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PHARMACOGNOSY AND CHROMATOGRAPHIC ANALYSIS OF *NILAPANAI CHOORANAM*, A SIDDHA POLYHERBAL FORMULATION

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ABSTRACT

The Indian subcontinent is enriched by a variety of flora – both medicinal and aromatic plants. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional systems of medicine. This study aims at exploring the pharmacognosy, phytochemistry, physico-chemical and TLC analysis of a Siddha polyherbal formulation *Nilapanai chooranam*. The raw materials were authenticated by a pharmacognosist. The macroscopic characters and the powder microscopy of the chooranam revealed the presence of all those ingredients in the final product. The preliminary phytochemical analysis of *Nilapanai choornam* revealed the presence of Glycoside, Tannins, alkaloids, Triterpenes, Flavonoids. In Thin layer chromatographic analysis the solvent front was standardized as Petroleum ether: Chloroform: Methanol 1:0:5:0.1. The methanol, chloroform and ether extracts were fractionized.

Keywords: Polyherbal, Nilapanai, Phytochemistry, Microscopy

INTRODUCTION

Man has been using herbs and plants products for combating diseases since time immemorial. The Indian subcontinent is enriched by a variety of flora – both medicinal and aromatic plants. This is due to the wide diversity of climatic conditions in India ranging from deserts to swamplands. Numerous types of herbs have been well recognized and cataloged by botanists from the high ranges of the Himalayan tract up to the sea shore of Kanyakumari. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional systems of medicine[1]

It is notable that World Health Organization is showing great interest on drugs from natural sources especially from traditional systems and folklore medicines. Among the natural sources, plants are economic and they are the source for abundant chemical intermediates to produce new drugs with fewer side effects.[2]

Plants are the only economic source of a number of well established and important drugs. In addition they are also the source of chemical intermediates needed for the production of some drugs[2].

Indian systems of medicine have a deep root in our cultural heritage and cater to the Medicare of large sections of our population. These systems mainly use herbs. In recent times, there has been a marked shift towards herbal cures because of the pronounced cumulative and irreversible ill effects of many modern drugs.

In Siddha system of medicine, Siddhars have mentioned 4448 diseases based on derangement in three humors due to changes in diet, environment and pathological changes and diagnosed by naadi. Among them they have dealt few diseases affecting the females. Among them the most commonly seen in females is the Vellai Noi. Among the siddha polyherbal formulations, *Nilapanai Choornam* is used to treat Vellai noi (Leucorrhoea).[3] The present study aims at the evaluation of pharmacognostical, phytochemical, physicochemical and TLC analysis of the formulation.

MATERIALS AND METHODS

Preparation of *Nilapanai choornam*[4]

Contents

1. Nilapanai (Root of *Curculigo orchioides*)
2. Nerunji mul (Fruit of *Tribulus terrestris*)
3. Nelli vatral (Dried fruits of *Phyllanthus emblica*)
4. Periya poornaikkali vidhai (Seeds of *Mucuna pruriens*)
5. Seenthil chakkarai (*Tinospora cardifolia*)
6. Mul Ilavam pisin (Gum of *Salmalia malabarica*)
7. Karkandu (*Saccharum officinarum*)

Reference

C. Kannusamy Pillai, Kannusamy parambarai vaidhyam, V Edition, Pg 111

Procurement and authentication of drugs

The dried raw drugs were procured from raw drug store from Chennai. The identity and authenticity of the drugs was confirmed by pharmacognosist, Siddha Central Research Institute (SCRI), Chennai

Purification of drugs

The Poonaikkali seeds were boiled in milked and the seed coat removed and dried. The other drugs were dried under sunlight and the dusts and foreign matter are removed.

Preparation of the drug

The contents were powdered and mixed in the ratio as per the literature. The powder was sieved by the traditional method adopted by Siddhars (Vasthirakayam).The choornam was purified by pittaviyal method using Avi Iyandhiram and stored in a clean glass airtight container.

Microscopic study of *Nilapanai choornam*

The macroscopy of the ingredients and microscopic study was done at Department of pharmacognosy, SCRI, Chennai.

PROCEDURE

Macroscopy of the ingredients and organoleptic and powder microscopy of the choornam were studied. Powder was analyzed microscopically after clearing it in chloral hydrate solution and Jeffery's reagent. The powder was mounted in alcohol, water and Smiths' starch reagent. It was also treated with iodine dissolved in potassium iodide solution.

Physico chemical analysis of *Nilapanai choornam*

The physico chemical parameters like total ash, water soluble ash, acid insoluble ash and loss on drying of the NP choornam was determined by standard methods[5]

Preliminary phytochemical analysis of *Nilapanai choornam*

The *Nilapanai choornam* (10 g) was extracted with the solvents namely methanol, ethyl acetate and chloroform. The extracts were filtered and the concentrated under vacuum, followed by drying (40 °C). The extracts were screened for the presence of phytochemicals

like alkaloids, flavonoid, carbohydrates, glycosides, saponins, tannins and triterpenoids by standard methods[6]

Thin layer chromatographic study of *Nilapanai choornam*

TLC was used to identify compounds and its purity. As a stationary phase a special fine pre coated silica gel plates (MERCK TLC plates) were used to analyze the extract. 5 microlitre of the extracts (methanol, chloroform and petroleum ether) were spotted and developed with various solvents as follows.

