deterioration of the formulation. It is because of this reason, Siddhars might have determined the shelf life period of Manappagu for six months.

The total ash content is the measure of inorganic constituents and earthy materials present in the drug. The total ash content of the syrup was 5.66%± 1.69% indicating the high amount of organic matter and minerals. The Acid insoluble ash value of the drug gives the amount of siliceous matter. If the acid insoluble ash is low, the quality of the drug is better. The Acid insoluble ash of the madhulai manappagu was found to be 0.83% ± 0.24 % which indicate its quality. The Water soluble ash is a part of Total ash content of the drug which is soluble in water. The Water soluble ash in this manappagu preparation was found to be 0.93% ± 0.20%.

Extractive values of the drug give the approximate amount of chemical constituents present in the formulation. The alcohol soluble extractive of madhulai manappagu was found to be 38.8% ± 2.2% where as the water soluble extractive was amount to 50.47% ± 4.1%. The stability of the syrup is evaluated by p<sup>H</sup> and it was found to be 7.2 % ± 0.6% for Madhulai manappagu.

**Results of Phytochemical Analysis - Qualitative**

The Phytochemical analysis of Madhulai Manappagu resulted as given below.

Compound	Steroids	Alkaloids	Flavonoid	Saponins	Tannins	Phenol	Protein	Carbohydrate	Glycosides
Madhulai Manappagu	+	+	+	-	+	+	-	+	+

+ Presence                      - Absence

Qualitative phytochemical analysis of the trial drug Madhulai Manappagu revealed the presence of higher percentage of bioactive phytoconstituents such as steroids, alkaloids, flavonoids, tannins, phenols, glycosides and carbohydrates.

**Results of Phytochemical Analysis – Quantitative**

Phytoconstituents	Madhulai Manappagu
Total flavonoids (quercetin mg/gm)	2.80 ± 0.11
Total alkaloids(mg/gm)	0.632 ± 0.04
Total tannins(mg/gm) (Tannic acid mg/gm)	0.37 ± 0.03
Total Phenol (Gallic acid equivalents (GAE) mg/g)	0.97 ± 0.05

Mean with 3 replicates ± SD.

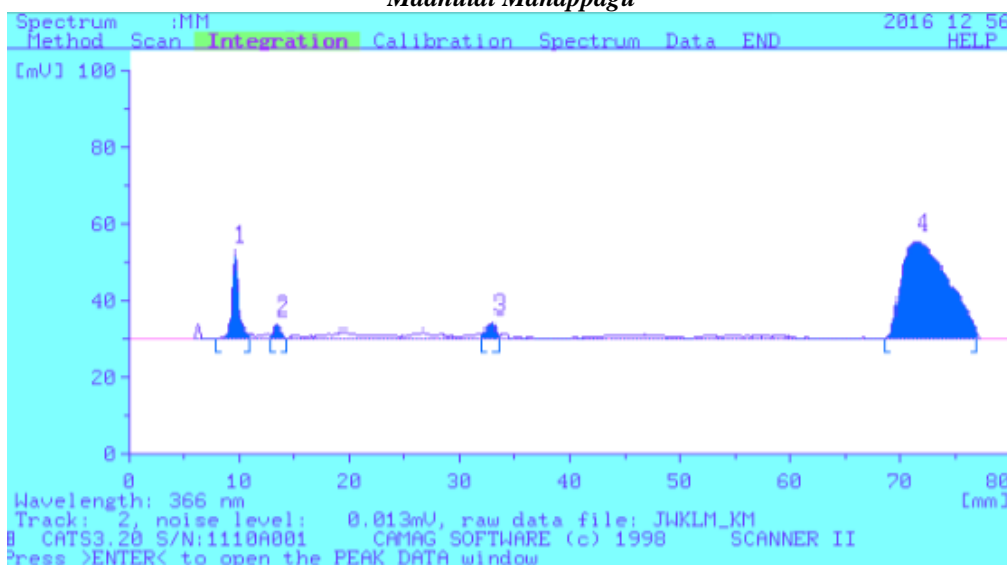
The quantitative estimation of total phenols, total flavonoids, total alkaloids and total tannins in the syrup were found to be 0.97, 2.80, 0.63 and 0.37 respectively.

