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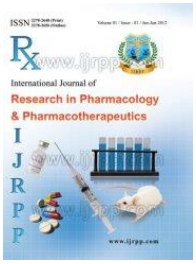

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Standardization of siddha formulation 'Pereechangai Nei' – medicated ghee of date fruit

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ABSTRACT

Drug standardization of herbal formulations is essential for their acceptance in this scientific medical world. In this article Physicochemical, Phytochemical and other standardization methods of one such polyherbal medicated ghee *Pereechangai nei* are discussed. *Pereechangai nei* has indication for *Madhumegam* as per siddha text *Therayar Maha Karisal*. The drug is prepared as per the method mentioned in the Siddha literature. The organoleptic characteristics, pH, Iodine number, Saponification value, Peroxidase value were studied. Physicochemical evaluation; ash values, namely total ash, acid-insoluble ash, water-soluble ash, alcohol soluble extractive value, and loss on drying were determined. Preliminary phytochemical screening was done for the presence of carbohydrates, proteins, flavonoids, saponins, diterpines, fat and fixed oils, HPTLC finger printing, Heavy metal analysis, Pesticide residues, Microbial and specific pathogen load ,Aflotoxin contamination were studied as per the Pharmacopial laboratory standards of Indian medicine. In results, it was found that the specified drug is containing various phytochemicals and is free from microbial contamination, Aflotoxin and pesticide residues. The heavy metals such as Arsenic, Mercury, Cadmium and Lead are not detected.

Keywords: Physicochemical analysis, Siddha, HPTLC, *Pereechangai nei.*

INTRODUCTION

Since ancient times medicinal plants are considered to be the most important source of therapeutic remedies for various ailments to mankind [1].The World Health Organization (WHO) estimated that about majority of human population depend on traditional herbal medicines for their primary health care needs [2]. Hence, here comes the importance of standardization of drugs to exhibit conformation of

its identity and determination of its purity, safety, potency and efficacy for safer practice [3].The Standardization of any drug is done by stepwise quality control methods as prescribed by Pharmacopial laboratory standards of Indian medicine [4].

Siddha pharmacopeia has given preparations in various forms as *Chooranam* (powder), *Maathirai*(tablet), *Manapagu* (syrup), *Nei*(medicated ghee) for the treatment of different diseases.Here the herbal medicated ghee, which is lipid based formulation has the ability to cross blood-brain barrier thereby having beneficial effects on the brain [5]. Also drugs prepared with ghee are easily digested and quickly absorbed. The lipid solubulized drugs are quickly distributed in the intra and extracellular spaces in our body [6].

Pereechangai Nei is one such Siddha polyherbal medicated ghee chosen from the text *Therayar Maha Karisal* is indicated for *Madhumegam* (Diabetes mellitus) [7]. Till date, no standards are available for *Pereechangai nei*. Hence, the current study has been carried out to assess its Physico-chemical, Phyto-chemical and other standardization parameters as a part of their scientific validiation.

MATERIALS AND METHODS

Identification of raw drugs

The herbal ingrediants were authenticated from Botanist, National Institute of Siddha, Tambaram sanatorium, Chennai.

Ingredients of Pereechangai nei: (Medicated ghee of Date fruit)

1.Pereechangai (Phonex dactilifera, Linn), 2.Peraamutti (Pavonia odorata,.Willd), 3.Kodiveli (Plumbago zeylanica,.Linn), 4.Peipudal (Trichosanthus cucumerina, Linn), 5.Nannari (Hemidesmus indicus,.R.Br), 6.Sirupeelai (Aerva lanata,.Linn), 7.Kondrai (Cassia fistula, Linn) , 8.Senbagam (Michelia champaca, Linn), 9.Balli poondu (Strigus lutea, Linn), 10.Chukku (Zingiber officinale,Rosc), 11.Milagu (Piper nigrum,Linn) , 12.Thippili (Piper longum, Linn), 13.Yelam (Elettaria cardamomum, Maton), 14. Lavangam (Syzygium aromaticum, Linn), 15.Pasu Nei (Cow's ghee)[7].

Method of Purification

Purification of raw drugs were done as per the methods given in Siddha text *Sigichaa rathna deepam* [8].

Method of Drug Preparation

Pereechangai Nei was prepared according to the procedure mentioned in Siddha classical text *Therayar Mahakarisal* [7].

