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CHEMICAL STANDARDSATION OF NILAPPANAI CHOORANAM - A POLY HERBAL FORMULATION USED IN SIDDHA FOR THE MANAGEMENT OF OLIGOSPERMIA

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ABSTRACT

Siddha system of medicine (SSM) is one of the oldest traditional systems of medicine, which has been originated from India and is practised mostly in the southern part of the country for treating various diseases including even chronic conditions. Man has been using plants and plant based medicines for combating diseases since time immemorial. The plant materials and other raw materials are sold in the crude drug market in different vernacular names, which leads to confusion of one drug to the other. Adulteration and substitution of the genuine drug with drug of similar morphological characters which belong to different species or genus, sometimes entirely different family is being offered in the market. Moreover the percentage compositions of the plant constituents vary due to climatic conditions, terrestrial conditions and maturity of the plant. Standardization embodies total information, and controls that are necessary to guarantee consistency of composition of the product ensuring their quality. The aim of the present study is to standardize a Siddha formulation, Nilappana chooranam based on the evaluation of pharmacognostical, physicochemical, toxicological and HPTLC parameters of the formulation. Nilappanai chooranam was prepared with genuine drugs and analysed for fixing standards for the drug. Different parameters like organoleptic characters, physico-chemical parameters, HPTLC profiles, heavy metals and other metallic components, microbial contamination, pesticide residues and aflatoxins were determined for the purpose of quality evaluation and standardisation. The results obtained may be used as a diagnostic tool to identify and to determine the quality and purity of the drug.

KEYWORDS: Nilappanai Chooranam, Physico-Chemical Parameters, HPTLC Profiles, Microbial Contamination, Pesticide Residues.

INTRODUCTION

Ayurveda, Siddha and Unani (ASU) systems of healthcare are largely based on drugs of plant origin and so many herbs are used as single drugs, and as raw materials for the preparation of compound formulations. These materials are sold in the crude drug market in different vernacular names, which leads to confusion of one drug to the other. Adulteration and substitution of the genuine drug with drug of similar morphological characters which belong to different species or genus, sometimes entirely different family is being offered in the market. Moreover the percentage compositions of the plant constituents vary due to climatic conditions, terrestrial conditions and maturity of the plant. Standardisation of drug means confirmation of its identity, quality and purity by the determination of certain nationally or internationally accepted properties. For any quality medicine, the quality control methods are important. Standardisation and Quality control of ASU drugs includes Botanical identification, Chemical analysis and Toxicological evaluation.

Siddha system of medicine (SSM) is one of the oldest traditional system of medicine, which has been originated from India and is practised mostly in the southern part of the country for treating various diseases including even chronic conditions.[1] Siddha system of medicine is a comprehensive health system that includes diagnostic, preventive and curative aspects. Man has been using plants and plant based medicines for combating diseases since time immemorial. Indian systems of medicine have a deep root in our cultural heritage and provide healthcare benefits to large sections of our population. Effectiveness, easy availability, low cost and comparatively less toxic effects are responsible factors to popularize herbal remedies. But it is observed that there is no uniformity in the aspects of quality, efficacy and safety with respect to the single drugs as

well as compound preparations manufactured by various pharmaceuticals due to various reasons.

Worldwide, the herbal drug market at present is increasing at the rate of 15% per annum. One of the major bottlenecks in the wider acceptance of herbal drugs from developing countries is the inadequacy or lack of standardization for the raw materials and for the finished products. It necessitates the need for standardization of ASU drugs and medicinal preparations. Standardization embodies total information and controls that are necessary to guarantee consistency of composition of the product ensuring their quality.

The aim of the present study is to standardize a Siddha formulation, Nilappana chooranam following WHO and FDA guidelines based on the evaluation of pharmacognostical, phytochemical, physicochemical and HPTLC parameters of the formulation.[2] Nilapanai chooranam is used to treat *Vidhanu kuraivu* (oligospermia) and *Vellai noi* (leucorrhoea).[3] Chooranam is a fine powder of one or more drugs. The drugs are separately powdered, sieved, weighed separately and mixed together. The powders of the drugs should pass through 180 µm IS sieve (sieve no.85). The required quantities by weight are taken and thoroughly mixed to uniformity. It should be kept in clean, dry, airight glass containers.

