Research Article

Standardization of Siddha Poly Herbal Formulation Saruva Thitha Nirgundi Thailam

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ABSTRACT

The global shift toward evidence-based herbal medicine has highlighted the need for standardized, safe, and efficacious traditional formulations. Siddha medicine is a classical Indian system offers several therapeutic preparations for chronic inflammatory and infectious disorders, yet their global acceptance depends on specific scientific validation and standardization. The present study focuses on *Saruva Thitha Nirgundi Thailam* is indicated for *Virana Silethumam* (Chronic Tonsilltis) and aims to establish its quality profile following PLIM guidelines. Comprehensive physicochemical and phytochemical analysis were performed to ensure identity, purity, and stability. The organoleptic characteristics revealed a reddish-brown viscous oil with a characteristic order and smooth consistency. Viscosity, acid value, peroxide value, refractive index and Iodine value and HPTLC fingerprinting exhibited the presence of multiple bioactive phytocompounds responsible for the drug's pharmaceutical action. Microbial and heavy metal analysis confirmed the absence of contamination, with no detectable bacteria, fungi, aflatoxins, Mercury or Arsenic, and Lead and Cadmium found below quantifiable limits. These findings validate formulation's safety for human use. Traditionally the formulation is prescribed to pacificy Kaba dosha correlating with its modern anti-inflammatory and anti-microbial and immune modulatory activities beneficial in managing Tonsillitis. The study supports its potential clinical application as a safe and effective alternative therapy for paediatric tonsillitis and provides a foundation for further clinical validation bridging siddha medicine with the modern phytotherapeutic standards.

Keywords: *Saruva Thitha Nirgundi Thailam, Virana Silethumam,* Chronic Tonsillitis, Standardization, Siddha.

INTRODUCTION

Globally, the use of standardized herbal medicines is expanding as safer, evidence-based alternatives gains public trust. However, ensuring quality control, consistency and regulatory harmonization remain a persistent challenge due to variations in raw materials and manufacturing methods.[1] Hence modern analytical protocols integrating physicochemical, phytochemical and toxicological evaluations are essential to validate traditional formulations and ensure reproducible safety and efficacy.

Within India's traditional medical systems, Siddha medicine has long utilized diverse herbal and Herbo-mineral formulations for chronic inflammatory and infectious conditions. To enhance global credibility siddha formulations are increasingly standardized following the PLIM and AYUSH guidelines, which emphasize identity, purity and physicochemical stability.[2] Such studies justify the scientific integration of traditional siddha drugs with modern quality assurance methods.

Clinically acute and chronic tonsillitis represents a major pediatric problem, affecting nearly 1.1% of U.S. children annually, 35% presenting chronic forms.[3] In a hospital-based study from Tiruchirapalli, Tamil Nadu, assessing pediatric ENT morbidities, tonsillitis ranked among the most common upper respiratory disorder in children attending outpatient clinics, highlighting its regional burden in south India.[4] Conventional management relies on antibiotics for bacterial cases and tonsillectomy for recurrent infections.[5] Yet increasing antibiotic resistance and surgical risks have justified the need for safe, effective, and non-invasive alternatives. Recent randomized trial supports standardized herbal formulations significantly improved pharyngotonsillitis in children.[6]

The selected experimental drug for thisstudy *Saruva Thitha Nirgundi Thailam* is indicated for *Annakku Thooru Thabitham* in the siddha textbook of *Therayar Thailavarga Surukkam*.[7] The various ingredients of *Saruva Thitha Nirgundi Thailam* possess heat potency to reduce the elevated *Kabam*, so that it will be very effective in reducing the symptoms of *Virana Silethumam.*

Budding on this evidence, siddha polyherbal formulations may provide comparable efficacy due to their multi targeted anti-inflammatory, antimicrobial, and immunomodulatory actions. However, without proper standardization, their safety and reproducibility remain uncertain. Hence, the present study aims to standardize the siddha formulation Saruva Thitha Nirgundi Thailam, traditionally used for *Virana Silethumam* (Chronic Tonsillitis), through comprehensive Pharmacogenetic, physicochemical, phytochemical, and safety evaluations following PLIM guidelines, this approach will justify its safe clinical application and strengthen the scientific foundation for siddha formulations within global phytomedicine standards.