ANALYTICAL STUDY

The organoleptic characteristics, pH, Iodine number, Saponification value, Peroxidase value Heavy metal analysis (such as Arsenic, Mercury, Cadmium and Lead) , Pesticide residues were studied as per standard operation procedures at VS clinical research & hospitals, Taramani, Chennai. Physicochemical evaluation; ash values, alcohol soluble extractive value, loss on drying, and Preliminary phytochemical screening: for the detection of carbohydrates, proteins, flavonoids, saponins, diterpines, fat and fixed oils, were studied at The Tamil Nadu Dr. MGR Medical University, Anna Salai, Guindy, Chennai-600032.

HPTLC finger printing, Microbial and specific pathogen load, Aflotoxin contamination were studied as per the WHO standards at in Regional Research Institute of Unani Medicine (RRIUM), Royapuram, Chennai-600013[11]. The results found were discussed below.

Organoleptic characteristics

The sensory characters of the drug were carefully noted and the interpretation illustrated in Table 1.

Colour

The *Pereechangai nei* was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour

The *Pereechangai nei* was smelled twice individually with an interval of 2 minutes.

Taste

Small amount of *Pereechangai nei* was kept over the tip of the tongue.

Table 1. Organoleptic Parameters of *Pereechangai Nei*

1.	Colour	Yellow
2.	Odour	Characteristic
3.	Taste	Sour
4.	Touch	Sticky
5.	Appearance	Turbid

PHYSICO CHEMICAL ANALYSIS

Loss On Drying

An accurately weighed 1g of *Pereechangai nei* was taken in a glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

Determination of total ash

Weighed accurately 2g of *Pereechangai nei* was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

Determination of acid insoluble ash

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of water soluble ash

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash was determined by drying the filtrate.

Determination of alcohol soluble extractive

1 gm. of air dried drugs, coarsely powdered *Pereechangai nei* was macerated with 20 ml. alcohol in closed flask for 24 hrs. With frequent shaking. It was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Determination of pH

Sample being oily in nature the direct litmus evaluation method was adopted to check the pH of the sample.

Determination of Iodine value

About 20 gm of oil 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. The 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(weight equivalent to oil) 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. 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 Peroxide Value

5 g of substance was weighed accurately into a 250ml glass-stoppered conical flask and allowed to dissolve completely in 30 ml mixture, 3 volumes of glacial acetic acid and 2 volumes of chloroform. To this 0.5ml saturated potassium iodide solution was added, mixed properly and kept for 1 minute with occasional shaking. Later after adding 30ml of

distilled water it was titrated against 0.01M sodium thiosulphate until the yellow colour almost disappears. Titration process was continued on addition of 0.5ml starch solution and the titrant volume required for the disappearance of blue colour was noted and labeled as 'a'. Similarly volume required for the same operation omitting the substance being examined labeled as'b'. In blank determination the volume of 0.01M sodium thiosulphate was carefully chosen such that it should be within0.1ml.

Peroxide value was calculated using the following formula:

Peroxide value = 10 (a – b)/w [where w = weight of the substance in g]

Table 2: Physicochemical interpretation results

S.no	Parameters	Percentage
1	Loss on drying	0%
2	Total ash value	0.9%
3	Acid insoluble ash	0.5%
4	Water soluble ash	0.45%
5	Alcohol soluble extraction	18%

Table 3: Interpretation of results

S.No	Specific Test	Values
1.	pH	6
2.	Refractive index	1.46
3.	Iodine value (mg I2/g)	103
4.	Peroxide value (Meq/kg)	10
5.	Saponification Value (mg of KOH to saponify 1gm of fat).	207

PHYTOCHEMICAL ANALYSIS

Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

Detection of Saponins

Froth Test

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of Flavonoids

Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Detection of Proteins

Xanthoprotein Test

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

Detection of Diterpenes

Copper Acetate Test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Test for Fixed oils and Fats

Spot test

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Table 4: Phytochemical interpretation of results:

S.No.	Phytochemicals	Test Name	H2O Extract
1	Carbohydrates	Molisch's Test	+ve
2	Saponin	Froth Test	+ve
3	Flavonoids	Alkaline Reagent Test	+ve
4	Proteins	Xanthoprotein Test	+ve
5	Diterpenes	Copper Acetate Test	+ve
6	Fat & Fixed Oil	Spot Test	+ve

