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STANDARDIZATION OF THANNEERVITTAN NEI BY PHYSICOCHEMICAL, PHYTOCHEMICAL AND HPTLC ANALYSIS

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ABSTRACT

Thanneervittan Nei is a traditional *Siddha* herbal medicated ghee used for the treatment of gynecological disorders, especially Menorrhagia. The present study was carried out to evaluate the physicochemical properties, phytochemical constituents, and HPTLC profile of the formulation. Physicochemical parameters such as pH, specific gravity, acid value, peroxide value, and saponification value were analysed using standard methods. Preliminary phytochemical screening was performed to identify the presence of active compounds. HPTLC fingerprint analysis was also carried out for standardization of the formulation. The study revealed the presence of important phytoconstituents such as flavonoids, tannins, phenols, alkaloids, and terpenoids. The HPTLC profile showed distinct bands indicating the presence of various bioactive compounds. The results of the study help in the quality evaluation and standardization of *Thanneervittan Nei*.

KEYWORDS: *Thanneervittan Nei*, Menorrhagia, Physicochemical, Phytochemical, HPTLC

INTRODUCTION

Siddha Medicine is one of the oldest traditional systems of medicine practiced in South India. Herbal formulations used in *Siddha* medicine are known for their therapeutic benefits and minimal side effects. *Thanneervittan Nei* is a polyherbal formulation stated for Perumbadu

(Menorrhagia) in the *Siddha* literature ***"The Siddha formulary of India Part 1"***[1]. It is a lipid-based formulation prepared using ghee and herbal ingredients, primarily indicated in the management of Menorrhagia and other gynaecological disorders. Standardization of herbal formulations is important to ensure their quality, purity, and safety. Physicochemical analysis helps determine the stability and quality of the formulation through parameters like pH, acid value, peroxide value, and specific gravity. Phytochemical analysis is useful for identifying active chemical constituents responsible for therapeutic activity. High-Performance Thin Layer Chromatography (HPTLC) is a simple and reliable analytical technique used for fingerprint profiling of herbal medicines. It helps in the identification and authentication of phytoconstituents present in the formulation. Therefore, the present study was undertaken to evaluate the physicochemical properties, phytochemical constituents, and HPTLC fingerprint profile of *Thanneervittan Nei* for its standardization and quality assessment.

MATERIALS AND METHODS

Collection of the drug

The fresh plant was collected from Tirunelveli district, and the raw drugs were purchased from authenticated raw drug store in Chennai.

Identification and Authentication of the drug

All the drugs were recognized and verified by the experts of Gunapadam in Government Siddha medical College, Chennai.

Preparation of *Thanneervittan Nei*

Table-1 Ingredients of the *Thanneervittan Nei.*

S. No	Name of the drug	Botanical name	Quantity
1.	Thanneervittan kizhangu juice	*Asparagus racemosus*	600ml
2.	Nilappanai kizhangu juice	*Curculigo orchioides*	150ml
3.	Pasu nei	*Bos taurus*	300ml
4.	Saathikkai	*Myristica fragrans*	2g
5.	Ilavangapattai	*Cinnamomum verum*	2g
6.	Ilavangam	*Syzygium aromaticum*	2g
7.	Saathipathiri	*Myristica fragrans*	2g
8.	Kunkuma poo	*Crocus sativus*	1g

Procedure

The raw drugs were purified as per the *Siddha* literature *Sarakkugalin suddhi sei muraigal.*[2] The drug was prepared according to the given *Siddha* literature.

The above listed raw drugs of specified quantity were taken and ground using Thanneervittan kizhangu juice into a paste. Then Nilappanai kizhangu juice and ghee were mixed with this paste and boiled to make nei. Kunkuma poo was powdered and added to this preparation.

Drug profile

Route of administration: Oral

Dose : 10-15ml (Twice a day)

Indication : Perumbadu (Menorrhagia), Neersurukku, Irumal, Iraippu

Shelf life : 6 Months

METHODOLOGY

PHYSICOCHEMICAL ANALYSIS.[3]

1.Loss on Drying

The test sample was accurately weighed in a dry Petri dish and dried in a hot air oven at 105°C until constant weight was obtained. It was cooled in a desiccator and reweighed. The loss in weight represented the moisture content. The percentage loss on drying was calculated.