MATERIALS AND METHODS

Source of the sample:

The essential raw materials required for the production of *Saruva Thitha Nirgundi Thailam* were purchased from a reputed supplier specializing in raw medicinal ingredients and was authenticated by medicinal botanist of NIS. After proper purification the medicine was prepared in the Gunapadam Laboratory of National Institute of Siddha.

Ingredients:

1.	Vennotchi ilai juice *[Vitex negundo Linn.]*	- *4 padi (*6080 ml*)*
2.	Semmanithakkali juice [*Solanum nigrum]*	- *4 padi (*6080 ml*)*
3	Vasambu [*Acorus calamus Linn.*]	- 1 balam (35 grams)
4	Murungai vithai [*Moringa oleifera Linn.*]	- 1 balam (35 grams)
5.	Vattathirupi [*Sida acuta]*	- 1 balam (35 grams)
6	Chukku [*Zingiber officinale]*	- 1 balam (35 grams)
7.	Parumunnai ver [*Premna corymbosa]*	- 1 balam (35 grams)
8.	Kumkumapoo [*Crocus sativus]*	- 1 balam (35 grams)
9	Thippili [*Piper longum]*	- 1 balam (35 grams)
10.	Siruthekku [*Cleodendrum serratum Linn]*	- 1 balam (35 grams)
11.	Venkadugu [*Brassica alba]*	- 1 balam (35 grams)
12.	Sesame oil	- 1 padi (1520 ml)

Purification of raw drugs:

Vennotchiiliai - Washed with a pure water.

Semmanithakkali - Washedwith a pure water.

Vasambu - Burnt and made into charcoal (ash).

Murungaivithai - Outer coat is removed and washed in warm water and then dried.

Vattathirupppi - Made into pieces and dried in sunlight.

Chukku - Outer coat is removed and made to dried.

Parumunnai ver - Made into pieces and dried in sunlight.

Kumkuma poo - Heated in a flame and to be made as a roasted texture

Thippili - Washed by purified water and dried.

Siruthekku - Outer coat is removed and dried in sunlight.

Venkadugu - Dri in the sunlight for 2 hours.

Physiochemical Evaluation

Determination of Iodine value

About 20 gm weight equivalent of test sample was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30 mins and refrigerated for an hour. T About 10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow color. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking sample and note the corresponding reading for blank titration.

Determination of saponification value

About 2 gm weight equivalent of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions were added to the round bottomed flask. Repeat the same procedure without taking the sample for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

Determination of Viscosity value

Viscosity determination has 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.

Determination of Refractive Index

Determination of RL was carried out using Refractometer.

Determination of Weight per ml

Weight per ml was determined using the comparative weight calibration method, in which the weight of 1ml of the base of the formulation was calculated and then weight of 1 ml of finished formulation have been calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

Acid Value

Accurately 5 g weight equivalent of the test sample was weighed and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink color indicated the end point. The volume of consumed KOH solution was determined, and the titration of test sample was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression.

Acid value = Titter Value X 0.00561X 1000 / Wt of test sample (g)

Table 1: Physiochemical Evaluation

State	Liquid
Nature	Viscous
Odor	Characteristic
Touch / Consistency	Greasy
Flow Property	Free Flowing
Appearance	Reddish Brown

Table 2: Solubility Profile

S. No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Soluble
2	Ethanol	Insoluble
3	Water	Insoluble
4	Ethyl acetate	Soluble
5	DMSO	Insoluble

Peroxide value

5 g weight equivalent of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow color almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue color just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Peroxide value = 10 (a-b)/w

Table 3: Analytical Report

Parameter	SNT
Viscosity at 50°C (Pa s)	56.16
Refractive index	1.88
Weight per ml (gm/ml)	0.81
Iodoine value (mg I2/g)	119.44
Saponification Value (mg of KOH to saponify 1gm of fat)	221.37
Acid Value mg KOH/g	0.62
Peroxidase Value mEq/kg	4.43

TLC Analysis

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 was 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 the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm

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 analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning was brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

HPTLC finger printing analysis of the sample reveals the presence of nine prominent peaks corresponds to the presence of versatile phytocomponents present with in it. The major Rf value of the peaks ranges from 0.09 to 0.64.

