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Research Article

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Formulation and Standardization of Siddha drug-Amirthathi Nei

Bakkialakshmi R[1], Victoria S[2], Shanmugapriya C[3], Manju Hemamalini N[4]
[1]Post Graduate, Department of Kuzhanthai Maruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.
[2]HOD, Department of Kuzhanthai Maruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.
[3]Lecturer, Department of Kuzhanthai Maruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.
[4]Lecturer, Department of Kuzhanthai Maruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.

Abstract

Background: Siddha medicines is composed of ideology of nature remedy in the path of treating various ailments and chronic diseases. It is believed as one of the ancient and traditional medicinal practice followed and created by siddhars more specifically for childrens. Among them amirthathi nei which is mentioned in agasthiyar 2000 which is used in treatment of sinusitis in children. This formulation of Siddha drug composed of 11 herbal drugs. These Siddha preparations are clinically attempted on reverse pharmacology by using modern standardization methods proceed for proven the purity, identity, shelflife of the preparation of drug. samples are collected and subjected to standardization on the basics of physiochemical analysis its result obtained from this study play a vital role in data research. This paper is attempt to describe the standardization and efficacy of the drug.

Methods: The drug was screened for physiochemical, phytochemical analysis and HPTLC to estimate the quality of the drug.

Results: The results obtained from standardization and physiochemical analysis clearly reveals that the volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.Further we need to proven the medicinal uses and phytochemical and bioactivity studies to prove their therapeutic properties. its also useful in further reference of medicinal plants by go through the Siddha texts and related articles .the results of phytochemical analysis indicates the formulation AMIRTHATHI NEI contains alkaloids, coumarins, saponins, tannins, glycosides, Triterpenoids, anthocyanin, carbohydrate, protein. The results of HPTLC analysis shows phytoconstituents present in the sample and has no traces of heavy metals metals Cadmium, whereas the sample shows the presence of Lead at 6.43 ppm, Arsenic at 0.49 ppm and Mercury at 0.50 ppm.

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, Aflatoxin G2. 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.

Conclusion: From the results of the study it was evident that the Siddha formulation amirthathi nei complies with the standard and may be used for clinical management of surapeenisam. But further studies need to carried out to exact role of phytotherapeutics present in the formulation might responsible for the expected pharmacological action in animals and human as well.

Keywords: Siddha medicine for surapeenisam, Amirthathi nei, phytochemical activity, HPTLC for Siddha drugs.

Introduction

Siddha medicine is believed as one of the potential way of treatment on curing certain ailments. Though it is curable but this system of medicine still trying to proven their drug effectiveness through the standardization methods. In this study amirthathi nei was selected and screened for standardization methods as per procedure the medicine composed of drugs such as *Tinospora cordifolia, Indigofera aspalathoides, Solanum procumbens, Andrographics paninculata, Curculigo orchioides, Gmelina asiatica,Sida cordifolia, Cyperus rotundus, Alternanthera sessilis, Nelumbo nucifera, Pavonia odorata*. These drug possess the anti inflammatory, anti microbial, anti oxidant property. It is effective against in the treatment of surapeenisam (sinusitis in children).It is most common upper respiratory tract infection affected from the age of 7 years the frontal sinuses are developed it occurs with the symptoms of headache,sneezing repeatedly at early morning, facial odema, pain present in the face fever. It classified in to acute and chronic sinusitis due to dust allergy, food allergy, immune suppressed, family history with allergy are highly at risk to develop this condition.

Materials and Methods

2.1 Selection of drug:

The drug Amirthathi nei was collected from the classical Siddha literature.

2.2 Collection and authentication of the drug:

The raw materials included in the formulation are *Tinospora cordifolia, Indigofera asapalathoides, Solanum procumbens, Andrographics paniculata, Curculigo orchiodes, Gmelina asiatica, Sida cordifolia, Cyperus rotundus, Alternanthera sessilis, Nelumba nucifera, Pavonia odorata* were procured from the country drug shop at Chennai, Tamilnadu. They were identified and authenticated by the Botanist, Govt. Siddha Medical College, Arumbakkam,Chennai-106.

2.3 Purification of drug:

The purification of drugs was done by procedures mentioned in Siddha literature. This Amirthathi nei is polyherbal medicine .the aim of this study was carried out to standardized the drug Amirthathi nei by evaluating physiochemical properties.