Hexane: chloroform: methanol 1:0.5:0.1

Chloroform: methanol: ether 1:0.5:0.1

Petroleum ether: chloroform: methanol 1:05:0.1

Petroleum ether: chloroform: methanol 1:05:0.1 was optimized for the TLC separation of the extracts and the spots were visualized in Iodine chamber and UV light at 240 nm and 360 nm. R_f Values were calculated.

RESULTS

Microscopic study of *Nilapanai choornam*

Fig. 1: Nilapanai choornam

Macroscopy of the Ingredients

1. Nilappanai kizhangu (Rhizome) – *Curculigo orchioides* Gaertn.

The colour of the external surface is blackish brown and the cut surface is cream coloured. It appears as transversely cut pieces of 2.5cm long. The surface showed numerous shallow wrinkles and transverse cracks. Few rootlets and scars were seen. Nodes and internodes were prominent; taste mucilaginous and slightly bitter.

2. Cintil tantu (Stem) – *Tinospora cordifolia* (Willd.) Miers.

The thickness of pieces varies from 0.6 to 5cm in diameter. The colour was green in young stems and light brown in older ones. Swelling at nodes was seen in smooth surfaces of young stems. The transverse surface showed radial structure with medullary rays traversing porous tissues. The taste is bitter.

3. Poonaikkali (Seed) – *Mucuna prurita* Hook., Syn. *M.pruriens* Baker

The length of the seeds varied from 1.2 to 1.8cm and width 0.8 to 1.2cm. The seeds are ovoid in shape, hard and smooth to touch and not easily breakable. The seeds are slightly compressed laterally.

4. Neruncil mul (Fruit) – *Tribulus terrestris* L.

The colour fruits appeared light to greenish yellow. They are five ribbed and spherical in shape. They are covered with 5 pairs of prominent stiff spines pointing downwards. They are 0.5cm in length. The spines form a pentagonal appearance around the fruits. Taste of the fruits is slightly astringent.

5. Nelli vatral (Dried fruit) – *Phyllanthus emblica* L.Syn. *Emblica officinalis* Gaerth.

The dried fruits appeared as curled pieces of pericarp. The length varied from 1 to 2 cm. The colour is grey to black. The external surface is convex to somewhat concave and is wrinkled. It also shows a few white specks. The transverse lateral surface is wrinkled. The texture is rough and cartilaginous to touch, tastye being sour and astringent.

6. Mul ilavum pisin (gum) - *Bombax malabaricum* .L

The shape of the gum appeared as round to oval. The colour is dark reddish brown. The surface is hard and having a short fracture. There is no characteristic taste and odour.

Powder Microscopy of *Nilappanai Choornam*

Powder is pale brown in colour with characteristic odour and astringent taste.

Microscopy of *Nilapanai Choornam*

Vessels with annular and spiral thickenings, starch grains round to oval shaped measuring 4 to 21 µm in dia., a few acicular calcium oxalate crystals, colorless mass mucilage showed the presence of *Nilapanai kizhangu* – Tuberous root of ***Curculigo orchioides*** Gaertn in the choornam.

Simple, elliptic- ovoid starch grains measuring 6-23-36 µm in dia.; hilum centric and appear as a line; striations are not distinct; smaller and larger prismatic calcium oxalate crystals measuring 20 to 30 µm, revealed the presence of seenthil sarkarai- starch of ***Tinospora cordifolia*** (Willd.) Hk. F. & Th. in the powder.

The presence of fragments of testa with palisade like cells, thin-walled parenchyma, vessels which are pitted and reticulated, aleurone grains, starch grains measuring 6- 41 µm in dia confirmed the presence of seeds of ***Mucuna prurita*** Hook.

Abundance unicellular trichomes, rectangular epidermal cells, rosettes of calcium oxalate crystal and smaller prismatic calcium oxalate crystal showed the presence of Nerunjil – fruits of ***Tribulus terrestris*** L.

Distinct features like uniformly thickened straight walled epidermis and isodiametric parenchyma cells with irregular thickened walls, fibres or tracheids, showed the presence of Nellivatral dried pericarp of ***Phyllanthus emlica*** L.

When the choornam was mounted in alcohol, small angular fragments were noticed and on the addition of water the particles began to swell and their edges became more indefinite and formed a structureless jelly like mass and in solution of chloral hydrate, the swollen cell walls were evident. This showed the presence of Mul llavam pisin – gum of ***Bombax malabaricum*** L.

Table 1: Physico-chemical properties of *Nilapanai choornam*

S.	Parameter	Results
	Physical properties	
1	pH at 25°C (1:10 Ratio)	3.05
2	Ash Value @ 550°C (%)	12.0
3	Water soluble (%)	54.0
4	Alkalinity as $CaCO_3$ in water soluble ash (%)	1.18
5	Acid Insoluble Ash, (%)	3.0
6	Loss of drying @ 105°C (%)	0.21

Preliminary phytochemical analysis of *Nilapanai choornam*

The phytochemical analysis of *Nilapanai choornam* showed the presence of the following active constituents.