Figure 1: TLC Visualization of SNT at 366 nm

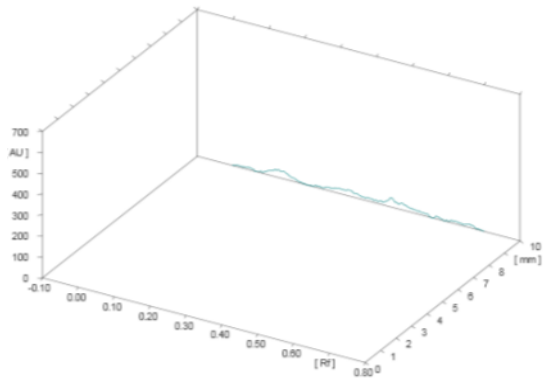

Figure 2: 3D Chromatogram

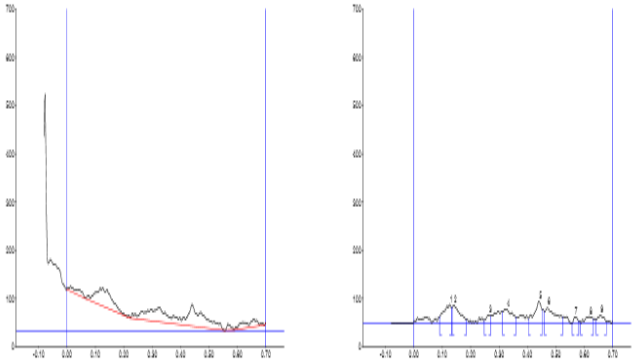

Figure 3: HPTLC finger printing of SNT

Table 4: Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	13.0	0.13	38.0	15.80	0.13	31.5	764.3	16.43
2	0.14	31.9	0.14	37.5	15.60	0.19	6.0	724.0	15.56
3	0.25	5.9	0.27	17.2	7.15	0.27	14.3	199.6	4.29
4	0.31	23.2	0.33	28.9	12.03	0.36	12.3	671.5	14.43
5	0.40	9.0	0.44	44.8	18.64	0.45	27.4	806.3	17.33
6	0.46	23.5	0.47	31.0	12.87	0.53	12.3	852.2	18.32
7	0.56	0.4	0.57	12.9	5.37	0.58	4.4	122.9	2.64
8	0.59	1.2	0.62	13.6	5.67	0.63	8.2	254.8	5.48
9	0.64	6.1	0.66	16.5	6.87	0.68	1.8	256.2	5.51

Test for Heavy metal Analysis

Method of Analysis Instrument Extraction Solvent

Model: AA 240 Series HCl and HNO3

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 HNO_3.

Standard preparation

As & Hg- 100 ppm sample in 1mol/L HCl & Cd & Pb- 100 ppm sample in 1mol/L HNO_3

Report and Inference

Results of the present investigation have clearly showed that the sample has no traces of heavy metal such as Mercury and Cadmium were as the sample evident the presence of Arsenic and Lead at 0.96 and 4.51 ppm levels as listed in the table 5.