**Result analysis of HPTLC**

**TLC Histogram of the Sample  
Madhulai Manappagu**



**HPTLC Chromatogram of  
Madhulai Manappagu**



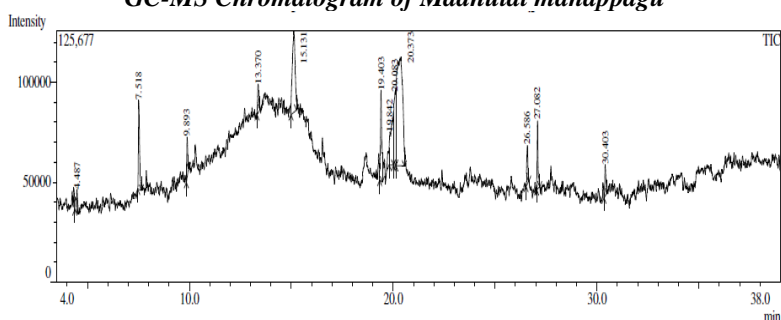
TLC developed plate loaded with Madhulai Manappagu shows 4 spot when eluted in the solvent system Toluene: Ethylacetate: Formic acid (5:3.5:1.5) with the Rf value ranging from 0.12 to 0.8.

**Result Analysis of GCMS**

Result Analysis of GC-MS Study of Madhulai Manappagu reveals the presence of 12 prominent peak in which the 11<sup>th</sup> prominent peak at 27.08 retention time with percentage peak area of 3.94% reveals the presence of ascorbic acid.



**GC-MS Chromatogram of Madhulai manappagu**



**Peak Table of GCMS analysis**

Peak#	R.Time	Area	Area%	Name
1	4.487	56391	2.06	2-Hydroxy-gamma-butyrolactone
2	7.518	180620	6.60	2-Butanone, 4-hydroxy-3-methyl-
3	9.893	60432	2.21	5-Hydroxymethylfurfural
4	13.370	56709	2.07	(Z)1-Allyl-2-methylcyclohexanol
5	15.131	385182	14.07	XANTHOSINE
6	19.403	195683	7.15	1,3,4,5-TETRAHYDROXYCYCLOHEXANOL
7	19.842	242644	8.86	1-Hexadecanethiol
8	20.083	238984	8.73	CYCLOHEXANEPROPANOIC ACID, METHYL ESTER
9	20.373	1078873	39.40	MOME INOSITOL
10	26.586	74393	2.72	Trehalose
11	27.082	107986	3.94	l-(+)-Ascorbic acid 2,6-dihexadecanoate
12	30.403	60041	2.19	2-Pentadecyn-1-ol
		2737938	100.00	

GC-MS analysis shows the presence of sugar moieties with highest peak area of 42.12%. Other chemical constituents include nucleoside, vitamin c, mercaptan compound, fatty acid, alkane and some volatile compounds. Some of these compounds are reported to have antiviral, anticancer and antioxidant activities.

**Result Analysis of ICP – MS (Heavy Metals analysis)**

Element	Concentration (mg/L)	Upper Limit (mg/L)
Arsenic (As)	0.0018	2.99
Lead (Pb)	0.027	9.98
Mercury (Hg)	BDL	0.998
Cadmium (Cd)	0.015	0.299

Heavy metal analysis of Madhulai Manappagu shows that presence of heavy metals such as Arsenic, Lead and cadmium are within the prescribed limit, whereas the level of mercury is below the detective level.

**Results of Aflatoxin Assay By TLC (B1,B2,G1,G2)**

Aflatoxin	Sample MM	AYUSH Specification Limit
B1	Not Detected – Absent	0.5 ppm
B2	Not Detected – Absent	0.1 ppm
G1	Not Detected – Absent	0.5 ppm
G2	Not Detected – Absent	0.1 ppm

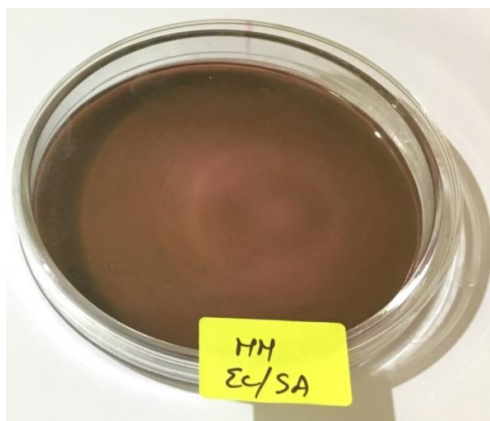
The results show that there were no spots been identified in the test sample loaded in TLC plated when compared to the standard indicate that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