Table 2:

Phytochemicals	Test used	Chloroform	Methanol
Alkaloids	Dragendroff	-	+
Flavanoids	Shinado	+	+
Glycosides	Legal's test	+	+
Saponins	Foam test	-	-
Tannins	Ferric chloride	+	-
Phytosterol	Liberman	-	-
Triterpenoids	Noller's test	+	-

Thin layer chromatographic analysis of *Nilapanai choornam*

The TLC separation of *Nilapanai Choornam* and the R_f values of the visible compounds and compounds at 360nm from bottom to top are as follows.

Visible compounds

Table 3:

Solvent front	R_f Values	
	Ether extract	Chloroform extract
	0.03	0.05

Fig. 2: TLC of Visible compounds of *Nilapanai choornam*

Compounds at 360nm

Table 4:

Solvent front	R_f Values		
	Methanol	Chloroform	Ether
Petroleum	0.14	0.21	0.32

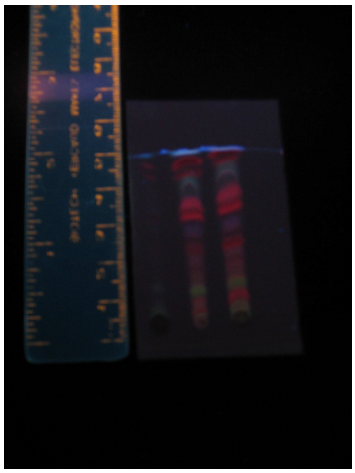

Fig. 3: TLC of Fluorescent compounds of *Nilapanai choornam at 360nm*

DISCUSSION

In Siddha system of medicine, the line of treatment starts from herbal drugs only. It can be well understood from the famous quote "Ver parru thazhai paru, minjinakkal parpa chendhooram pare". "Unave marunthu marunthe unavu" is the basic concept of Siddhars. Hence by this saying, it is well understood that the diet habits are the major cause of derangement in the 3 humors and hence diseases are produced. It is confirmed by Siddhar Thiruvalluvar by the saying "Miginum kuraiyinum noi seyyum noolor Valimudhala enniya moondru".

Vellai noi as mentioned earlier is mainly caused by the derangement of pitha humour. Hence in treating vellai noi the drug should be aimed to possess characters that reduces the pitha humour and reduces the symptoms of vellai noi.

Nilapanai choornam is a compound drug containing Nilapanai kizhangu, Nerunji mul, Nelli, Ponnaikkali vidhai, Mul Ilavam pisin, Seenthil chakkarai and Karkandu as ingredients.

Nilapanai itself is indicated in Ozhukku vellai and it is said to possess immunostimulatory effect, estrogenic activity and antioxidant properties. Nerunji mul is a coolent, astringent and is indicated in vellai noi. It is also useful in associated burning micturition and dysuria since it is a diuretic. It also dissipates the heat produced in the body. Nelli vattral is a good astringent and indicated in pitha disorders.

Poonaikkali is an astringent and possess antimicrobial anti inflammatory and antioxidant properties. Seendhil chakkarai is a good alterative, tonic and mild diuretic. So it may help weak patients. Mul Ilavam pisin is an astringent and indicated for Thandhu megam. Karkandu is an antiseptic and a demulcent. Hence the drugs, individually has good potentials to treat vellai noi.

Firstly the macroscopic characters of the ingredients and the microscopy of the choornam were done to ascertain the presence of the ingredients and authenticate them. The macroscopic study of the ingredients revealed the authenticity of the ingredients. The microscopic study of the choornam revealed the presence of the individual ingredients in the compound drug as a whole. This may pave way in future to use the same authenticated ingredients and develop standards for the choornam.

The physical characterization of the drug revealed its pH to be acidic and the other parameters like ash value etc may help to authenticate the drug in future too. The preliminary phytochemical analysis of Nilapanai choornam revealed the presence of Glycoside, Tannins, alkaloids, Triterpenes, Flavonoids that supports the literature study of the individual ingredients of the drug. Tannin containing drugs will precipitate protein and have been used traditionally as styptics and internally for the protection of inflamed surfaces. Flavanoids are good antioxidants.

In Thin layer chromatographic analysis the solvent front was standardized as Petroleum ether: Chloroform: Methanol 1:0:5:0.1. The methanol, chloroform and ether extracts were fractionized. The ether and chloroform extracts contained more number of visible compounds. There were no visible compounds at methanol extraction. Instead 4 compounds fluoresced at 360nm. Apart from the visible compounds the chloroform and ether extracts showed 5 and 4 more fluorescing compounds at 360nm respectively.

CONCLUSION

To conclude, the present study has revealed the microscopic, phytochemistry, physicochemical and the TLC fingerprint of the drug. Further studies should be carried out to generate the HPTLC and HPLC fingerprinting of the drug for maintaining the quality control of the drug.

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