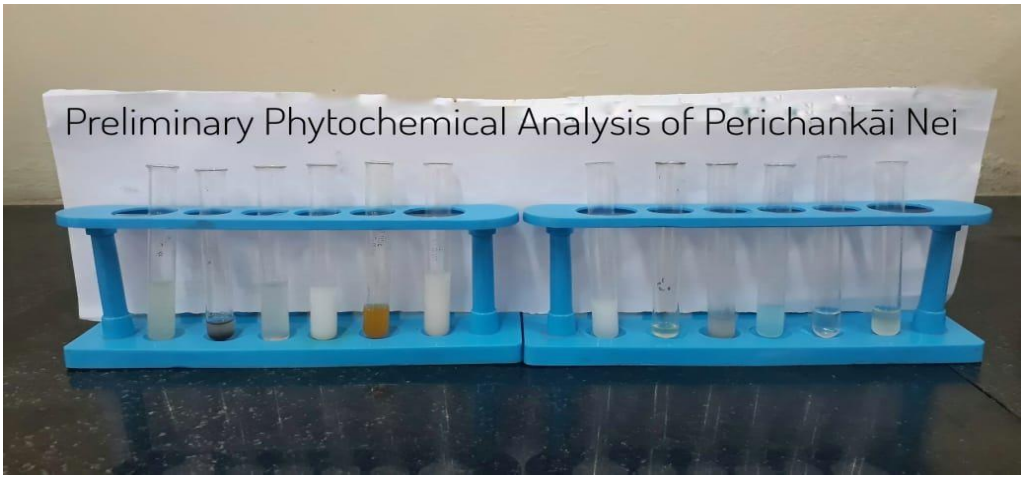


Figure: 1

TLC/HPTLC analysis

The procedures recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996.

Instrument Details

Name of the Instrument	: CAMAG (CAMAG - Automatic TLC sampler, Scanner and Visualiser)
Spray Gas	: N2
Lamp used	: Deuterium and Tungsten Lamp

The sample was applied for the Thin Layer Chromatography and High Performance Thin Layer Chromatography study with suitable solvent systems. After development the plate was allowed to dry in air and examined under UV – 254nm, 366nm and visible light after derivatised using vanillin – sulphuric acid.

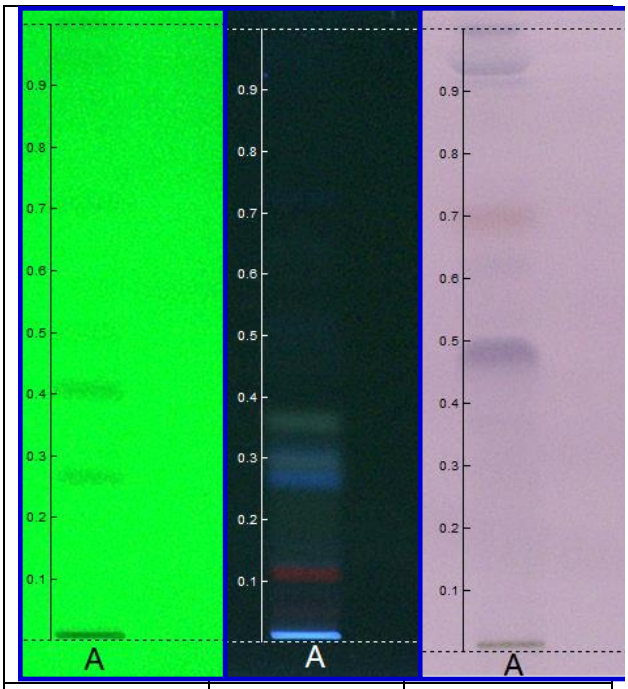
HPTLC finger print of *Pereechangai Nei* in Methanol Extract

TLC plate was developed using Toluene: Ethyl acetate: Formic acid (8.2: 1.8: 0.1) as mobile phase. After development allow the plate to dry in air, record the finger print and densitometric chromatogram of the two batch samples of the single compound scanned at 254 and 366 nm.

The results of HPTLC fingerprint of *Pereechangai nei* in 254nm UV (figure.4) shows that the peak correspond to the Rf values 0.24 has maximum peak area of 11444.9 Au. This peak (area % is 65.18%) could serve as a marker.

