Phytochemical Standardization of *Serankottai nei* (a Siddha drug from milk extract of *Semecarpus Anacardium* nuts) and its *in-vitro* antitubercular activity against H37Rv strain

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ABSTRACT

Background: *Serankottai nei* is a popular Siddha drug, used in the treatment of lung infections including tuberculosis, autoimmune joint diseases (rheumatoid arthritis, degenerative osteoarthritis), cancers and neurological pain. **Objective:** To standardize *serankottai nei* and to screen its *in-vitro* antitubercular activity of in H37Rv strain. **Materials and Methods:** *Serankottai nei*, a medicated ghee preparation was procured from the SKM Siddha and Ayurveda Co India Ltd, Erode, Tamil Nadu. Unsaponifiable matter (USM) from the ghee preparation was separated and preliminary phytochemical screening was done. Further, USM was dissolved in 10 ml of chloroform and 3 and 6 µl of the above sample was applied for HPTLC fingerprint, which was developed in toluene: ethyl acetate (9.0:1.0). The developed plates were scanned under UV 254 nm, 366 nm, 540 nm and 620 nm post derivatization. R_f, colour of the spots and densitometric scan were recorded. Different doses of USM were screened for *in-vitro* antitubercular activity against H37Rv strain using Alamar Blue Dye method. **Results:** Phytochemical screening of 8% w/w USM obtained from *serankottai nei* showed presence of alkaloid, phenol, steroid and terpenoid. In HPTLC, there were 15, 5 and 7 peaks at 254 nm, 366 nm and 620 nm respectively. The Minimum Inhibitory Concentration (MIC) against H37Rv strain of pyrazinamide, streptomycin, ciprofloxacin were 3.125, 6.25 and 3.125 µg/ml respectively. Whereas, the MIC of *serankottai nei* was 1.6 µg/ml, which was almost 25 to 50% of standard drugs. **Conclusion:** *Serankottai nei* has shown promising antitubercular activity in *in-vitro* study. Thus, *Semecarpus anacardium* could be a suitable candidate for a new herbal based antitubercular drug.

Key words: *Serankottai nei,* Siddha Medicine, *Semecarpus anacardium*, Tuberculosis, Antitubercular.

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INTRODUCTION

Siddha system of Medicine is one of the ancient traditional medical systems in India. The classical Siddha literatures describe lakhs of herbal formulations for the management of human and animal diseases. *Serankottai nei* (SN) is a popular Siddha drug, used in the treatment of lung infections including tuberculosis, rheumatoid arthritis, degenerative osteoarthritis, cancers, skin diseases and neurological pain.[1] In this preparation, *Semecarpus anacardium* nut was boiled in cow milk and cow ghee, thus also called as milk extract of *Semecarpus anacardium*. Earlier studies have proven the

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protective role of SN on cell membranes in aflatoxin b1 induced hepatocellular carcinoma bearing rats.[2] In Chronic myeloid leukemia mice model, anticancer activity of SN was comparable to standard drug imatinib.[3] The beneficial role of SN in rheumatoid arthritis rat model was proven in earlier studies.[4] SN has anticancer activity against hepatocarcinoma, antioxidant activityin animal models.[5,6] Thirty days administration of SN to rats did not show toxicity, thus SN is considered as safe.[7] The phytochemical standardization and antitubercular activity of SN have not been done so far. This study was aimed to standardize the *Serankottai nei* and to screen its antitubercular activity against H37Rv strain.

MATERIALS AND METHODS

Chemicals

Serankottai nei was procured from SKM Siddha and Ayurveda Co India Ltd, Erode, Tamil Nadu. *Mycobacterium tuberculosis* (H37Rv strain, No-27294) was purchased from ATCC, USA and maintained in the microbiology lab. All other chemicals used in the study were of analytical quality purchased from the local vendor.

