Research Article

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STANDARDISATION OF MURUNGAI NEI: A SIDDHA FORMULATION

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ABSTRACT

Murungai Nei (Moringa ghee) is a multi-herbal formulation indicated as an aphrodisiac and spermatogenic in Siddha literature. The study aims to standardise the Murungai Nei's quality, purity, and safety through its physicochemical, microbiological, chromatographic, and biochemical parameters. Physicochemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, alcohol-soluble extractive, and pH were found to be 0.03%, 0.0499%, 0.0199%, 0.07%, 0.1934%, and 18.49% respectively, which denote the purity and quality of the drug in this study. Qualitative preliminary phytochemical analysis reveals the presence of major phytochemicals: alkaloids, carbohydrates, saponin, phenols, tannins, flavonoids, diterpenes, quinones, gum, and mucilage. High-performance thin layer chromatography (HPTLC) fingerprinting analysis of the test drug reveals the presence of seven eminent modes relevant to the presence of seven different phytocomponents within it, whose Rf values range from 0.03 to 0.24. Biochemical analysis reports the presence of carbonate, sulphides, phosphate, ferrous, and magnesium. The results obtained from all the analyses of this study provide data on the pharmacogenetic (physical, chemical, and biochemical) properties of the Siddha poly-herbal formulation Murungai Nei. In the future, these data can be utilised as references for standardising the drug Murungai Nei.

Keywords: Murungai Nei, Siddha, Polyherbal formulation, Standardisation.

INTRODUCTION

The Siddha system of medicine is a holistic medical system. Standardisation is essential to ensure the safety and efficacy of herbal drugs in the healthcare system. As herbal drugs are composed of many components from different sources and the quality of plant materials, they are subjected to more influenceable factors than synthetic drugs, which often affect the quality of herbal drugs. A particular combination of medications in the Siddha School of Medicine can treat male infertility. According to the World Health Organization (WHO), it is indicated by the inability to conceive a child after 12 months or more of continuous, unprotected sexual activity[1]. Murungai Nei is a semisolid medication indicated as an aphrodisiac and spermatogenic in Siddha literature[2]. This study aims to standardise Siddha's poly-herbal formulation, Murungai Nei.

Aims and Objective: This study aims to explore the physicochemical, phytochemical, and HPTLC analysis of the Siddha polyherbal formulation "Murungai Nei" in ghee form for its potential in treating male infertility.

MATERIAL AND METHODS

Selection of Drug: In the Siddha text, "numerous formulations are mentioned with various formulations and indications. Murungai Nei is one of them. This herbal preparation is known to treat male infertility.

Ingredients: The drug comprises six ingredients, five herbals and one animal product (Table 1).

Collection of Raw Materials: The herbal drugs were procured from farmers and reputed traditional raw drug stores around the Madurai district. The authenticity of the plant materials was identified and verified by both the botanist and the head of the Department of Gunapadam, Government Siddha Medical College, Chennai, Tamil Nadu (certificate voucher number GSMC/MB-565-569).

Table 1: Ingredients of Murungai Nei

Drug Name	Botanical Name	Family	Part Used	Quantity
Murungai Poo	*Moringa oleifera* Lam.	Moringaceae	Flower	100 palam (3500 g)
Saranai Ver	*Trianthema decandra* Linn.	*Aizoaceae*	Root	1 palam (35 g)
Purified Seviyum	*Piper nigrum* L.	*Piperaceae*	Root	1 palam (35 g)
Maavilangu Ver	*Crateva magna* Lour.De.	*Capparaceae*	Root	1 palam (35 g)
Thippili Moolam	*Piper longum* L.	*Piperaceae*	Root	1 palam (35 g)
Nei	Cow's ghee	-	-	1 padi (1.3 litre)

Purification of the Raw Drugs: Herbal drugs were purified as mentioned in "Sikitcha Ratna Deepam Ennum Vaidhiya Nool"[3].