Results of HPTLC fingerprint of *Pereechangai nei* in 366nm UV (figure.5) shows the peak correspond to the Rf values 0.24 has maximum peak area of 5705.4 Au. This peak (area % is 29.95%) could serve as a marker, which can be responsible for expression of its Pharmacological and clinical actions

Methanol Extract



UV – 254 nm	UV – 366 nm	V - S Reagent
Solvent System: Toluene : Ethyl acetate : Formic acid (8.2:1.8:0.1)		
Track 1: 5 µl; Track 2: 10 µl		

Figure 2.Thin Layer Chromatography

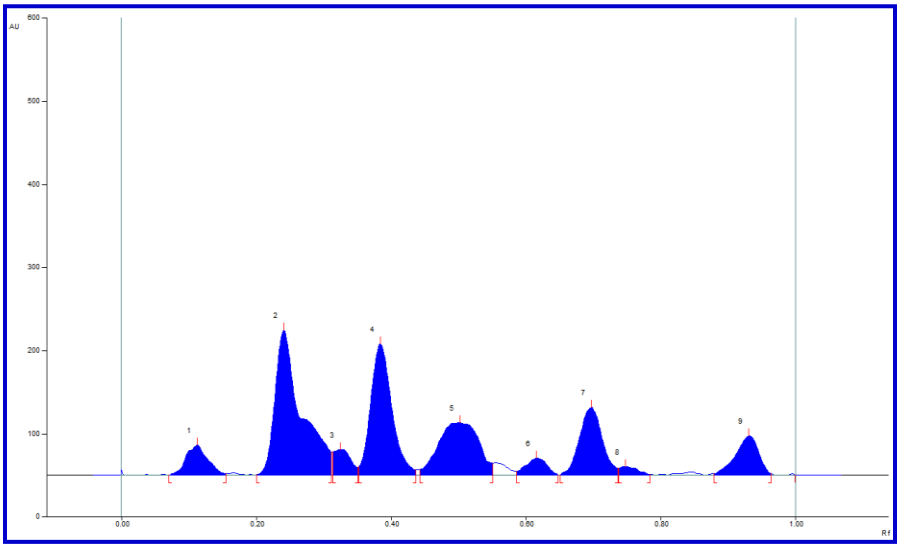


Figure 3.HPTLC finger print of methanol extract at 254 nm

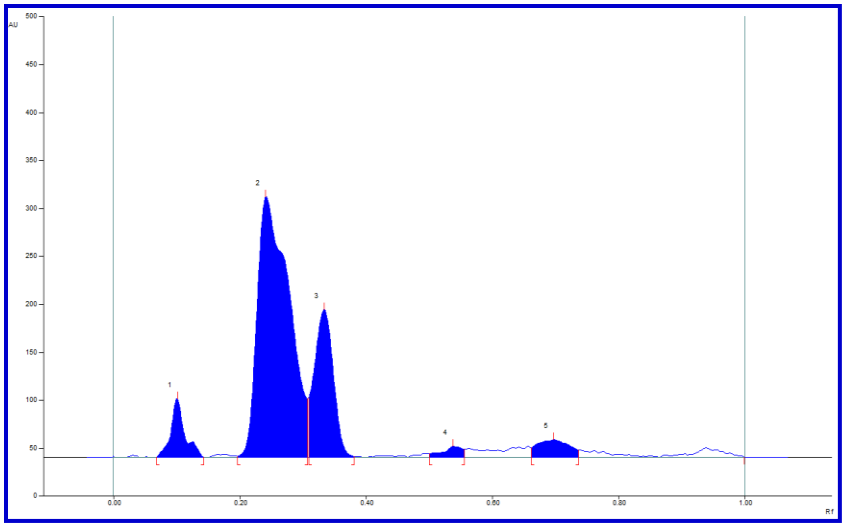


Figure 4.HPTLC finger print of methanol extract at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.2 AU	0.10 Rf	61.2 AU	11.82 %	0.14 Rf	0.3 AU	1121.0 AU	6.38 %
2	0.20 Rf	1.1 AU	0.24 Rf	271.9 AU	52.53 %	0.31 Rf	60.9 AU	11444.9 AU	65.18 %
3	0.31 Rf	61.8 AU	0.33 Rf	154.4 AU	29.82 %	0.38 Rf	0.9 AU	3906.9 AU	22.25 %
4	0.50 Rf	3.9 AU	0.54 Rf	11.6 AU	2.24 %	0.56 Rf	8.6 AU	302.1 AU	1.72 %
5	0.66 Rf	9.9 AU	0.70 Rf	18.6 AU	3.59 %	0.74 Rf	7.7 AU	783.0 AU	4.46 %