Seperation of unsaponifiablematter[8]

Weighed 100 g of the *serankottai nei* was taken in a 2 litre round bottomed flask. Added 1 litre of alcoholic KOH in to the sample. Boiled gently but steadily under reflux condenser for one hour. The condenser was washed with 10 ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 1 litre of water was added to the separating funnel followed by an addition of 250 ml of petroleum ether. The stopper was inserted and shaken vigorously for 1 min and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 250 ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 100 ml of aqueous alcohol and shaken vigorously, drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 100 ml of water until the water no longer turns pink on addition of a few drops of phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Placed the flask in an air oven at 85°C for about 1 h to remove the last traces of ether. Ten ml of acetone was added and evaporated to dryness on a water bath. Cooled in a desicator to remove last traces of moisture and then weighed. Percentage of unsaponifiable matter determined with respect to weight of sample taken.

Preliminary phytochemical screening[9,10]

The preliminary phytochemical screening was done according to the standard procedure. The brief of the procedure are as follows;

Tests for alkaloids

Dragendroff's test: To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Wagners's test: To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

Mayer's test: To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's test: To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Tests for carbohydrates

Molisch's test: To the extract, 1 ml of α-naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's test: A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's test: To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

Test for steroids

Libermann-Burchard test: To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few

drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

Salkowski test: The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for saponins

To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for tannins

To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for flavonoids

Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for coumarins

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for triterpenoids

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for carboxylic acid

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin

Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of turbidity.

Test for quinine

A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinine.

High Performance Thin Layer Chromatography[11,12]

Unsaponifiable matter of *Serankottai nei* was dissolved in 10 ml of chloroform and 3 and 6 µl of the above sample was applied on to a pre-coated silica plate to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in toluene: ethyl acetate (9.0:1.0). The developed plates were visualized in UV 254, 366, 540 (White light) and scanned under UV, 366 nm, 540 nm and 620 nm post derivatization. R_f, colour of the spots and densitometric scan were recorded.

In-vitro anti tubercular activity[13]

The anti-mycobacterial activity of Unsaponifiable matter of *Serankottai nei* was assessed against *Mycobacterium tuberculosis* (H37Rv strain from ATCC, No-27294) using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of test drug were made directly on plate. The final drug concentrations tested were 100 to 0.8 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. Pyrazinamide, streptomycin, ciprofloxacin and *serankottai nei* were added in different concentrations. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The Minimum Inhibitory Concentration (MIC) was defined as lowest drug concentration which prevented the color change from blue to pink.

Table 1: The observations duringphytochemical tests of *Serankottai nei*

Tests	Color if positive	*Serankottai nei*	Presence / absence
	Alkaloids		
Dragendrof's test	Orange red precipitate	Orange red precipitate	+
Wagners test	Reddish brown precipitate	Reddish brown precipitate	+
Mayers test	Dull white precipitate	Dull white precipitate	+
Hagers test	Yellow precipitate	Yellow precipitate	+
	Steroids		
Liebermann-buchard test	Bluish green	Bluish green	+
Salkowski test	cherry red color in chloroform layer and bluish green fluorescence in acid layer	cherry red color in chloroform layer and bluish green fluorescence in acid layer	+
	Carbohydrate		
Molish test	Violet ring at junction	Violet ring at junction	-
Fehlings test	Brick red precipitate	Blue color	-
Benedicts test	Red precipitate	Dark blue color	-
	Tannin		
With $FeCl_3$	Dark blue or green or brown	Yellow color	-
	Flavanoids		
Shinoda's test	Red to pink	Colorless solution	-
	Saponins		
With $NaHCO_3$	Stable froth	No stable froth	-
	Triterpenoids		
Tin and thionyl chloride test	Pink	Pink color	+
	Coumarins		
With 2 N NaOH	Yellow	Colorless solution	-
	Phenols		
With alcoholic ferric chloride	Blue to blue black, brown	brown color	+
	Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No effervescence	-
	Resin		
With aqueous acetone	Turbidity	No turbidity	–
	Quinone		
5% NaOH	Pink/purple/red	Colorless solution	–
	Amino acids		
Ninhydrine reagent	Purple color	Colorless solution	–