2. Determination of Fat Content

The test sample was weighed, and the fat was extracted using petroleum ether in a Soxhlet apparatus for 4–6 hours. The solvent was evaporated and the extract was dried to constant weight. The remaining residue represented the fat content. The result was expressed as percentage w/w.

3. Determination of Acid Value

The test sample was weighed and dissolved in neutralized alcohol-ether mixture. It was titrated against standard potassium hydroxide using phenolphthalein indicator until a faint pink colour appeared. The acid value was calculated from the titre value obtained.

4. Determination of Saponification Value

A known quantity of test sample was refluxed with alcoholic potassium hydroxide for about 30 minutes. After cooling, the excess alkali was titrated with standard hydrochloric acid using phenolphthalein indicator. A blank was conducted simultaneously. The saponification value was calculated**.**

5. Determination of Iodine Value

The test sample was dissolved in carbon tetrachloride and iodine monochloride reagent was added. The mixture was kept in the dark for 30 minutes, then potassium iodide and distilled water were added. The liberated iodine was titrated with sodium thiosulphate using starch indicator. The iodine value was calculated.

6. Determination of Peroxide Value

The test sample was dissolved in acetic acid and chloroform mixture. Potassium iodide solution was added, and the reaction was allowed to occur in the dark. The liberated iodine was titrated with sodium thiosulphate using starch indicator. The result was expressed as peroxide value.

7. Refractive Index

A drop of the test sample was placed on the prism of a refractometer maintained at 25°C. The instrument was allowed to stabilize, and the refractive index reading was noted directly.

8. Rancidity Test

The test sample was mixed with concentrated hydrochloric acid and phloroglucinol solution. The mixture was shaken well and observed for the development of pink colour. The appearance of pink colour indicated rancidity

9. Specific Gravity

A clean, dry specific gravity bottle was taken and weighed empty. It was then filled with the test sample and weighed again, and the procedure was repeated using distilled water. The specific gravity was calculated by comparing the weight of the sample with the weight of an equal volume of water at the same temperature.

10. Melting Point

A small amount of the test sample was filled in a capillary tube and placed in the melting point apparatus. It was heated gradually, and the temperature at which the sample completely melted was noted. The melting range observed was recorded**.**

11. Determination of pH

Prepare a suitable solution/suspension of the test sample in distilled water. Calibrate the pH meter using standard buffer solutions. Immerse the electrode into the sample and record the pH value directly.

PHYTOCHEMICAL ANALYSIS[4]

Phenols

2 ml of the test sample was taken, and a few drops of 5% ferric chloride solution were added. The mixture was mixed gently, and the colour change was observed. The formation of a deep blue, green, or black colour indicated the presence of phenols.

Tannins

To 2 ml of the test sample, ferric chloride reagent was added. The mixture was shaken well and allowed to stand for a few minutes. A blue-black or greenish-black colour confirmed the presence of tannins.

Flavonoids

A few drops of concentrated hydrochloric acid and magnesium turnings were added to the test sample. The mixture was warmed gently for 2–3 minutes. The development of a pink, red, or orange colour indicated the presence of flavonoids.

Triterpenoids

The test sample was mixed with chloroform, and concentrated sulphuric acid was added carefully along the side of the test tube. The solution was allowed to stand undisturbed. A reddish-brown colour at the interface confirmed the presence of triterpenoids.

Proteins

A few drops of Biuret reagent were added to the test sample. The mixture was shaken thoroughly and kept for 2 minutes. The appearance of a violet or purple colour indicated the presence of proteins.

Glycosides

The test sample was hydrolysed with dilute hydrochloric acid and neutralized with sodium hydroxide. Fehling's solution was added, and the mixture was heated gently. A brick-red precipitate indicated the presence of glycosides.

Reducing Sugars

To the test sample, equal volumes of Fehling's solution A and B were added. The mixture was heated in a water bath for a few minutes. The formation of a brick-red precipitate confirmed the presence of reducing sugars.