**Results of Specific Pathogen Test**

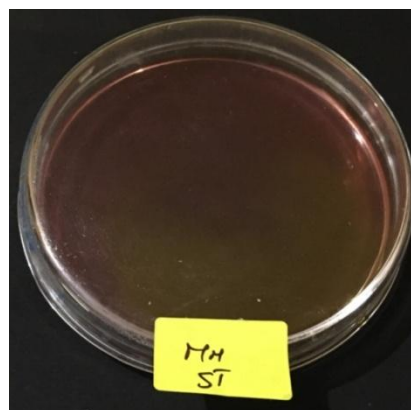
No growth was observed after incubation period. Reveals the absence of specific pathogen

Organism	Specification	Result	Method
<i>E-coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus Aureus</i>	Absent	Absent	
<i>Pseudomonas Aeruginosa</i>	Absent	Absent	

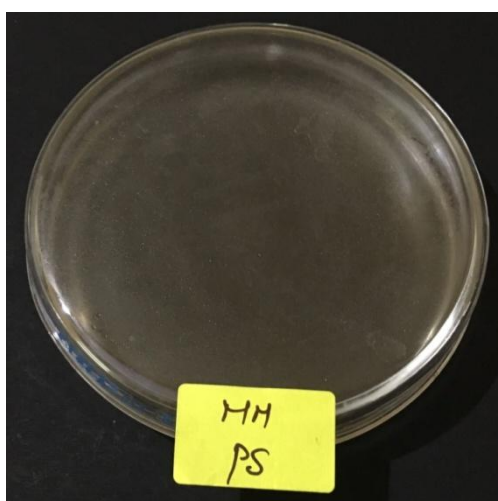
No growth / colonies were observed in any of the plates inoculated with the test sample.



Culture plate with E-coli and Salmonella specific medium



Culture plate with Staphylococcus Aureus specific medium



Culture plate with Pseudomonas Aeruginosa specific medium

**Result of Sterility Test by Pour Plate Method**

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

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10 <sup>3</sup> CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10 <sup>3</sup> CFU/g	

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



**Results of Pesticide Residue Analysis of Madhulai Manappagu**

Pesticide Residue	Sample MM	AYUSH Limit (mg/kg)
<b>I.Organo Chlorine Pesticides</b>		
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
<b>II.Organo Phosphorus Pesticides</b>		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
<b>III.Pyrethroid</b>		
Cypermethrin	BQL	1mg/kg
<b>IV. Organo carbamate</b>		
Carbofuran	BQL	2mg/kg

BQL- Below quantification Limit

The results showed that there were no traces of pesticide residues such as Organo chlorine, Organo phosphorus, Organo carbamate and Pyrethroids in the sample Madhulai Manappagu. It further shows the above mentioned residues were not been detected in the sample Madhulai Manappagu provided for analysis.

Since herbal drugs are of natural origin, microbial contamination is also an important issue. Pharmacopoeias have established limits for HERBAL drugs. In addition to microbial contamination, heavy metals, and pesticide residues, and aflatoxins is also to be checked<sup>28</sup>.

The present study of standardization of Madhulai Manappagu in respect to aflatoxin assay, sterility test for microbial contamination, test for specific pathogens and pesticide residue analysis revealed the absence of microorganisms, aflatoxins and pesticides in the syrup preparation and ensures the safe usage of the formulation. Safety studies of herbal drugs and other products used in traditional medicines have become mandatory in order to ensure their quality and risk free therapeutic application

#### IV. Conclusion

From this current study of preclinical standardization of Madhulai Manappagu indicated for Pandu Noi a syrup formulation which is mentioned in Siddha texts shows that the drug is a viscous reddish brown liquid with sweet taste. The specific gravity of the drug is 1.2670 while the p<sup>H</sup> is 7.2. The phytochemical analysis shows the presence of bioactive phytoconstituents such as steroids, alkaloids, flavonoids, tannins, phenols, glycosides and carbohydrates. Result Analysis of GC-MS study reveals the presence of ascorbic acid which may enhance the erythropoiesis in our body. Heavy metal analysis shows that presence of heavy metals such as Arsenic, Lead and Cadmium are within the prescribed limit and the level of mercury is below the detective level there by ensures its safe usage. In addition to these, the study reveals that the syrup is sterile and free of bacteria, fungi, and specific pathogens like E-coli, Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa and pesticide residues. As a result, Madhulai Manappagu, Siddha syrup formulation<sup>29</sup> is subjected to many studies to validate its efficacy and safety through the defined standardization procedure and it is recommended to take the formulation to the next level of investigation like pharmacological studies and clinical trials. It is the need of the hour to validate the concept of “Unavae Marunthu; Marunthae Unavu”<sup>30</sup> – the famous paradigm of Siddha System to the modern materialistic world by ensuring the efficacy of this syrup formulation.

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