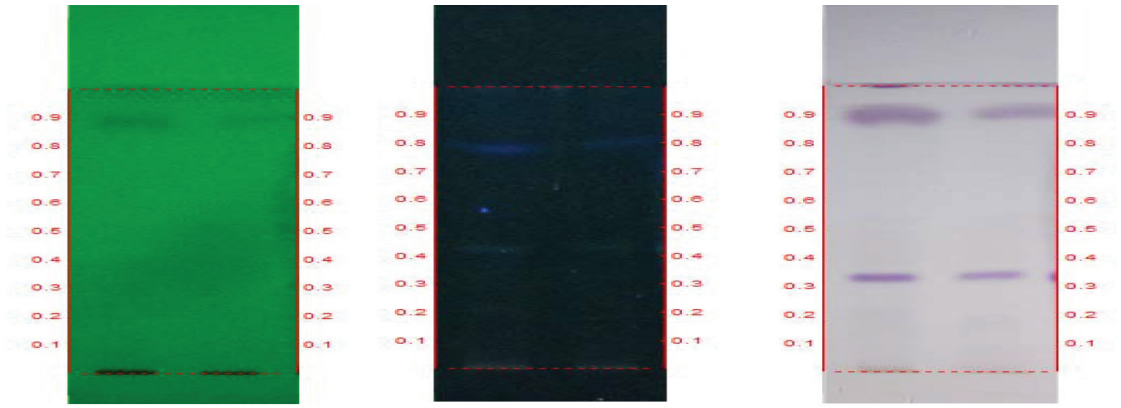
Table 2: R_f values of *Serankottai nei*

254 nm	366 nm	Post derivatisation
-	0.21 (FL. blue)	-
-	-	0.33 (D. purple)
-	0.44 (FL. blue)	-
-	0.80 (FD. blue)	-
0.90 (L. green)	-	0.90 (D. purple)
-	-	-

*L-Light; D-Dark; F-Fluorescent

Table 3: Minimum inhibitory concentration (MIC) of standard drugs and *Serankottai nei* against H37Rv strain

Drug name	Minimum inhibitory concentration (MIC)
Pyrazinamide	3.125 µg/ml
Ciprofloxacin	3.125 µg/ml
Streptomycin	6.25 µg/ml
Serankottai nei	1.6 µg/ml



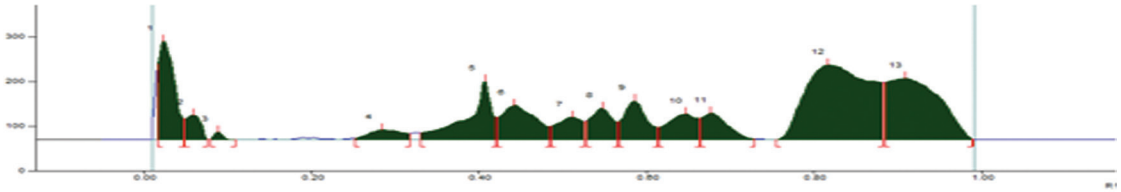
At 254 nm At 366 nm After derivatisation