2.4 Preparation of the drug:

Seenthil kodi, Sivanarvembu, Thoothuvelai ver, Nilavembu, Nilapanaikilanghu, Nilakumil ver, Sitamutti, Peramutti, Koraikilanghu, Poonaganni ver, Thamaraielaisurool are taken in the equal quantity and made in to fine powder. Then powder is soaked in to the cow milk (20 ser).Then it boiled until it reduced into 8 ser kudineer and filter it and mix the ghee before it get cooled. The nei were stored in the clean air tight container.

State	Liquid
Nature	Viscous
Odour	Characteristic
Touch	Greasy
Flow Property	Free Flowing
Appearance	Yellowish

Physiochemical evaluation:

Solubility profile:

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

Determination of Iodine value

About 20 gm 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 colour. 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 of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeatthe 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 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 bellow 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 were 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 of 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)

Peroxide value

5 g 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 soluton. 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 colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour 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.

Analytical report:

S.No	Parameter	***ATN***
1	Viscosity at 50^{o}C (Pa s)	61.26
2	Refractive index	1.52
3	Weight per ml (gm/ml)	0.82
4	Iodoine value (mg I2/g)	85.73
5	Saponification Value (mg of KOH to saponify 1gm of fat)	152.24
6	Acid Value mg KOH/g	0.72
7	Peroxidase Value mEq/kg	4.06

Phytochemical Analysis

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols:

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

A. Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

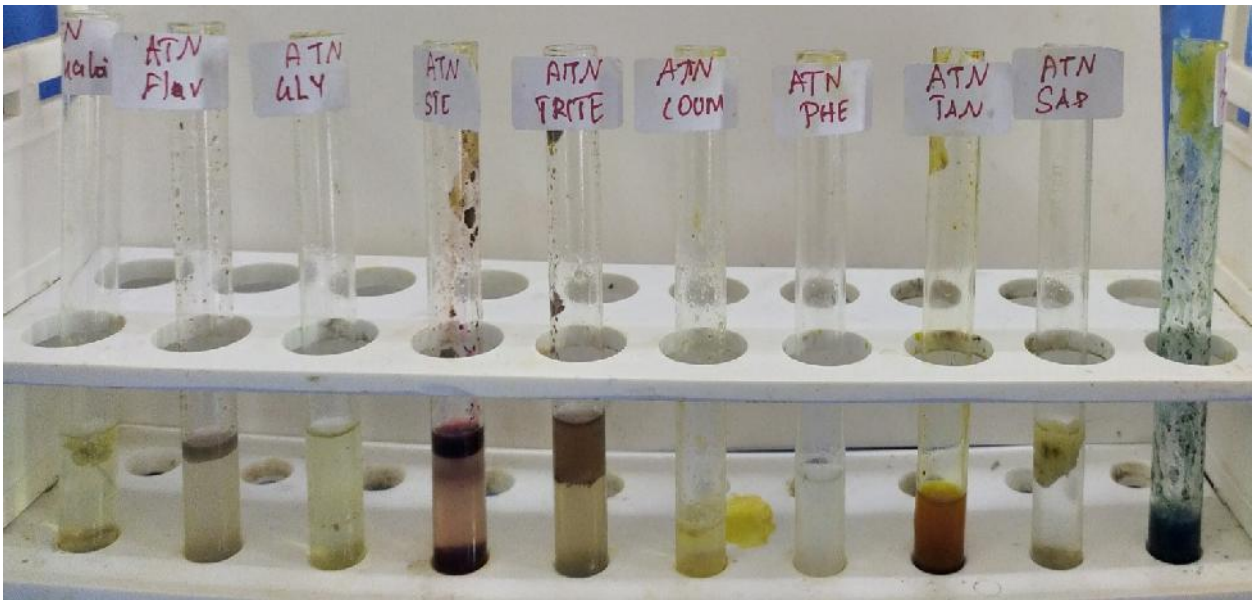
To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Results

Qualitative Phytochemical Investigation



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 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 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.

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. 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 phytotherapeutics.

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 were 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.

Methodology for heavy metals analysis

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 HNO_3

Name of the Heavy Metal	Absorption MaxΛ max	Result Analysis	Maximum Limit
Lead	217.0 nm	6.43 PPM	10 ppm
Arsenic	193.7 nm	0.49 PPM	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.50 PPM	1 ppm

BDL- Below Detection Limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metals Cadmium, whereas the sample shows the presence of Lead at 6.43 ppm, Arsenic at 0.49 ppm and Mercury at 0.50 ppm.

