



PHARMACOGNOSTICAL INVESTIGATION OF A POLYHERBAL SIDDHA FORMULATION- NEERIZHIVU CHOORNAM

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ABSTRACT

Neerizhivu Choornam is a polyherbal Siddha formulation useful in the treatment of Diabetes. Efficacy of formulation depends on their genuineness of herbs used. Authentication of herbs is first and fundamental step for standardization of herbal formulation. In this paper Pharmacognostic investigations like Macroscopic, Microscopic and preliminary phyto-chemical examination of Neerizhivu Choornam is reported. The standard methods recommended in "Quality Control Methods for Medicinal Plant Materials" by WHO, 1998 was followed. Macro-microscopic, preliminary phyto-chemical and physico-chemical constants of the formulation has been documented. Findings of the study helpful in standardization of polyherbal Siddha formulation Neerizhivu Choornam, which will promote global acceptance of the formulation and reputation of the Siddha system.

Keywords: Anti-diabetic, Siddha herbal formulation, Neerizhivu Choornam, Standardization, Pharmacognosy.

INTRODUCTION

Diabetes mellitus, a metabolic disorder, is becoming a serious threat to health of people. The prevalence of diabetes mellitus is expected to reach up to 4.4% in the world by 2030 [1]. The people with diabetes in the world are expected to approximately double between 2000 and 2025. India leads the world with largest number of diabetic subjects being termed as 'diabetes capital of the world' [2,3]. Plants have been used since time immemorial for medicinal purposes and form the origin of much of modern Pharmacotherapy. Many plants are reported to be useful in the treatment of Diabetes mellitus.

Neerizhivu Choornam is a polyherbal Siddha formulation useful in the treatment of Diabetes. Neerizhivu Choornam (Table.1) consists of fine powders of Kadukkai - *Terminalia chebula* (Pericarp, 2 parts), Karuveppilei - *Murraya konigii* (Leaves, 2 parts), Nelli vatral - *Emblia officinalis* (Pericarp, 2 parts), Navalkottai - *Syzygium cumini* (Seed, 1 part), *Tinospora cordifolia* (Seenthil, 1 part), Kizanelli - *Phyllanthus amarus* (Whole plant, 1 part) and Korai kizhangu - *Cyperus scariosus* (Rhizome, 1 part).

Polyherbal formulation in powdered form where the botanical ingredients are not more than ten can be identified microscopically [4]. The hypoglycemic activity of alcoholic extract of individual ingredients of *Cyperus*

scariosus [5], *Terminalia chebula* [6], *Emblia officinalis* [7], *Phyllanthus amarus* [8], *Syzygium cumini* [9], *Murraya konigii* [10] and *Tinospora cordifolia* [11] is already reported. In the present investigation Macro-microscopic, preliminary phyto-chemical and physico-chemical constants of the formulation were carried out.

MATERIALS & METHODS

The ingredients of Neerizhivu Choornam were purchased from local raw material traders and the raw materials were authenticated by comparing with the in-house standards of Captain Srinivasa Murthi Research Institute of Ayurveda and Siddha Drug Development, Arignar Anna Government Hospital Campus, Arumbakkam, Chennai - 600 106. The samples are cleaned and shade dried, organoleptic characters of individual raw drugs were recorded, powdered and passed through sieve no. 80 [12]. Each one of the powder is weighed separately and each powder are mixed together. Powder of individual ingredients *Terminalia chebula* (Pericarp), *Emblia officinalis* (Pericarp), *Murraya konigii* (Leaves), *Syzygium cumini* (seed), *Tinospora cordifolia* (Stem), *Phyllanthus amarus* (Whole plant) and *Cyperus scariosus* (Rhizome) and the compound formulation were

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analyzed microscopically after clearing them in Chloral hydrate solution.

A few mg of powder treated with iodine in potassium iodide solution and mounted in glycerin for observation of starch. A few mg of powder treated with solution of phloroglucinol, allowed to dry, added a few drops of hydrochloric acid and mounted in glycerin to observe lignified tissues. Quantitative analysis for total ash, water soluble ash, acid insoluble ash, ethanol soluble extractive value, water soluble extractive value and loss on drying at 105^oc were carried out in triplicate for the polyherbal Siddha formulation Neerzhivu Chooranam according to the method recommended in "Quality Control Methods for Medicinal Plant Materials" by WHO, 1998. Preliminary phytochemical analysis and Fluorescence analysis were also carried out.

Preparation of extracts for TLC

2g of drug samples were soaked in Alcohol: Water (80:20 V/V) for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made up to 5ml in a standard flask separately.