Track 1-*Semecarpus anacardium*–3 µl

Track 2-*Semecarpus anacardium*–6 µl

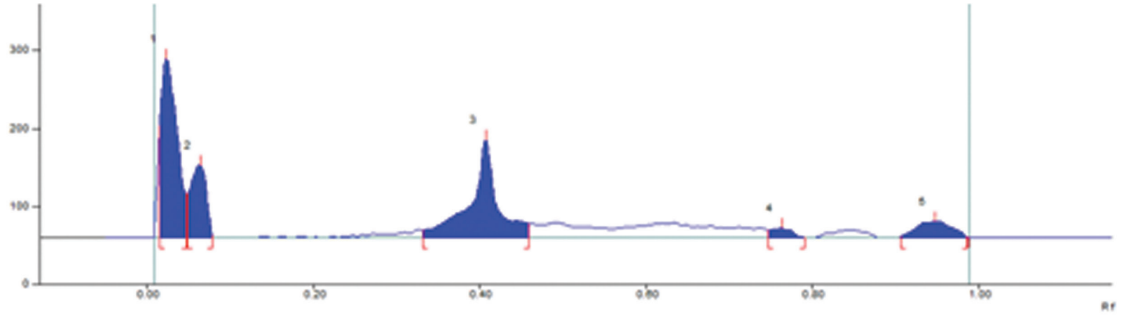
Solvent system: Toluene: Ethyl acetate (9.0:1.0)

Figure 1: HPTLC photo documentation of ethanol extract of *Serankottai nei*.



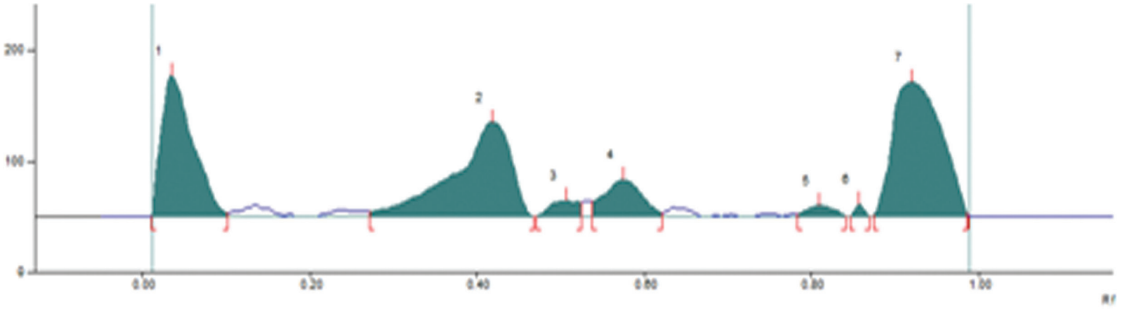
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	168.7 AU	0.02 Rf	219.6 AU	19.12 %	0.05 Rf	46.4 AU	2962.0 AU	9.31 %
2	0.05 Rf	46.5 AU	0.06 Rf	54.8 AU	4.77 %	0.08 Rf	2.1 AU	695.4 AU	2.19 %
3	0.08 Rf	1.8 AU	0.09 Rf	17.2 AU	1.49 %	0.11 Rf	0.0 AU	128.5 AU	0.40 %
4	0.25 Rf	2.8 AU	0.29 Rf	22.4 AU	1.95 %	0.32 Rf	13.2 AU	638.9 AU	2.01 %
5	0.33 Rf	15.6 AU	0.41 Rf	131.0 AU	11.40 %	0.42 Rf	49.1 AU	2668.0 AU	8.39 %
6	0.42 Rf	49.2 AU	0.44 Rf	76.8 AU	6.69 %	0.49 Rf	28.8 AU	2253.7 AU	7.09 %
7	0.49 Rf	29.1 AU	0.51 Rf	50.3 AU	4.38 %	0.53 Rf	40.9 AU	1099.7 AU	3.46 %
8	0.53 Rf	41.9 AU	0.55 Rf	69.7 AU	6.07 %	0.57 Rf	39.5 AU	1317.4 AU	4.14 %
9	0.57 Rf	39.9 AU	0.59 Rf	86.6 AU	7.54 %	0.61 Rf	27.2 AU	1707.6 AU	5.37 %
10	0.62 Rf	27.4 AU	0.65 Rf	57.5 AU	5.01 %	0.66 Rf	47.4 AU	1433.7 AU	4.51 %
11	0.67 Rf	47.8 AU	0.68 Rf	59.3 AU	5.16 %	0.73 Rf	0.9 AU	1287.4 AU	4.05 %
12	0.76 Rf	0.4 AU	0.82 Rf	166.9 AU	14.53 %	0.88 Rf	27.2 AU	9344.4 AU	29.38 %
13	0.89 Rf	127.4 AU	0.91 Rf	136.5 AU	11.88 %	0.99 Rf	1.8 AU	6271.8 AU	19.72 %

Figure 2: Densitometric scan of *Serankottai nei* (6 µl) at 254 nm.