Preparation of the Drug: Three and a half kilograms of purified Murungai poo were added with 21.5 litres of water. The combination was reduced to 2.68 litres in an 8:1 ratio, and then 1.3 litres of ghee was added. Alongside, all the other ingredients (each 35 grams) that are powdered were made into a semisolid consistency by mixing with warm water, and this mixture was added and boiled. When it reached its consistency, the flame was switched off and filtered. It was preserved in a glass container.

Murungai Nei's phytochemical screening and physicochemical analysis were conducted at the Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai.

Standardisation Parameters

The various standardisation parameters, organoleptic properties, preliminary phytochemical screening, physicochemical analysis, and HPTLC analysis were studied.

Organoleptic Characters: The colour, odour, taste, and consistency of the drug were noted.

Preliminary Phytochemical Screening of Murungai Nei: Qualitative preliminary Phytochemical analysis reveals the presence of major phytochemicals: alkaloids, carbohydrates, saponins, phenols, tannins, flavonoids, diterpenes, quinones, gum, and mucilage. This was carried out using standard methods.

Loss on Drying

An accurately weighed 1 gm of Murungai Nei formulation was taken in a tarred glass bottle. The crude drug was heated at 105 °C for 6 hours in an oven until it reached a constant weight. The percentage moisture content of the sample was calculated with reference to the shade-dried material.

Determination of Total Ash

Weighed accurately, 2 gm of Murungai Nei formulation was added in a crucible at a temperature of 600 °C in a muffle furnace until carbon-free ash was obtained. It was calculated with reference to the air-dried drug.

Determination of Acid-Insoluble Ash

The ash above was boiled for 5 minutes with 25 ml 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 burned to a constant weight in a muffle furnace. The percentage of acid was calculated with reference to the air-dried drug.

Determination of Water-Soluble Ash

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

Determination of Water-Soluble Extractive

Five grams of air-dried Murungai Nei was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The solution was filtered, and 25 ml of the filtrate was evaporated in a tarred flat-bottom shallow dish, further dried at 100 °C, and weighed. The percentage of water-soluble extractives was calculated with reference to the air-dried drugs.

Determination of Alcohol Soluble Extractive

One gram of air-dried Murungai Nei was macerated with 20 ml of alcohol in a closed flask for 24 hours. With frequent shaking, it was filtered rapidly, taking precautions against loss of alcohol; 10 ml of filtrate was then evaporated in a tarred flat-bottom shallow dish, dried at 100 °C, and weighed. The percentage of alcohol-soluble extractives was calculated with reference to air-dried drugs.

Physicochemical Evaluation: The "Pharmacopeial Laboratory for Indian Medicine" (PLIM guidelines)[4] standard procedures were followed for the physico-chemical evaluation of Murungai Nei.

Detection of Alkaloids

Ethanol extracts were dissolved individually in diluted Hydrochloric acid and filtered.

- Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow precipitate indicates the presence of alkaloids.
- Dragendorff's Test: Filtrates were treated with Dragendorff's reagent (Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids.
- Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of brown/reddish precipitates indicates the presence of alkaloids.

Detection of Carbohydrates

Ethanol 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 an ethanol extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well, and a few drops of concentrated sulphuric acid are slowly added along the sides of the test tube. A violet ring indicates the presence of carbohydrates.
- Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. An orange-red precipitate indicates the presence of reducing sugars.

Detection of Saponins

Foam Test: 0.5 gm of ethanol extract was shaken with 2 ml of water. If the foam produced persists for ten minutes, it indicates the presence of saponins.

Detection of Phenols Ferric Chloride Test

Ethanol extracts were treated with 3-4 drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of phenols.

Detection of Tannins Gelatin Test

The ethanol extract is dissolved in 5 ml of distilled water, and 2 ml of a 1% solution of Gelatin containing 10% NaCl is added. A white precipitate indicates the presence of phenolic compounds.