Table 5: Heavy metal Analysis

Test Report Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	4.51	10 ppm
Arsenic	193.7 nm	0.96	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

BDL- Below Detection Limit

STERILITY TEST BY POUR PLATE METOD

Objective

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

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 and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

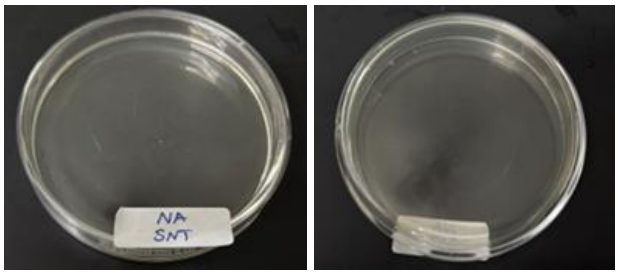

Figure 4: Sterility Test

Observation

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

Test for Specific Pathogen

Methodology

Test sample was directly inoculated into 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 colour with respect to pattern of colony formation in each differential media.

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.

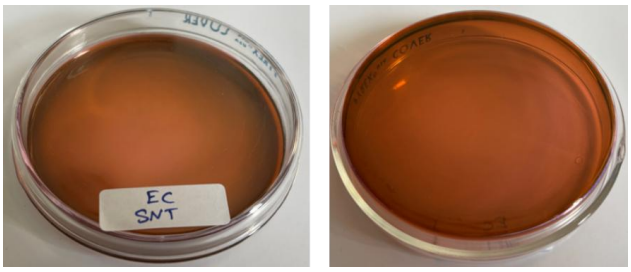

Figure 5: Culture plate with E-coli (EC) specific medium

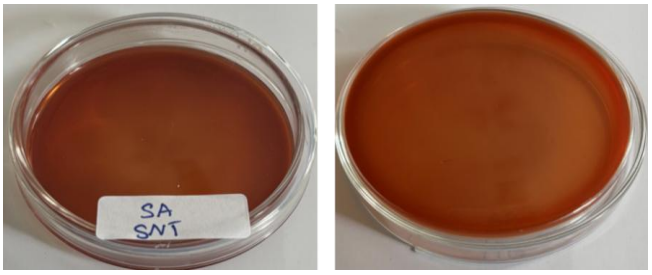

Figure 6: Culture plate with Salmonella (SA) specific medium

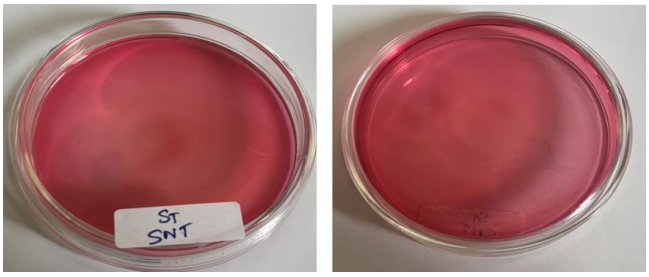

Figure 7: Culture plate with Staphylococcus Aureus (ST) specific medium

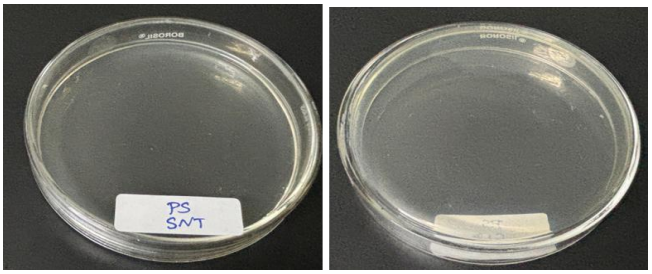

Figure 8: Culture plate with Pseudomonas Aeruginosa (PS) specific medium

Table 6: Test for Specific Pathogen

Organism	Medium	Specification	Result	Method
E-coli	EMB Agar	Absent	Absent	As per AYUSH specification
Salmonella	Deoxycholate agar	Absent	Absent	
Staphylococcus Aureus	Mannitol salt agar	Absent	Absent	
Pseudomonas Aeruginosa	Cetrimide Agar	Absent	Absent	

Test for Organochlorine pesticide, organophosphorus pesticide and pyrethroids

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 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 and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Table 7: Organochlorine pesticide, organophosphorus pesticide and pyrethroids

Pesticide Residue	Sample SNT	AYUSH Limit (mg/kg)
I. Organo Chlorine Pesticides		
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
Dichlorvos	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.