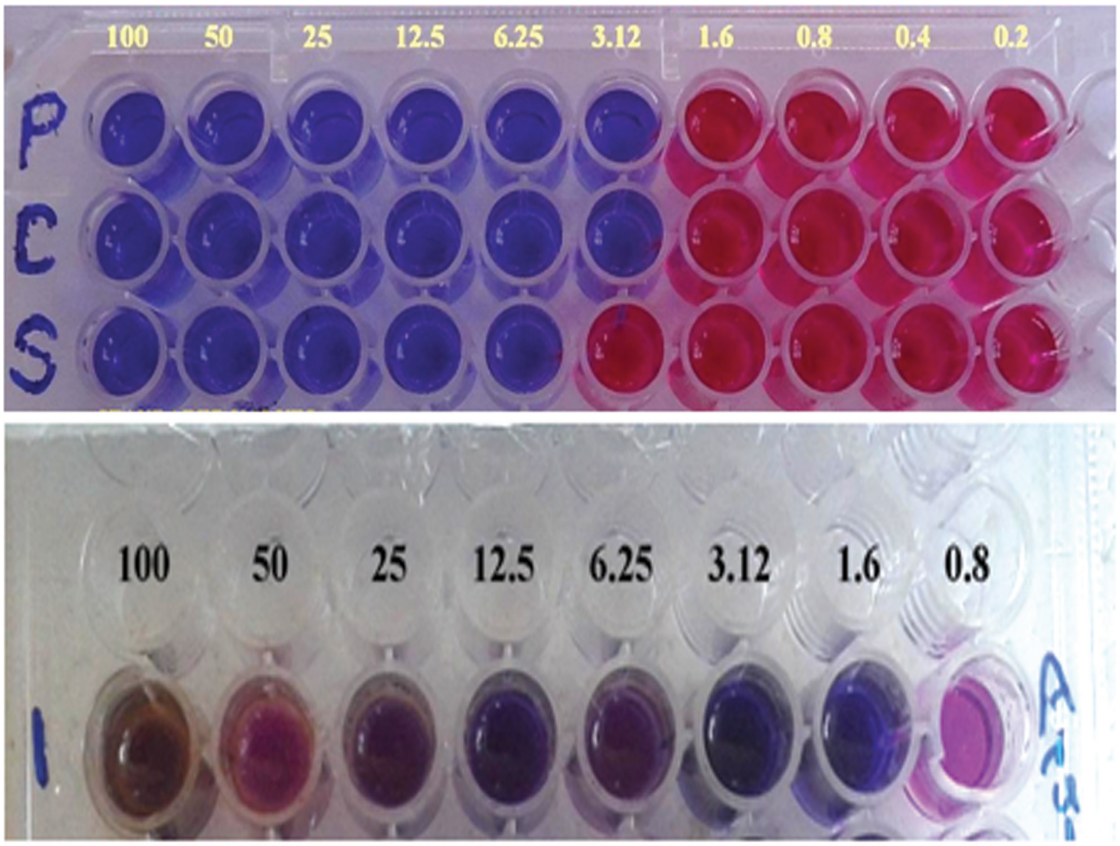
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	143.2 AU	0.02 Rf	229.4 AU	47.72 %	0.05 Rf	55.8 AU	3187.8 AU	39.27 %
2	0.05 Rf	57.4 AU	0.06 Rf	93.0 AU	19.34 %	0.08 Rf	2.7 AU	1223.9 AU	15.08 %
3	0.33 Rf	9.7 AU	0.41 Rf	125.6 AU	26.12 %	0.46 Rf	18.1 AU	2854.5 AU	35.16 %
4	0.75 Rf	9.7 AU	0.76 Rf	11.8 AU	2.46 %	0.79 Rf	0.4 AU	213.9 AU	2.63 %
5	0.91 Rf	1.7 AU	0.95 Rf	21.0 AU	4.36 %	0.99 Rf	0.7 AU	638.1 AU	7.86 %