Detection of Flavonoids

- Alkaline Reagent Test: Ethanol extracts were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow colour, which becomes colourless with the addition of dilute acid, indicates the presence of flavonoids.
- Lead Acetate Test: Ethanol extracts were treated with a few drops of lead acetate solution. The formation of a yellow colour precipitate indicates the presence of flavonoids.

Detection of Diterpenes Copper Acetate Test
Ethanol extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. The formation of an emerald green colour indicates the presence of diterpenes.

Test for Quinones
Ethanol extract was treated with sodium hydroxide; a blue or red precipitate indicates the presence of quinones.

Gum and Mucilage
To 1 ml of ethanol extract, add 2.5 ml of absolute alcohol, stirring constantly. Then, the residue was dried in the air and examined for its swelling properties. The swelling was observed, indicating the presence of gum and mucilage.

High-Performance Thin Layer Chromatography Analysis: The HPTLC method is a modern, sophisticated, and automated separation technique derived from TLC. Pre-coated HPTLC-graded plates and an autosampler were used to achieve qualitatively and quantitatively precision, sensitiveness, and significant separation. High-performance thin-layer chromatography (HPTLC) is a valuable quality assessment tool for efficiently and cost-effectively evaluating botanical materials. The HPTLC method offers high selectivity, sensitivity, and rapidity combined with single-step sample preparation. Thus, this method can be conveniently adopted for routine quality control analysis. It provides a chromatographic fingerprint of phytochemicals, which is suitable for confirming the identity and purity of phytotherapeutics[5].

Chromatogram Development
Murungai Nei was subjected to HPTLC analysis. It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried[5].

Scanning
Plates were scanned under UV at 366 nm. The data obtained from scanning was integrated through CAMAG software. A chromatographic fingerprint was developed to detect phytoconstituents in each sample, and their respective Rf values were tabulated.

Table 2: Organoleptic character of Murungai Nei

State	Semisolid
Odour	Characteristics
Touch	Greasy
Flow propertry	Non-free flowing
Appearance	Light yellow

Table 3: Physicochemical analysis of Murungai Nei

Parameters	Percentage (%)
Loss on drying	0.03
Total ash value	0.0499
Acid-insoluble ash	0.0199
Water-soluble ash	0.07
Water-soluble extraction	0.19
Alcohol-soluble extraction	18.49

Table 4: Phytochemical analysis of Murungai Nei

Phytochemicals	Test Name	Ethanol Extract
Alkaloids	Mayer's Test Dragendorff's Test Wagner Test	Negative Positive Positive
Carbohydrates	Molisch's Test Benedict Test	Positive Negative
Saponin	Foam Test	Positive
Phenols	Ferric Chloride Test	Negative
Tannins	Gelatin Test	Negative
Flavonoids	Alkaline Reagent Test Lead Acetate	Negative Negative
Diterpenes	Copper Acetate Test	Positive
Quinones	Test for Quinones	Negative
Gum & Mucilage	Test for Gum & Mucilage	Negative

Table 5: Peak Table

Peak	Start RF	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	5.2	0.06	255.1	45.31	0.09	13.1	2854.7	28.31
2	0.09	17.3	0.10	22.1	3.92	0.13	0.3	217.5	2.16
3	0.14	0.6	0.19	45.6	8.11	0.22	2.3	694.4	6.89
4	0.24	4.9	0.32	240.2	42.66	0.42	0.9	6315.9	62.64

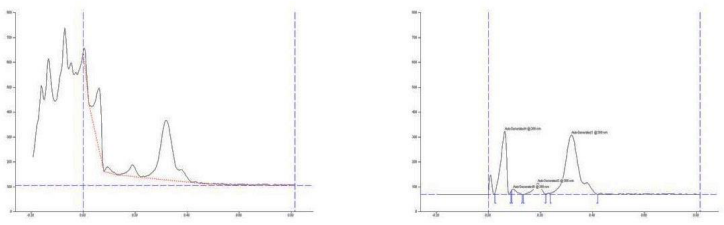

Figure 1: HPTLC finger printing of sample Murungai Nei

RESULTS

Siddha Specification of Murungai Nei

According to Siddha literature, Nei must be in semisolid preparation and yellowish. Murungai Nei has the consistency of a semisolid with a light yellow colour and a non-free-flowing property that is bitter (Table 2).