R_f values of methanol extract at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.1 AU	0.11 Rf	36.3 AU	5.85 %	0.16 Rf	2.0 AU	968.3 AU	5.08 %
2	0.20 Rf	0.6 AU	0.24 Rf	174.5 AU	28.08 %	0.31 Rf	27.8 AU	5705.4 AU	29.95 %
3	0.31 Rf	28.0 AU	0.32 Rf	31.1 AU	5.00 %	0.35 Rf	9.6 AU	667.3 AU	3.50 %
4	0.35 Rf	10.1 AU	0.38 Rf	157.6 AU	25.36 %	0.44 Rf	6.5 AU	4133.6 AU	21.70 %
5	0.44 Rf	6.9 AU	0.50 Rf	63.0 AU	10.13 %	0.55 Rf	14.3 AU	3169.5 AU	16.64 %
6	0.59 Rf	4.6 AU	0.62 Rf	20.4 AU	3.29 %	0.65 Rf	0.3 AU	524.8 AU	2.76 %
7	0.65 Rf	0.1 AU	0.70 Rf	81.3 AU	13.08 %	0.74 Rf	8.4 AU	2295.2 AU	12.05 %
8	0.74 Rf	8.4 AU	0.75 Rf	10.2 AU	1.65 %	0.78 Rf	0.6 AU	229.1 AU	1.20 %
9	0.88 Rf	1.4 AU	0.93 Rf	47.0 AU	7.56 %	0.97 Rf	1.6 AU	1353.6 AU	7.11 %

R_f values of methanol extract at 366 nm

Microbial load

The procedures recommended for analysis of microbial load as per the guideline (WHO, 2007). 10 g or 10 ml of the preparation being examined was homogenized with 5 g of polysorbate 20 or polysorbate 80. Based on the nature of drug taken heat was applied at a temperature not more than 40ºC and mixed carefully without changing the

temperature. Later 85 ml of buffered sodium chloride-peptone solution pH 7.0 or any other suitable medium with no antimicrobial activity under the conditions of the test was added after raising its temperature to not more than 40º and maintained for the shortest time necessary for formation of an emulsion and in any case for not more than 30 minutes. Based on the test condition pH was also adjusted to about 7.

For bacteria

For determining bacterial count petri dishes 9 to 10 cm in diameter was plated with 15 ml of liquefied casein soya bean digest agar at not more than 45º along with1 ml of the pretreated preparation. Alternatively it was also spread plated with the pretreated preparation on the surface of solidified medium in a Petri dish of the same diameter. Based on the nature of drug the preparation was diluted so that a colony count of not more than 300 could be expected. Two petri plates were plated at same dilution and incubated at 30º to 35º for 5 days until a more reliable count was obtained in a shorter time. After incubation the results were chosen from the plate with greatest number of colonies but not more than 300 colonies.

For fungi

For determining fungal count petri dishes 9 to 10 cm in diameter was plated with 15 ml of Sabouraud dextrose agar with antibiotics at not more than 45º along with 1 ml of the pretreated preparation. Alternatively it was spread plated with the same on the surface of solidified medium in a petri dish of same diameter. Based on the nature of drug, preparation was diluted so that a colony count of not more than 300 could be expected. Two petri plates were plated at same dilution and incubated at 20º to 25º for 5 days until a more reliable count was obtained in a shorter time. After incubation the results were chosen from the plate with greatest number of colonies but not more than 100

Table 5: Interpretation of Microbial load results

S. No.	Parameters	Reference Limits as per WHO (2007)	Results	Remarks
1	Total Bacterial Count (TBC)	10^5CFU/gm	$2x10^2$ cfu/gram	Within permissible limits
2	Total Fungal Count (TFC)	10^3CFU/gm	Absent	
3	Enterobacteriaceae	10^3	Absent	
4	*Escherichia coli*	10	Absent	
5	Salmonella Spp	Absent	Absent	
6	*Staphylococcus aureus*	Absent	Absent	

Test for Aflatoxin

The procedures recommended for the detection of Aflatoxin as per WHO (2007).