Anthraquinones

Shake the test sample with benzene and separate the benzene layer. Add dilute ammonia solution to the benzene extract. A pink, red or violet colour in ammoniacal layer indicates anthraquinones.

Quinones

Concentrated sulphuric acid was added carefully to the extract of the test sample. The colour formation was observed immediately. The appearance of red or blue colour indicated the presence of quinones.

Alkaloids

The test sample was acidified with dilute hydrochloric acid and filtered. Dragendorff's reagent was added to the filtrate. The formation of an orange or reddish-brown precipitate confirmed the presence of alkaloids.

Saponins

The test sample was diluted with distilled water and shaken vigorously for 5 minutes. The solution was allowed to stand. Persistent froth formation indicated the presence of saponins.

Cardiac Glycosides

To the test sample add glacial acetic acid containing ferric chloride, followed by concentrated sulphuric acid along the side of the tube. Observe the interface carefully. Formation of brown ring confirms cardiac glycosides.

Steroids

The test sample was mixed with chloroform, and concentrated sulphuric acid was added slowly. The mixture was allowed to stand without shaking. The appearance of a red, green, or bluish colour indicated the presence of steroids.

Coumarins

Sodium hydroxide solution was added to the test sample. It was observed under UV light or fluorescence was noted. Yellow fluorescence indicated the presence of coumarins.

Acids:- Sodium bicarbonate solution was added slowly to the test sample. Effervescence due to the release of carbon dioxide was observed. The formation of bubbles indicated the presence of acidic compounds.

TLC/HPTLC

10, 15µl Applied sample extract on TLC plate using Camag's ATS4 applicator and developed by the Mobile phase: Toluene: Ethyl acetate: Acetic acid (7.5:2.5:0.5) up to 8 cm distance. After development, the plate was photo documented using Camag's TLC Visualizer under UV 254 nm and UV 366 nm. Scanned the plate using Camag's Scanner 4 at UV 254 nm (D 2 lamp/Absorption mode) fingerprint profiles of the extract were documented. Then the plate was dipped in vanillin-sulphuric acid reagent followed by heating at 105 o C till development of coloured spots. The plate was then photo documented in white light and scanned at 520 nm (W lamp/Absorption mode).

RESULTS

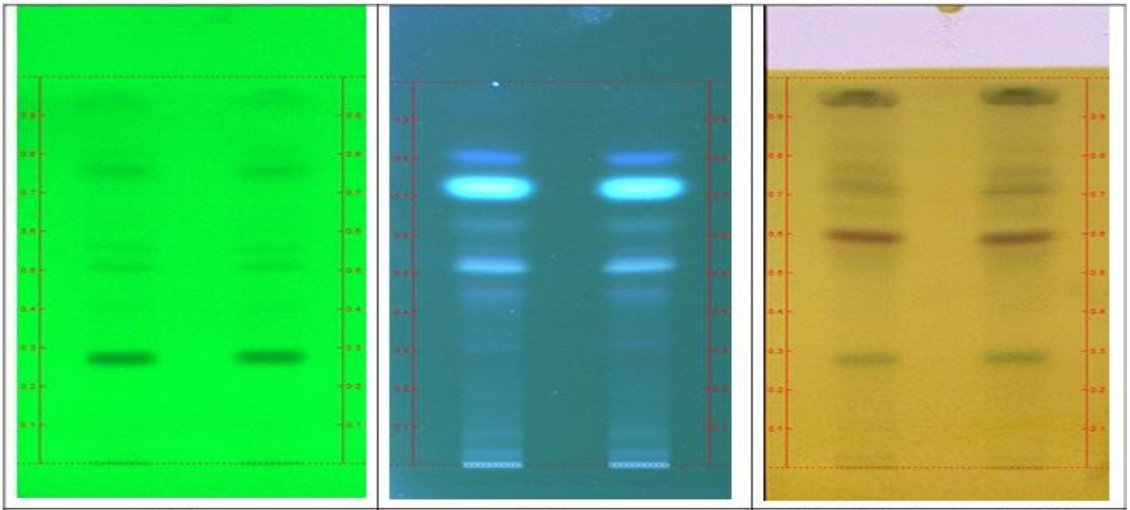
Physico-chemical analysis.