Physicochemical Properties of Murungai Nei

The analysis of physicochemical parameters determines that the percentage of loss on drying, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, alcohol-soluble extractive, and pH are 0.03%, 0.0499%, 0.0199%, 0.07%, 0.1934%, and 18.49%, respectively (Table 3).

Phytochemical Analysis of Murungai Nei

Phyto-chemical analysis reveals the presence of major phytochemicals alkaloids: carbohydrates, saponins, phenols, tannins, flavonoids, diterpenes, quinones, gum, and mucilage. The observed results are tabulated in Table 4.

High-Performance Thin Layer Chromatography Analysis

HPTLC fingerprinting analysis of the sample reveals the presence of four prominent peaks, which correspond to the presence of four versatile photo components. The Rf value of the peak ranges from 0.03 to 0.24 (Figure 1 and Table 5).

DISCUSSION

Physicochemical parameters determine the percentage of loss on drying, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, alcohol-soluble extractive, and pH as 0.03%, 0.0499%, 0.0199%, 0.07%, 0.1934%, and 18.49%.

It revealed that moisture content is as low as 0.03%. The total ash value of 0.0499% indicated the presence of a substantial quantity of inorganic residue in the formulation. The acid-insoluble ash value of 0.0199% was within the safety margin limit, suggesting the absence of contamination. The water-soluble ash value of 0.07% indicated the presence of water-soluble components in the formulation. Finally, the alcohol-soluble extract value of 18.49% indicated the exhaustion of the drug in the formulation.

Phytochemical screening showed the sample's presence of bioactive components such as alkaloids, carbohydrates, saponin, phenols, tannins, flavonoids, diterpenes, quinones, gum, and mucilage. Additionally, it demonstrated the absence of quinones, tannins, flavonoids, and phenols.

HPTLC fingerprinting analysis of the sample revealed the presence of five prominent peaks, corresponding to the presence of five versatile phytocomponents present in it. The Rf value of the peak ranged from 0.03 to 0.24 (Figure 1 and Table 5).

These studies have collectively established the therapeutic significance of these specific ingredients within the formulation. The current physicochemical, phytochemical, and HPTLC analysis of Murungai Nei yielded valuable evidence regarding the presence of certain compounds.

The present study on physicochemical parameters, TLC and HPTLC analysis, and heavy metal analysis provides essential information that can be used as a fingerprint of the herbal Siddha medicine Murungai Nei.

The following studies have looked into the sexual performance of *Moringa oleifera*[6] and the aphrodisiac effects of *Trianthema decandra.*[7], increases in libido parameters, black pepper's antioxidant and plasma testosterone-raising properties[8], *Crateva magna* had antioxidant activity[9], *Piper longum's* analgesic and aphrodisiac properties[10], and the aphrodisiac potential of cow's ghee[11]. These investigations have proven the therapeutic value of these particular formulation constituents. This establishes that the chemicals in Murungai Nei also possess the same aphrodisiac properties.

By identifying and quantifying these phytoconstituents, researchers can gain insights into the formulation's potential mechanisms of action and therapeutic properties. This standardisation process ensured the formulation consistency, safety, and efficacy, thus enhancing its value as a therapeutic intervention.

CONCLUSION

The standardisation process implemented in this study successfully confirmed the presence of bioactive phytocomponents in the formulation. These bioactive compounds enhance the therapeutic potential of Murungai Nei as an intervention for male infertility. However, it is important to note that further in vivo studies and clinical trials involving larger sample sizes are necessary to substantiate its efficacy as a preferred treatment for male infertility.

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Description

The English term for the Siddha herbal Murungai Nei is "Moringa Ghee". It is referred to as Murungai Nei throughout this article.

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