Figure 3: Densitometric scan of *Serankottai nei* (6 µl) at 366 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.04 Rf	127.6 AU	31.54 %	0.10 Rf	3.2 AU	3487.6 AU	23.78 %
2	0.27 Rf	4.9 AU	0.42 Rf	85.7 AU	21.17 %	0.47 Rf	0.0 AU	4210.2 AU	28.71 %
3	0.47 Rf	0.2 AU	0.51 Rf	14.7 AU	3.62 %	0.53 Rf	13.0 AU	369.4 AU	2.52 %
4	0.54 Rf	14.0 AU	0.58 Rf	33.4 AU	8.25 %	0.62 Rf	3.9 AU	1103.8 AU	7.53 %
5	0.78 Rf	2.2 AU	0.81 Rf	10.3 AU	2.54 %	0.84 Rf	0.3 AU	241.9 AU	1.65 %
6	0.85 Rf	0.2 AU	0.86 Rf	11.6 AU	2.86 %	0.87 Rf	0.3 AU	92.5 AU	0.63 %
7	0.88 Rf	1.2 AU	0.92 Rf	121.5 AU	30.03 %	0.99 Rf	4.1 AU	5160.9 AU	35.19 %

Figure 4: Densitometric scan of *Serankottai nei* (6 µl) at 620 nm.



P–pyrazinamide, C-ciprofloxacin, S–streptomycin, 1–*Serankottainei*

Figure 5: Antitubercular screening of *Serankottai nei* by Alamar Blue assayagainst H37Rv strain.

RESULTS

Preliminary phytochemical tests

We obtained 8% w/w USM from *serankottai nei*, which showed presence of alkaloid, steroid, terpenoid and phenol (Table 1).

High Performance Thin Layer Chromatography

On photo documentation at 254 nm, 366 nm and under white light (post derivatization with vanillin sulphuric acid), *serankottai nei* showed one spot (R_f0.90), three spots (R_f0.21, 0.44, 0.80) and two spots (R_f0.33, 0.90) respectively (Figure 1, Table 2).

On densitometric scan at 254 nm, *serankottai nei* showed 13 peaks; peak with R_f 0.76 and 0.89 being the major spots contributing to 29.38% and 19.72% area (Figure 2). At 366 nm, drug showed 5 peaks; peaks with R_f 0.02, 0.33 and 0.05 being the major spots of 39.27%, 35.16% and 15.08% area (Figure 3). At 620 nm, drug showed 7 peaks; peak with R_f 0.88, 0.27 and 0.01 being the major peak with 35.19%, 28.71% and 23.78% area (Figure 4). Thus, this protocol could be useful for fingerprinting the *serankottai nei.*

In-vitro anti tubercular activity

Different doses of USM of *serankottai nei* were screened for *in-vitro* antitubercular activity against H37Rv strain using Alamar Blue Dye method. The Minimum Inhibitory Concentration (MIC) of pyrazinamide, streptomycin, ciprofloxacin were 3.125, 6.25 and 3.125 µg/ml respectively. Whereas, the MIC of *serankottai nei* was 1.6 µg/ml, which was almost 25 to 50% of standard drugs. (Figure 5 and Table 3).

DISCUSSION

The preliminary analyses of chemical composition are one of the best methods to analyse quality of herbal formulations. The testsshowed presence of alkaloid, phenol, steroid and terpenoid. The HPTLC finger print

profile of *serankottai nei* was been obtained with suitable solvent system. Based on our study, HPTLC fingerprinting is an effective technique of screening the same herbal formulation for authenticity and quality.