S NO	Parameter	Exp I	Exp II	Mean %
1	LOD	0.34	0.24	0.29
2	Fat content%	93.159	93.159	93.159
3	Acid value	4.62	4.64	4.63
4	Saponification value	216.67	211.19	213.93
5	Iodine value	55.03	36.22	45.625
6	Peroxide value	2.95	2.78	2.86
7	Refractive index	1.4579	1.4579	1.4579
8	Rancidity	Absent	Absent	Absent
9	Specific gravity	0.907	0.907	0.907
10	Melting point	39oC	39oC	39oC
11	pH	6.23		

Phytochemical analysis.

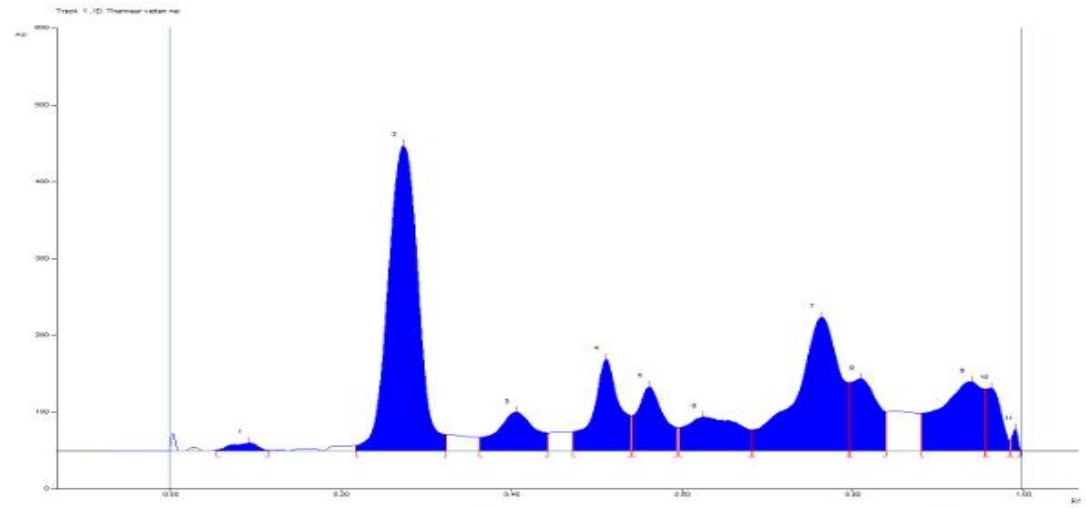
S.NO	Phytochemical Test	Result
1.	Test for phenols	Present
2.	Test for Tannin	Present
3.	Test for Flavonoids	Absent
4.	Test for Triterpenoids	Absent
5.	Test for Proteins	Absent
6.	Test for Glycosides	Absent
7.	Test for Reducing Sugar	Present
8.	Test for Anthraquinones	Absent
9.	Test for Quinones	Present
10.	Test for Alkaloids	Present
11.	Test for Saponins	Present
12.	Test for Cardiac glycoside	Absent
13.	Test for Steroids	Absent
14.	Test for Coumarin	Absent
15.	Test for Acids	Absent