Methodology for pesticide:

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

Results:

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.

Methodology of aflatoxin:

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5µL, 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 KMM AYUSH specifications limit

B1 Not Detected-Absent 0.5ppm
B2 Not Detected-Absent 0.1ppm
G1 Not Detected-Absent 0.5ppm
G2 Not Detected-Absent 0.1ppm

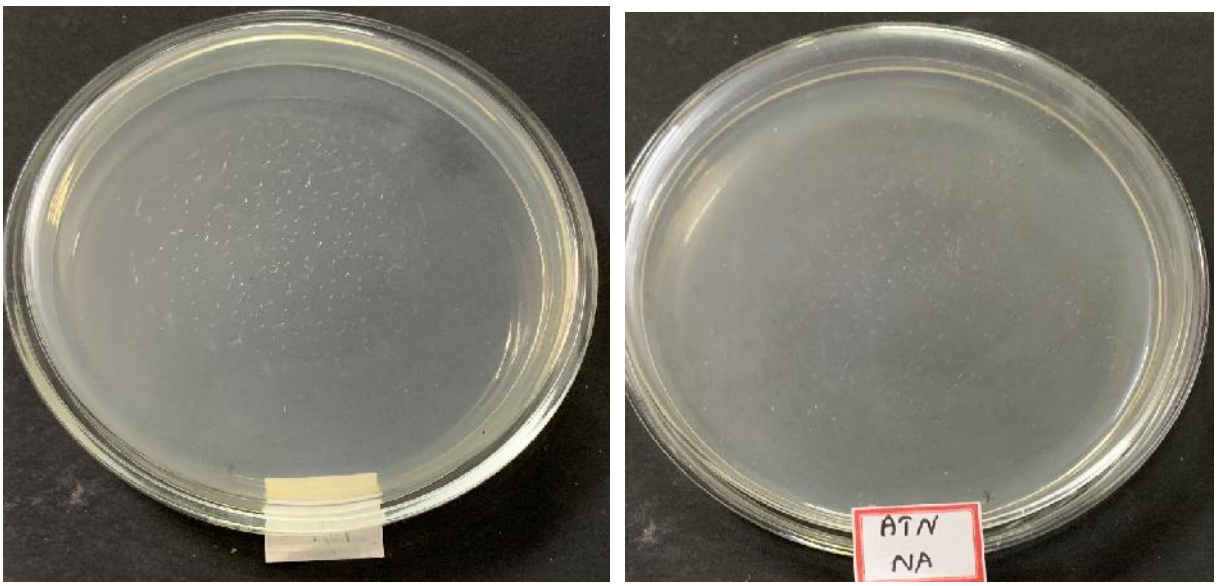
Result:

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, AflatoxinB2, Aflatoxin G1, Aflatoxin G2.

Test for sterility:

Methodology:

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}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^{o} 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.



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	

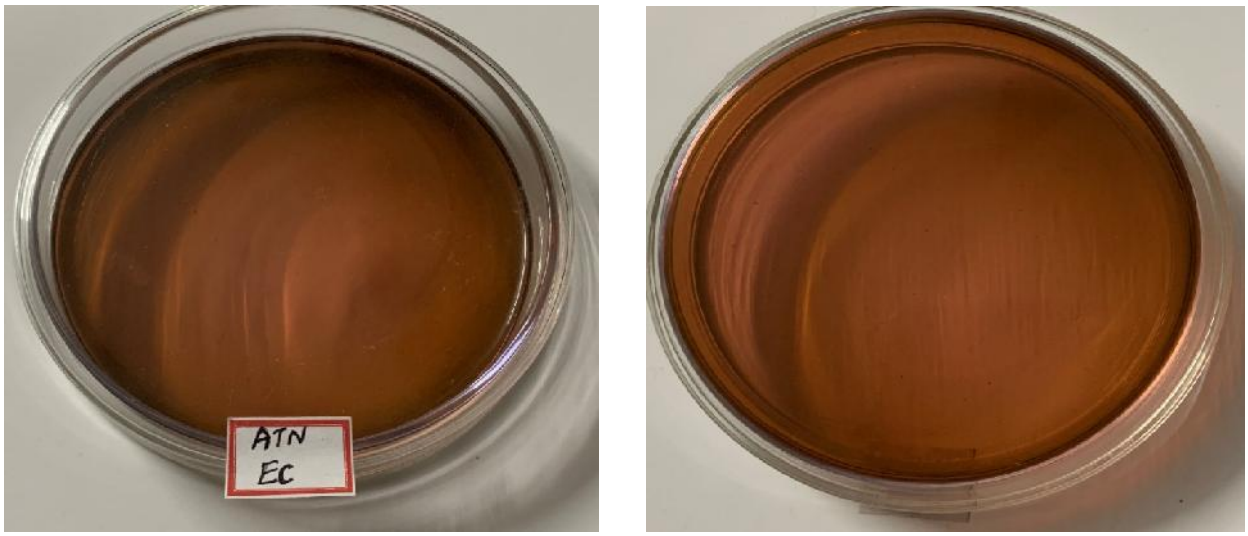
Methodology of specific pathogen:

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^{o}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.

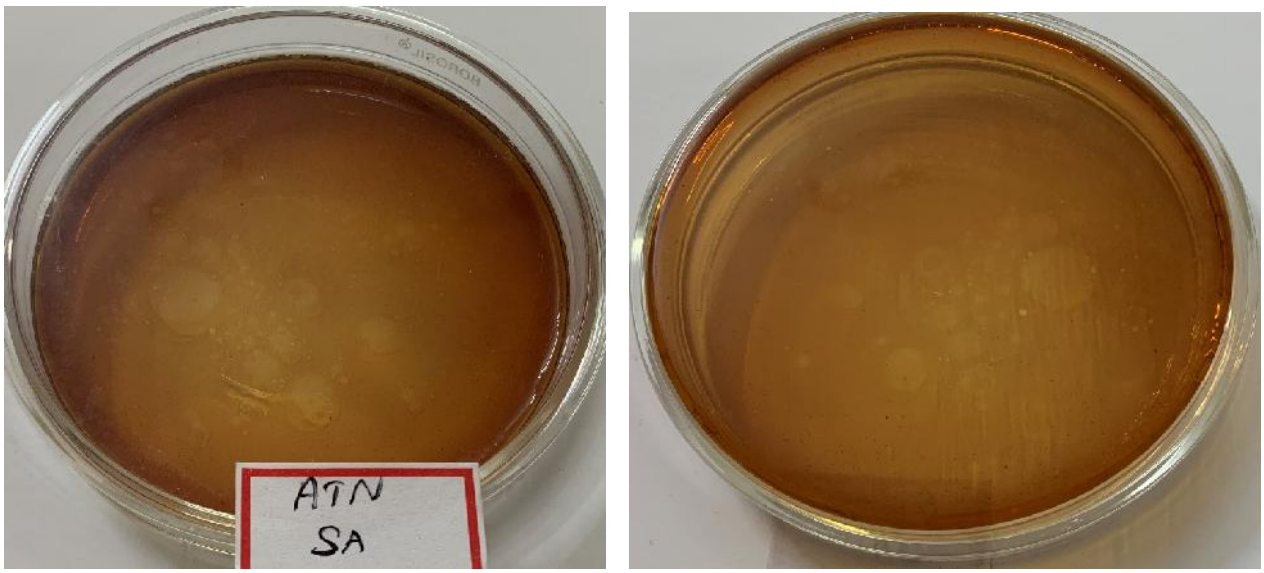
Result:

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

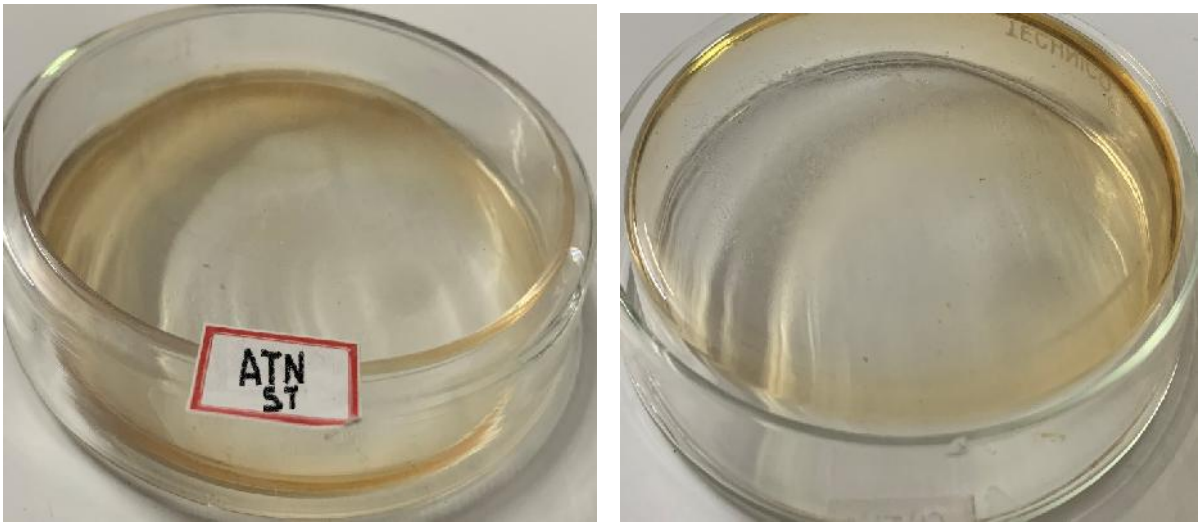
Organism	Specification	Result	Method
E. coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	



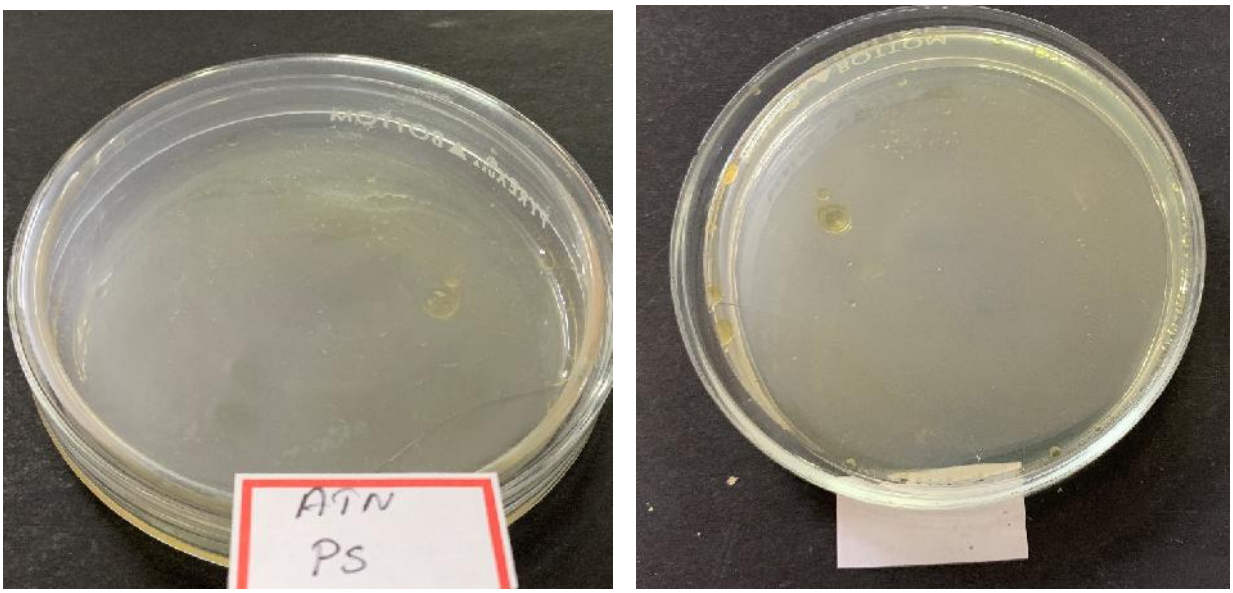
Culture plate with *E. coli* (EC) specific medium



Culture plate with Salmonella (SA) specific medium



Culture plate with *Staphylococcus aureus* (ST)specific medium



Culture plate with *Pseudomonas aeruginosa* (PS) specific medium

Discussion

The results obtained from standardization and physiochemical analysis clearly reveals that the volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml. Further we need to proven the medicinal uses and phytochemical and bioactivity studies to prove their therapeutic properties. Its also useful in further reference of medicinal plants by go through the Siddha texts and related articles .the results of phytochemical analysis indicates the formulation Amirthathi nei contains alkaloids, coumarins, saponins, tannins, glycosides, Triterpenoids, anthocyanin, carbohydrate, protein.

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