Method of developing for TLC

The aqueous alcoholic extract was applied on pre-coated silica gel 60 F₂₅₄ TLC plate (E-merck) as absorbent and the plate was developed using solvent system toluene : Ethyl acetate (1: 1). After developing, the plates were dried and observed the color spots at UV-254, UV-366 nm and vanillin-sulphuric acid spraying reagent (Wagner *et al.*, 1984).

RESULTS AND DISCUSSION

Fig.3 Powder microscopy of Neerzhivu Chooranam 1. Tyloses of bark (Seenthil) 2. Stomata with epidermal cells (Kizhanelli) 3. Palisade cells with epidermis (Kizhanelli) 4. Sclereids (Navalkottai, Kadukai) 5. Stone cells (Kariveppilei, Navalkottai, Kadukai) 6. Simple pitted vessels (Kizhanelli, Kariveppilei) 7. Rosette crystals (Kizhanelli) 8. Prismatic crystals (Kizhanelli, Kariveppilei) 9. Starch grains (Korai kizhangu,

Navalkottai) 10. Spiral vessels (Nellivatr, Navalkottai) 11. Cells with calcium oxalate crystals 12. Fibres (Nellivatr, Seenthil, Kizhanelli, kadukai, kariveppilei) 13. Crystal fibre (Seenthil) 14. Epidermal cells with collenchyma (Kadukai) 15. Fragment of testa in surface view (Navalkottai) 16. Fragment of testa in sectional view (Navalkottai) 17. Cells of testa in surface view (Kizhanelli) 18. Trichomes (Kariveppilei)

Tissue debris consisting of packed regular rows of fibre-sclereids of fairly uniform size, and narrow scalariform vessel showing laterally placed simple perforation, thick-walled trichomes, thick walled rounded to elongated parenchyma with brown content of tannin, numerous round, numerous oval to elongated starch grains and a few rosette crystals of calcium oxalate (Korai kizhangu); a few fragments of uniformly thickened straight walled epidermis, isodiametric parenchyma cells with irregularly thickened walls, occasionally short fibres and tracheids (Nellivatr); a few pitted and spiral vessels, epicarp of fruit with anisocytic stomata, prismatic and rosette crystals of calcium oxalate (Kizhanelli); crisscross layers of fibres, thin walled fibres and broad lumen with pegged tip, polygonal cells of epidermis showing slight beading and prismatic and druses of calcium oxalate crystals, pitted stone cells with thick lumen (Kadukkai); stone cells and sclereids in different shapes and sizes, a few fragments of testa in sectional and surface view (Navalkottai); a few bent or curved trichomes, epidermal cells with anomocytic stomata, portion of secretory canals, a few prismatic crystals of calcium oxalate (Kariveppilei); a few fragments of pericyclic fibres lignified with wide lumen and pointed ends, associated with a large number of crystal fibres containing a single prism in each chamber, a few fragments of vessels with bordered pits associated with tyloses, a few isolated or group of stone cells (Seenthil). The results of preliminary phytochemical analysis and Fluorescence analysis were given in the tables (Table 2, 3 & 4).

Thin Layer Chromatography: In UV - 254, 366 nm and visible light it shows 6, 7 and 6 spots respectively with different R_f values (Fig. 4, Table-5).

Table1. Neerzhivu Chooranam Formulation

S.No	Name of the drug	Parts used	Biological source & Family	Quantity
1	Kadukkai thol	Skin	<i>Terminalia chebula</i> (Combretaceae)	2 part
2	Kariveppilai	Leaves	<i>Murraya koenigii</i> (Rutaceae)	2 part
3	Nellivatr	Fruits	<i>Emblia officinalis</i> (Euphorbiaceae)	2 part
4	Navalkottai	Seeds	<i>Syzygium cumini</i> (Myrtaceae)	1 part
5	Seenthil	Stem	<i>Tinospora cordifolia</i> (Menispermaceae)	1 part
6	Keezhkkai nelli	Fruits	<i>Phyllanthus fraternus</i> (Euphorbiaceae)	1 part
7	Korai kizhangu	Rhizomes	<i>Cyperus rotandus</i> (Cyperaceae)	1 part

Table 2. Physicochemical analysis of Neerzhivu Chooranam [Values are mean of three determinations ±SD]

Parameters		
Ash value	Total ash	6.019 ± 0.072
	Water soluble ash	1.826 ± 0.074
	Acid-insoluble ash	1.241 ± 0.019

Extractive value	Water soluble extractive	21.460±0.202
	Alcohol soluble extractive	12.724 ± 0.183
Loss on drying	Loss on drying at 105 ^o C	9.174 ± 0.931

Table 3. Preliminary phyto-chemical analysis of Neerizhivu Choornam

Test	Presence/Absence in
Aqueous alcoholic extract	
Alkaloid	+
Amino acid	-
Coumarin	+
Flavonoid	+
Glycoside/Sugar	+
Phenol	+
Quinone	+
Steroid	+
Tannin	+
Triterpenoid	+
Saponin	+
Furanoid	-
Lignan	+
Carboxylic acid	-