TLC Photo documentation



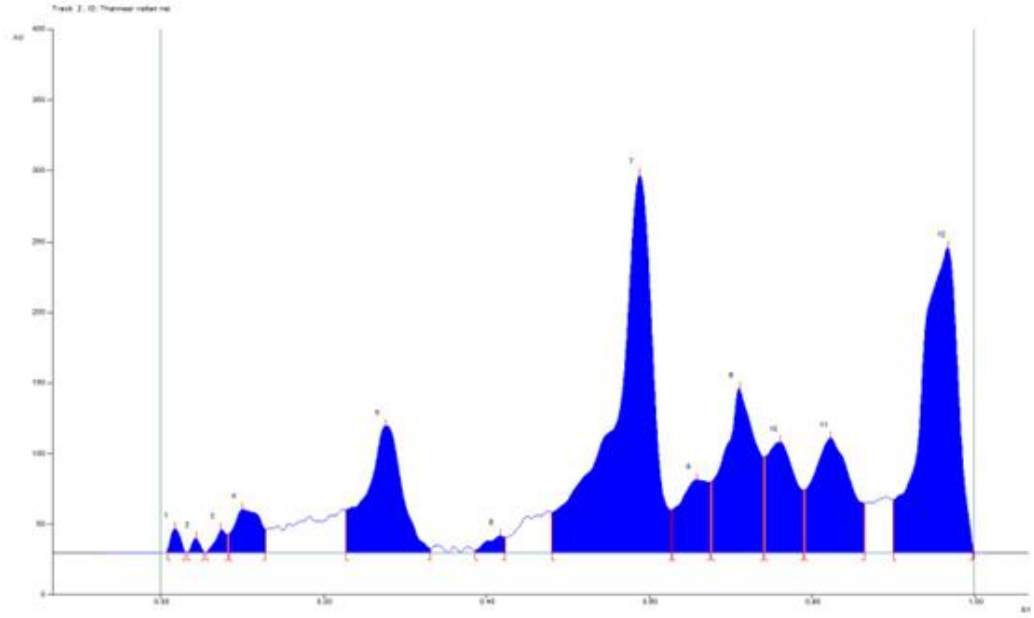
254 nm		366 nm		White light Derivatized plate	
R_f value	Color	R_f value	Color	R_f value	Color
0.27	Green	0.08	Blue	0.28	Green
0.51	Green	0.19	Blue	0.55	Purple
0.56	Green	0.30	Blue	0.59	Purple
0.76	Green	0.44	Blue	0.66	Blue
0.93	Green	0.52	Aqua	0.72	Blue
		0.62	Blue	0.76	Blue
		0.72	Aqua	0.95	Blue
		0.80	Blue		

Finger print profile at 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	1.2 AU	0.09 Rf	10.5 AU	0.89 %	0.12 Rf	0.4 AU	280.4 AU	0.74 %
2	0.22 Rf	6.9 AU	0.27 Rf	397.7 AU	33.87 %	0.33 Rf	21.1 AU	12080.7 AU	31.97 %
3	0.36 Rf	17.2 AU	0.41 Rf	50.1 AU	4.27 %	0.44 Rf	23.1 AU	1966.1 AU	5.20 %
4	0.47 Rf	24.6 AU	0.51 Rf	119.7 AU	10.20 %	0.54 Rf	46.1 AU	3173.6 AU	8.40 %
5	0.54 Rf	46.1 AU	0.56 Rf	83.5 AU	7.11 %	0.60 Rf	30.7 AU	2275.9 AU	6.02 %
6	0.60 Rf	30.8 AU	0.63 Rf	44.1 AU	3.76 %	0.68 Rf	27.6 AU	2382.2 AU	6.30 %
7	0.68 Rf	27.6 AU	0.77 Rf	174.0 AU	14.82 %	0.80 Rf	88.9 AU	7666.2 AU	20.29 %
8	0.80 Rf	89.1 AU	0.81 Rf	94.2 AU	8.02 %	0.84 Rf	50.2 AU	2578.6 AU	6.82 %
9	0.88 Rf	48.9 AU	0.94 Rf	90.3 AU	7.69 %	0.96 Rf	80.5 AU	3979.0 AU	10.53 %
10	0.96 Rf	80.7 AU	0.96 Rf	81.5 AU	6.94 %	0.99 Rf	13.0 AU	1236.9 AU	3.27 %
11	0.99 Rf	14.6 AU	0.99 Rf	28.5 AU	2.42 %	1.00 Rf	3.2 AU	167.1 AU	0.44 %