Instrument Details:

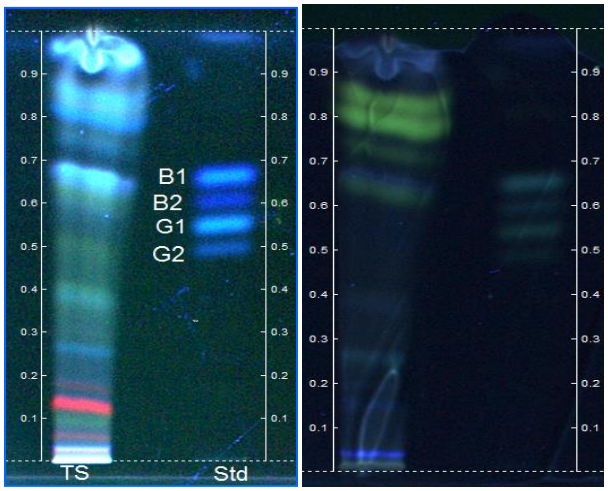
Name of the Instrument	: CAMAG (CAMAG - Automatic TLC sampler, Scanner and Visualiser)
Spray Gas	: N2
Lamp used	: Mercury (flourescent mode)

The samples were processed as per procedures recommended in WHO 2007 and applied for the Thin Layer Chromatography and High Performance Thin Layer Chromatography study with suitable solvent systems. After development the plate was allowed to dry in air and examined under UV 366nm. (figure 5)

The Standard (Std 20 µl) and the *Pereechangai*(TS) - 15µl were applied on TLC aluminium sheet silica gel 60 F 254 (E.MERCK) and plate was developed using the solvent system Chloroform : Acetone: Water (14 : 2 : 0.2).After development the plate was allowed to dry in air and examined under UV 366 nm.(figure 6)

Table 6: Interpretation of Aflotoxin results

S.No	Test Parameters	Results
1.	Aflotoxin B1	Absent
2.	Aflotoxin B2	Absent
3.	Aflotoxin G1	Absent
4.	Aflotoxin G2	Absent



A **B**

Figure 5. Pereechangai nei

A: Test sample: 20µl; Standard: 15 µl

B: Test sample: 20µl; Standard: 15 µl (isopropyl alcohol dipped)

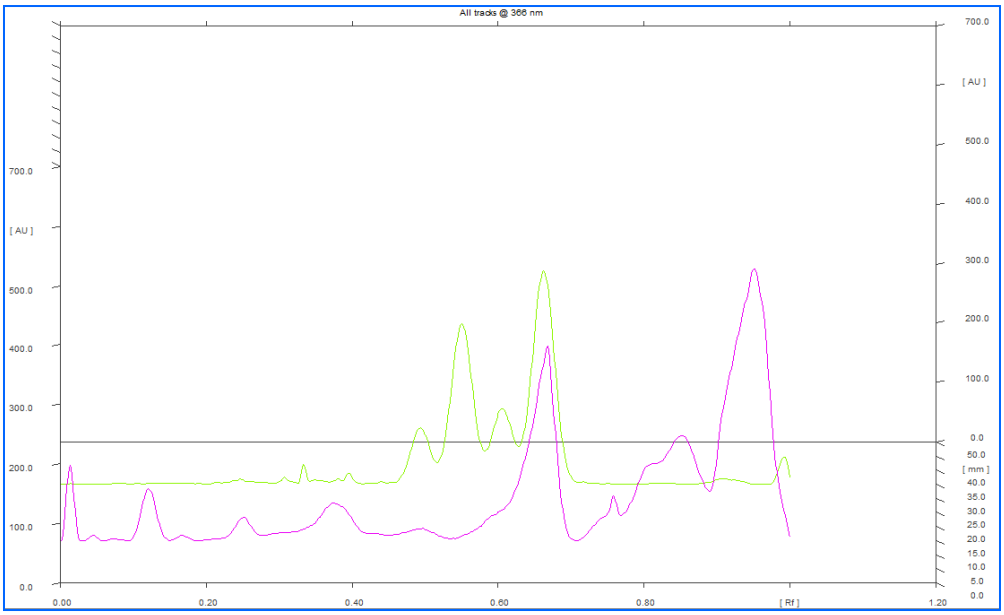


Figure 6. HPTLC Densitometric chromatogram (366nm)