Previous study on water extract of *Semicarpus anacardium* has shown promising antitubercular activity.[14] Our study also showed that the milk extract of *Semicarpus anacardium* (*serankottai nei*) has the potent antitubercular activity. Thus, there is rationale for using *serankottai nei* in the treatment of tuberculosis by Siddha physicians.

Serankottai nei has shown promising antitubercular activity in *in-vitro* study. Further studies should be focused on isolation of compound responsible for antitubercular activity and its molecular mechanism.

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AUTHORS CONTRIBUTION

Senthilvel G has conceived the idea, designed the study protocol, involved in writing the article and coordinated the entire study. Amuthan A and Kumar KNS were involved in designing the study protocol, phytochemical standardization and writing the article.

REFERENCES

1. Mudhaliayr KNK, Utthamarayan KS. Siddha Vaiddyathirattu (in Tamil). Directorate of Indian Medicine and Homeopathy, Chennai: India. 1996.p.256.
2. Premalatha B, Muthulakshmi V, Vijayalakshmi T, Sachdanandam P. Protective role of *serankottai nei*, a siddha preparation, on cell membranes, in aflatoxin b1 induced hepatocellular carcinoma bearing rats. Indian Drugs. 1997;34(7):384-9.
3. Dhanasekaran S, Palanivelu S, Panchanatham S. Restoration of energy metabolism in leukemic mice treated by a siddha drug-Semecarpus anacardium Linn. nut milk extract. Chemico-Biological Interactions. 2008;173(1):43-58.
4. Rajendran M, Palanivelu S, Panchanadam S. Efficacy of Siddha formulation Kalpaamruthaa in ameliorating joint destruction in rheumatoid arthritis in rats. Chemico-Biological Interactions. 2008;176(2-3):243-51.
5. Premalatha B, Sachdanandam P. Semecarpus anacardium L. nut extract administration induces the *in vivo* antioxidant defence system in aflatoxin B1 mediated hepatocellular carcinoma. J Ethnopharmacol. 1999;66(2):131-9.
6. Premalatha B, Sachdanandam P. Potency of Semecarpus anacardium Linn. nut milk extract against aflatoxin B(1)-induced hepatocarcinogenesis: reflection on microsomal biotransformation enzymes. Pharmacol Res. 2000;42(2):161-6.
7. Vijayalakshmi T, Muthulakshmi V, Sachdanandam P. Toxic studies on biochemical parameters carried out in rats with *Serankottai nei*, a siddha drug-milk extract of Semecarpus anacardium nut. J Ethnopharmacol. 2000;69(1):9-15.
8. The Ayurvedic Pharmacopoeia of India. 1st ed., Part I, Vol VI. Govt. of India, Ministry of Health and Family Welfare, Dept. of AYUSH, New Delhi, 2008, p. 233-91.
9. Brain KR, Turner T. The practical evaluation of Phytopharmaceuticals. Bristol: Wright-Scientechnica; 1975; p.10-12.
10. Harborne JB. Method of extraction and isolation in Phytochemical methods. 2nd ed., London: Chapman and Hall; 1998; p.60-66.
11. Sethi PD. High Performance Thin Layer Chromatography. 1st ed., New Delhi: CBS Publishers and Distributors; 1996; p.1-56.
12. Stahl I. Thin layer Chromatography, a laboratory hand book. Berlin: Springer-Verlag; 1969; 127(8):p.52-86.
13. Rodrigues FAR, Oliveira ACA, Cavalcanti BC, Pessoa C, Pinheiro AC, de Souza MVN. Biological Evaluation of Isoniazid Derivatives as an Anticancer Class. *Scientia Pharmaceutica*. 2014;82(1):21-8.
14. Singh R, Kakkar A, Mishra VK. Anti-tuberculosis activity and GC-MS analysis of water extract of *Semecarpus anacardium* nuts. Der Pharma Chemica. 2015;7(2):278-85.