Finger print profile at 520 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.0 AU	0.02 Rf	16.9 AU	1.72 %	0.03 Rf	0.3 AU	172.5 AU	0.49 %
2	0.03 Rf	0.0 AU	0.04 Rf	10.1 AU	1.02 %	0.05 Rf	0.4 AU	81.0 AU	0.23 %
3	0.06 Rf	0.1 AU	0.07 Rf	16.1 AU	1.64 %	0.08 Rf	12.9 AU	192.1 AU	0.55 %
4	0.08 Rf	13.1 AU	0.10 Rf	30.5 AU	3.10 %	0.13 Rf	16.1 AU	845.4 AU	2.40 %
5	0.23 Rf	30.2 AU	0.28 Rf	89.4 AU	9.09 %	0.33 Rf	2.8 AU	3373.2 AU	9.58 %
6	0.39 Rf	1.4 AU	0.42 Rf	11.8 AU	1.20 %	0.42 Rf	10.8 AU	223.2 AU	0.63 %
7	0.48 Rf	28.4 AU	0.59 Rf	267.0 AU	27.14 %	0.63 Rf	30.1 AU	10801.4 AU	30.67 %
8	0.63 Rf	30.5 AU	0.66 Rf	51.5 AU	5.23 %	0.68 Rf	49.8 AU	1591.6 AU	4.52 %
9	0.68 Rf	50.0 AU	0.71 Rf	115.9 AU	11.78 %	0.74 Rf	67.4 AU	3970.3 AU	11.28 %
10	0.74 Rf	67.5 AU	0.76 Rf	77.9 AU	7.92 %	0.79 Rf	44.2 AU	2420.3 AU	6.87 %
11	0.79 Rf	44.3 AU	0.82 Rf	81.1 AU	8.24 %	0.87 Rf	34.8 AU	3295.5 AU	9.36 %
12	0.90 Rf	37.3 AU	0.97 Rf	215.5 AU	21.91 %	1.00 Rf	4.0 AU	8246.1 AU	23.42 %

DISCUSSION

The physicochemical analysis of *Thanneervittan Nei* revealed acceptable quality parameters and stability characteristics. The low loss on drying value (0.29%) indicates minimal moisture content, which may help in preventing microbial contamination and improving shelf stability. The high fat content (93.159%) confirms the lipid-rich nature of the formulation, consistent with medicated ghee preparations. The acid value (4.63) and peroxide value (2.86) were within acceptable limits, suggesting minimal hydrolytic and oxidative deterioration of fats. Absence of rancidity further supports the freshness and stability of the formulation. The saponification value (213.93) indicates the presence of fatty acids with comparatively shorter chain lengths, while the iodine value (45.625) suggests moderate unsaturation of fatty constituents. The refractive index (1.4579), specific gravity (0.907), melting point (39°C),

and pH (6.23) demonstrate the characteristic physicochemical nature of the preparation and may serve as standard reference parameters for quality control. Phytochemical screening demonstrated the presence of important secondary metabolites such as phenols, tannins, reducing sugars, quinones, alkaloids, and saponins. Phenolic compounds and tannins are known for their antioxidant and therapeutic potential, which may contribute to the medicinal efficacy of the formulation. The presence of alkaloids and saponins suggests possible pharmacological activities including anti-inflammatory, adaptogenic, and immunomodulatory effects. Reducing sugars indicate carbohydrate components, while quinones may contribute to biological activity. HPTLC fingerprint profiling of *Thanneervittan Nei* showed multiple distinct spots under UV 254 nm, UV 366 nm, and after derivatization at 520 nm, indicating the presence of several phytoconstituents with varying polarities. The different Rf values observed at each wavelength confirm the complexity of the herbal constituents present in the formulation. The chromatographic fingerprint obtained may be considered as a characteristic profile for identification and standardization of *Thanneervittan Nei*. The presence of well-resolved bands also indicates good separation of phytochemical constituents and supports the quality and consistency of the formulation.

CONCLUSION

The present study established the physicochemical, phytochemical, and HPTLC profile of *Thanneervittan Nei*. The physicochemical parameters confirmed the stability, purity, and acceptable quality characteristics of the formulation. Phytochemical analysis revealed the presence of biologically active constituents such as phenols, tannins, alkaloids, quinones, reducing sugars, and saponins, which may contribute to its therapeutic activity. HPTLC fingerprint profiling demonstrated characteristic chromatographic patterns that can serve as a reference for identification and quality assurance. Overall, the findings support the standardization and authenticity of *Thanneervittan Nei* and provide baseline analytical data for future pharmacological and clinical studies.

Declaration by Authors

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Self

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Conflict of interest

The author has no conflict of interest.

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