Aflatoxin Assay by TLC

Standard

Aflatoxin B1

Aflatoxin B2

Aflatoxin G1

Aflatoxin G2

Solvent

Standard samples were dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1µg 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 2.5µL, 5µL, 7.5µL and 10µL. 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 8: Aflatoxin Assay

Aflatoxin	Sample SNT	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 compared to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2.

DISCUSSION

In the present study, *Saruva Thitha Nirgundi Thailam*, a standardized herbal preparation, its organoleptic properties, which include its reddish brown in colour, characteristic odour, viscous in nature with free-flowing property and greasy in consistency was characterized for its physical and chemical properties, confirming its quality and safety. The viscosity is 56.6c/pas. The acid value of, *Saruva Thitha Nirgundi Thailam* was found to be 0.62. The peroxide value is found to be 4.43 it indicates the good quality of the oil and the oil has long shelf life.

The refractive index is found to be 1.88 it interprets that there is no adulteration in the sample**.** The index of weight per ml is 0.81 gm/ml. The iodine value is 119.44 (mg I2/g) So the trial drug is rich in Poly Unsaturated Fatty Acids (PUFA) which is helpful in reducing LDL cholesterol.

The saponification value is 221.37 mg KOH/g to neutralize the fatty acids resulting from the complete hydrolysis of 1gm of sample. So, the saponification value is high percentage of Short Chain Fatty Acids (SCFA) in the sample drug which may improve colonic health and get easily absorbed and digested.

HPTLC finger printing analysis of the sample reveals the presence of nine prominent peaks corresponds to the presence of versatile phytocomponents present with in it. The major Rf value of the peaks ranges from 0.09 to 0.64. Further the peak 1 occupies the major percentage of area 40.10% and peak 5 occupies the second major percentage of area 20.63% which denotes abundant existence of such compound.

This study also reveals that the drug was sterile and free of bacteria, fungi and specific pathogen like Salmonella, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and pesticide residues. Thus, microbial analysis revealed no contamination, ensuring its suitability for human use. In heavy metal analysis there are no traces of Mercury and Arsenic. lead and cadmium in the sample seems to be Below Quantification limit. There were no spots of Aflatoxin. like B1, B2, G1, G2.

The ingredients of *Saruva Thitha Nirgundi Thailam* are traditionally indicated to reduce Kabam Dosha according to Siddha principles, and modern pharmacological perspectives also support its potential anti-inflammatory and soothing effects, which are relevant in the management of tonsillitis.

Given its traditional formulation and demonstrated safety, *Saruva Thitha Nirgundi Thailam* presents a promising therapeutic option for managing tonsillitis in children. The absence of microbial contamination and standardization of its composition further strengthens its potential application in clinical settings. Considering that *Saruva Thitha Nirgundi Thailam* is a Sastric preparation, it aligns with regulatory guidelines that may exempt it from preclinical toxicological studies prior to initiating clinical trials. This provides a rationale for progressing directly to clinical evaluation to assess its efficacy and safety in paediatric tonsillitis.

Future clinical trials are warranted to establish the therapeutic benefits, optimal dosing, and safety profile of *Saruva Thitha Nirgundi Thailam* in children with tonsillitis. Such studies will not only validate traditional knowledge but also potentially provide a complementary treatment option aligned with both modern and Siddha medical systems.

CONCLUSION

Saruva Thitha Nirgundi Thailam, a standardized Siddha herbal preparation, demonstrated acceptable physical, chemical, and microbial quality, confirming its safety for human use. Its traditional and pharmacological attributes suggest potential benefits in managing tonsillitis, particularly in children. As a Sastric formulation meeting regulatory standards, Saruva Thitha Nirgundi Thailam is suitable for direct clinical evaluation. Further clinical studies are recommended to validate its efficacy, optimize dosing, and confirm its safety profile, thereby bridging traditional wisdom with modern therapeutic approaches.

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