Test sample (TS) : Pereechangai Nei ; Standard (S) – G2, G1, B2 & B1

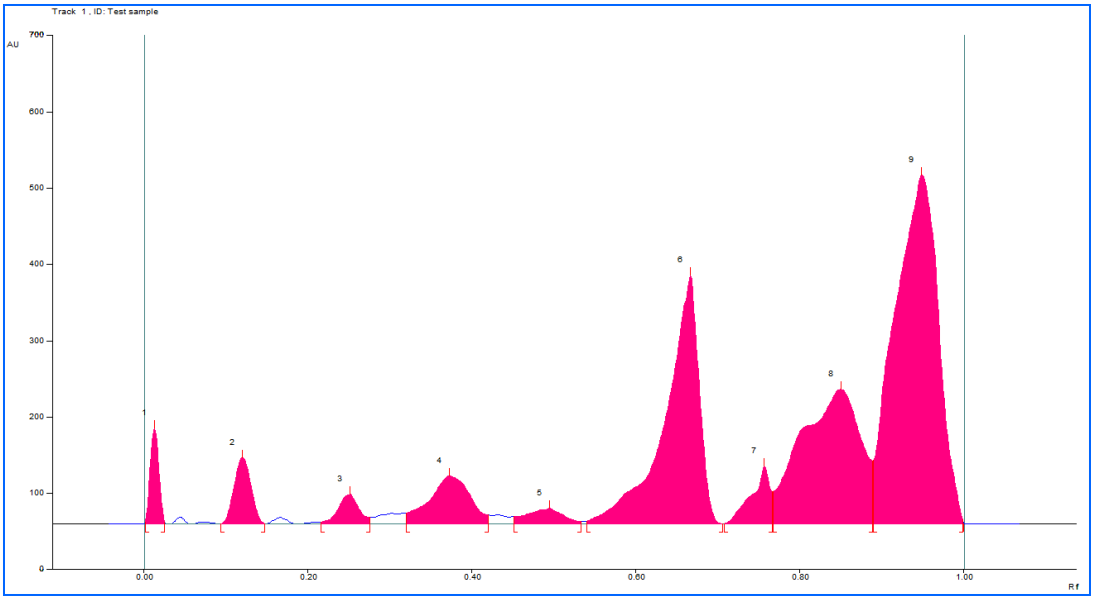


Figure 7. HPTLC finger print of Sample (TS) : Pereechangai nei at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.9 AU	0.01 Rf	125.8 AU	9.18 %	0.03 Rf	1.7 AU	1095.6 AU	2.18 %
2	0.09 Rf	0.0 AU	0.12 Rf	86.5 AU	6.32 %	0.15 Rf	0.3 AU	1486.8 AU	2.96 %
3	0.22 Rf	2.5 AU	0.25 Rf	38.5 AU	2.81 %	0.28 Rf	8.5 AU	811.4 AU	1.62 %
4	0.32 Rf	14.2 AU	0.37 Rf	62.7 AU	4.58 %	0.42 Rf	11.4 AU	2598.7 AU	5.17 %
5	0.45 Rf	9.2 AU	0.50 Rf	20.3 AU	1.48 %	0.53 Rf	3.4 AU	743.7 AU	1.48 %
6	0.54 Rf	3.4 AU	0.67 Rf	326.5 AU	23.83 %	0.71 Rf	0.1 AU	11131.5 AU	22.16 %
7	0.71 Rf	0.2 AU	0.76 Rf	75.7 AU	5.53 %	0.77 Rf	42.0 AU	1409.6 AU	2.81 %
8	0.77 Rf	42.3 AU	0.85 Rf	176.2 AU	12.86 %	0.89 Rf	82.9 AU	10644.0 AU	21.19 %
9	0.89 Rf	83.9 AU	0.95 Rf	457.6 AU	33.41 %	1.00 Rf	5.8 AU	20304.0 AU	40.43 %