(+ indicates Present, - indicates Absent)

Table 4-Fluorescence analysis of Neerizhivu Choornam

S.No	Reagents with powder	UV-254 nm	UV- 366 nm	Visible light
1.	Powder as such	Greenish Yellow	Greenish Yellow	Greenish Yellow
2.	n-hexane	Green	Light green	Yellowish green
3.	Chloroform	Dark green	Light green	Yellowish green
4.	Ethyl acetate	Yellowish green	Green	Green
5.	Ethanol	Green	Yellowish green	Green
6.	Acetone	Dark green	Yellowish brown	Yellowish green
7.	Water	Yellowish green	Light brown	Yellow
8.	1N Sodium hydroxide (Aqueous)	Violet	Red	Orange
9.	1N Sodium hydroxide (Alcohol)	Violet	Violet	Red
10.	1 N Hydrochloric acid	Yellowish green	Violet	Yellow
11.	1 N Nitric acid	Green	Pale brown	Orange
12.	1 N Sulphuric acid	Light green	Brown	Purple
13.	50% Sulphuric acid	Violet	Brown	Purple

Table 5. R_f Values of aqueous alcoholic extract of Neerizhivu Choornam

Solvent system	R _f Values		
	UV 254nm	UV 366nm	Visible Light
Toluene:Ethyl acetate (1 : 1)	0.93 Pink	0.88 Violet	0.94 Grey
	0.87 Green	0.82 Violet	0.88 Violet
	0.79 Light Pink	0.65 Yellowish green	0.80 Grey
	0.67 Yellowish green	0.60 Blue	0.67 Orange
	0.29 Green	0.45 Blue	0.58 Grey
	0.12 Green	0.29 Pink	0.10 Light grey
		0.14 Light pink	