Rf values of Pereechangai nei (TS) at 366nm

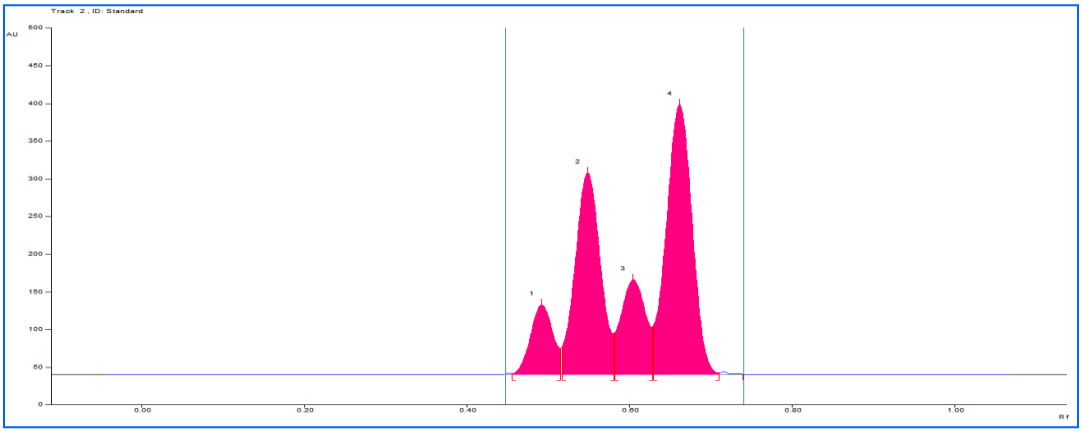


Figure 8. HPTLC finger print of Standard (S) at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.46 Rf	1.5 AU	0.49 Rf	92.9 AU	10.99 %	0.52 Rf	34.9 AU	2146.0 AU	9.87 %
2	0.52 Rf	36.2 AU	0.55 Rf	268.1 AU	31.71 %	0.58 Rf	54.4 AU	6949.3 AU	31.95 %
3	0.58 Rf	55.6 AU	0.61 Rf	125.9 AU	14.89 %	0.63 Rf	62.9 AU	3173.1 AU	14.59 %
4	0.63 Rf	64.4 AU	0.66 Rf	358.6 AU	42.41 %	0.71 Rf	2.0 AU	9481.1 AU	43.59 %

Rf values of Standard (S) at 366nm

Determination of Heavy metal analysis

Heavy metals may present in crude drugs through transmission from soil and atmospheric pollutions. But presence of these heavy metal residues in medicines above its permissible limits as prescribed by WHO/FDA guidelines are associated with serious adverse effects. Hence presence of heavy metals are need to be detected in these formulations as a part of drug standardization. The results of *Pereechangai nei* are given below

Table 7: Interpretation of Heavy metal analysis results

S.No	Test Parameters	Result	Unit
1	Arsenic (as As)	BLQ (LOQ:0.01)	mg/kg
2	Mercury (as Hg)	0.16 (BLQ)	mg/kg
3	Lead (as Pb)	BLQ (LOQ:0.08)	mg/kg
4	Cadmium (as Cd)	0.24 (BLQ)	mg/kg

Note: BLQ: Below Limit of Quantification; **LOQ**: Limit of Quantification.

Determination of Pesticide residues

For analysis reagents without any external components were chosen and the samples were analyzed using Gas chromatographic methods. Later the amount of different components such as organ phosphorus, organ chlorine and pyrethroid contents was recorded.

Table 8: Interpretation of Pesticide residue results

S.No	Parameters	Units	Results
1.	Organo Chlorine Pesticide(OCPS)	mg/kg	BDL(DL:0.1)
2.	OrganoPhosphorousPesticides(OPPs)	mg/kg	BDL(DL:0.1)
3.	Pyrethroids	mg/kg	BDL(DL:0.1)

Note: BDL: Below Dedectable Limit

DISCUSSION AND CONCLUSION

In the current scenario drug standardization is vital for even centuries old traditional Siddha formulations for its global acclimatization. Evaluation of parameters such as ash value, loss on drying, peroxide value, saponification, iodine values, HPTLC studies are determined, which signifies standard parameters to ensure the purity and quality of the drug. The phytochemical findings of the study confirms the presence carbohydrate, protein, saponins, flavonoids and diterpenoids has anti inflammatory, anti oxidant property with effective anti diabetic potential. The present study concludes that standardization of *Pereechangai nei* have exhibited significant results. With further therapeutical studies an effective herbal drugs can be developed for diabetes which is less expensive, less time consuming and more economic friendly than allopathic drugs is possible.

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Conflict of interest

Author declares none.

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