Fig.1. Neerizhivu Choornam



Fig.2. Ingredients of Neerizhivu Choornam



2.1 Korai kizhangu



2.2 Kadukkai



2.3 Kizhanelli



2.4 Seenthil



2.5 Kottainaval

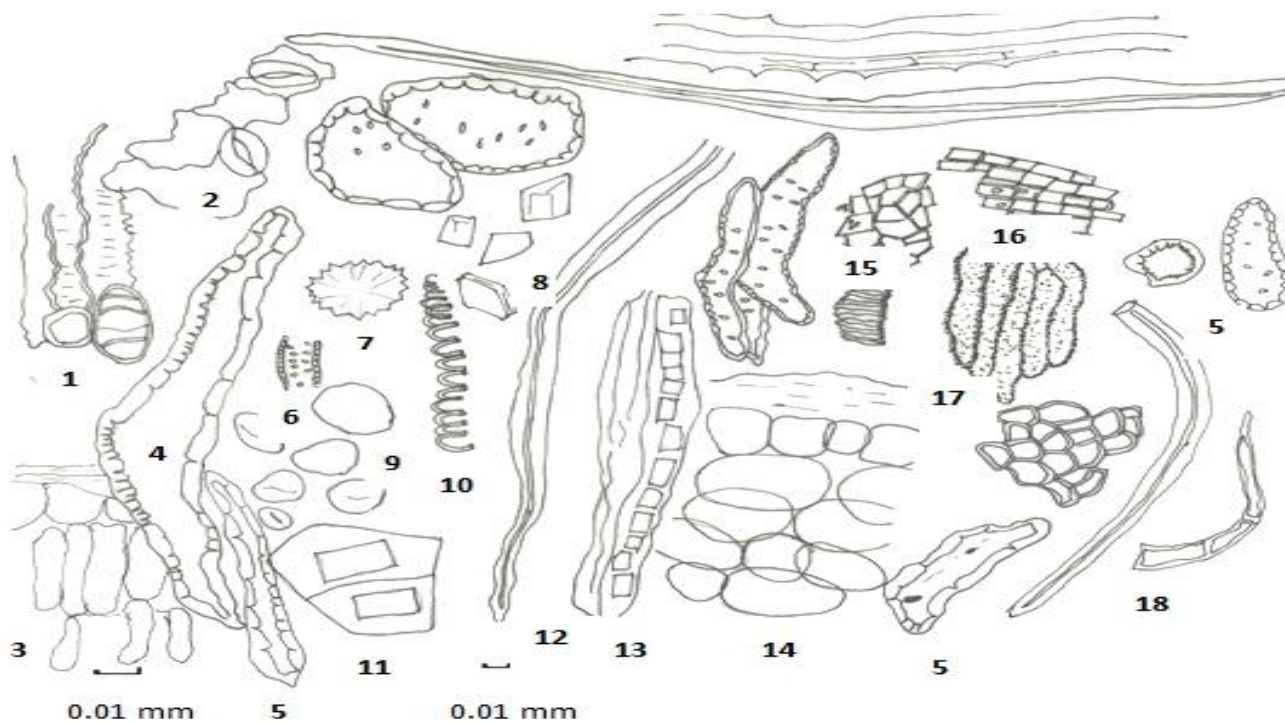


2.6 Karuveppilei



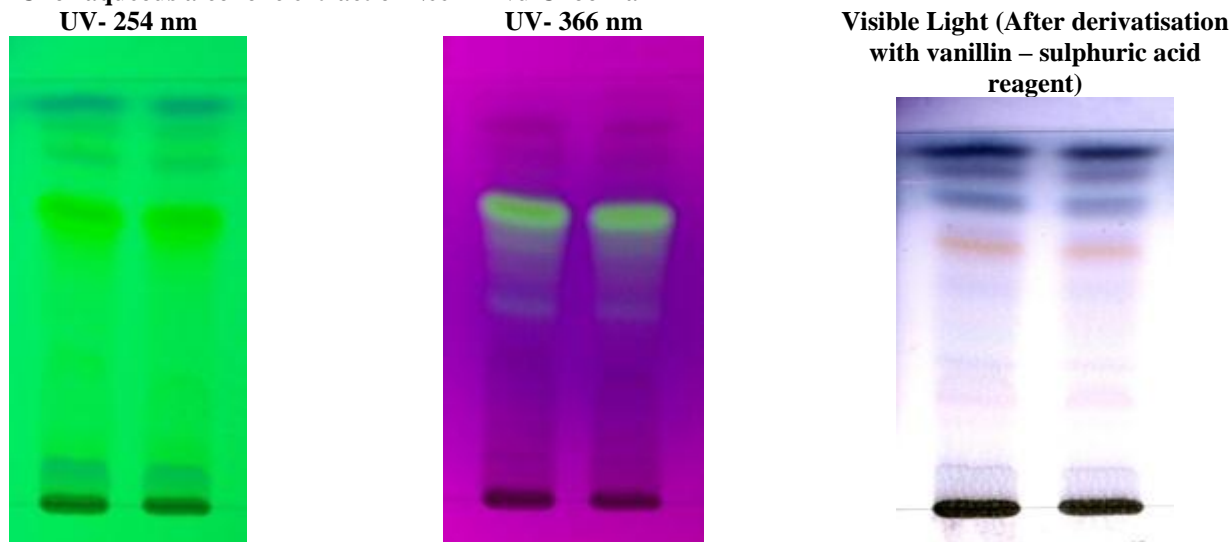
2.7 Nellivatral

Fig.3. Powder microscopy of Neerizivu Chooranam 1. Tyloses of bark (Seenthil) 2. Stomata with epidermal cells (Kizhanelli) 3. Palisade cells with epidermis (Kizhanelli) 4. Sclereids (Navalkottai, Kadukai) 5. Stone cells (Kariveppilei, Navalkottai, Kadukai) 6. Simple pitted vessels (Kizhanelli, Kariveppilei) 7. Rosette crystals (Kizhanelli) 8. Prismatic crystals (Kizhanelli, Kariveppilei) 9. Starch grains (Korai kizhangu, Navalkottai) 10. Spiral vessels (Nellivatral, Navalkottai) 11. Cells with calcium oxalate crystals 12. Fibres (Nellivatral, Seenthil, Kizhanelli, kadukai, kariveppillei) 13. Crystal fibre (Seenthil) 14. Epidermal cells with collenchyma (Kadukai) 15. Fragment of testa in surface view (Navalkottai) 16. Fragment of testa in sectional view (Navalkottai) 17. Cells of testa in surface view (Kizhanelli) 18. Trichomes (Kariveppilei)



Thin Layer Chromatography: In UV - 254, 366 nm and visible light it shows 6, 7 and 6 spots respectively with different R_f values (Fig. 4, Table-5).

Fig.4. TLC for aqueous alcoholic extract of Neerizhivu Chooranam



DISCUSSION

Pharmacognostic characters of herbal drugs play an important role since particular macro-microscopic features are unique for each plant. The macroscopic and microscopic studies of the herbs should be the first and

fundamental step to authenticate the botanical source. TLC profile of aqueous alcoholic extracts provides a suitable method for monitoring the identity and purity and also standardization of the drug.

CONCLUSION

The present study, analysed the macro-microscopic characters, preliminary phyto-chemical and physico-chemical constants of the formulation. The results obtained will help in standardization of polyherbal Siddha formulation Neerizhivu Choornam.

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