In the present study, different parameters like Organoleptic characters, Physico-chemical parameters, Phytochemical components, High performance thin layer chromatographic (HPTLC) profiles, Heavy Metals and other Metallic Components, Microbial Contamination, Pesticide residues and Aflatoxins were determined for the purpose of quality evaluation and standardisation The results obtained may be used as a diagnostic tool to identity and to determine the quality and purity. The chooranam was prepared with genuine drugs for analysing to fix standards for the drug.

MATERIALS AND METHODS

1. SOP (Standard Operating Procedures) of Nilappanai chooranam: Nilappanai chooranam is a compound formulation having seven ingredients and was prepared by the Clinical Research Section, Siddha Regional Research Institute, Poojappura, Thiruvananthapuram by the method described in Kannusamy parambarai vaidhyam.[4] The ingredients of the preparation are given in Table - I.

Table. 1: Formulation Composition.

Sl. No.	Name of raw drug	Botanical name	Part	Quantity
1	Nilappanai kizhangu	Curculigo orchiodes Gaertn.	Rhizome	100 gm
2	Poonaikali vithai	Mucuna pruriens Bak.	Seed	100 gm
3	Nerinjil mul	Tribulus terrestris Linn.	Fruit	100 gm
4	Nelli vatral	Emblica officinalis Gaertn.	Fruit (Dried)	100 gm
5	Mulillavam pisin	Bombax malabaricum DC.	Exudate	100 gm
6	Seenthil sarkarai	Tinospora cordifolia (Willd.) Miers	Stem (Extract)	100 gm
7	Panang karkandu	Borasses flabellifer Linn.	Fruit (Secondary product)	100 gm

The raw drugs were procured from Thiruvananthapuram, identified and authenticated by a botanist, University of Kerala, Thiruvananthapuram. First of all the foreign matter, if any, was removed. The Poonaikali vithai was purified by boiling in milk; the seed coat was removed and dried. All the other drugs were dried under sunlight. All the drugs were powdered separately and mixed in the ratio as given in the table. The powder was sieved by the traditional method and stored in a clean glass airtight container for further studies. Three batches of the chooranam were prepared as above.

2. Organoleptic Characters: The organoleptic characters such as colour, touch, taste and odour were noted.

3. Physico-chemical parameters: The Physico-chemical parameters such as total ash, acid insoluble ash, extractable matter in water and alcohol, loss on drying at 105°C, pH value, total sugar, reducing sugar and volatile oil were determined by standard methods.[5]

4. Preliminary phytochemical analysis: For preliminary phytochemical studies, 5g powdered Nilapanai chooranam was successively extracted using soxhlet apparatus with petroleum ether, chloroform, ethanol and water. The extracts were concentrated by distilling off the solvents under reduced pressure. The presences of different phyto-constituents were determined by standard procedures.[6,7]

5. High performance thin layer chromatographic analysis (HPTLC)

a. Sample preparation: Extract of the chooranam was prepared by boiling 1g of the drug in 10 ml ethanol. The filtrates were concentrated on a water bath to 1 ml. This extract was used for chromatographic studies.[8]

b. Development and documentation of HPTLC

Alcohol extract of the chooranam as prepared above was spotted in the form of bands with Camag microlitre syringe on a precoated silica gel 60 F254 (Merck) plate with Automatic TLC Sampler 4 (ATS4). Mobile phase used was Toluene: Ethyl acetate: Formic acid (5: 2: 0.1). Linear ascending development was done in 10 cm x 10 cm twin trough glass chamber saturated with mobile phase saturated with mobile phase. The plate was air dried and kept in CAMAG visualizer and the images

were captured under UV light at 254 nm and 366 nm. Densitometric scanning was performed using CAMAG TLC Scanner 4 which is operated by winCATS software. The sources of radiation utilized were deuterium lamp and mercury lamp. The bands were analyzed at a wavelength of 254 nm and 366nm. The slit dimensions used in the analysis were 8.00 x 0.40 mm, Macro. The R_f values and finger print profile were recorded. Concentrations of compound chromatographed were evaluated as peak areas. The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105ºC by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. After that the plate was densitometrically scanned for finger print profile study at 575 nm using tungsten light source.[9]

6. Heavy Metals and other Metallic Components: The heavy/toxic metals, lead, cadmium, arsenic and mercury and other metals manganese, potassium, magnesium, zinc, aluminium, iron, calcium and sodium were determined using Atomic Absorption Spectrophotometer (AAS).

7. Microbial Contamination: The microbial contaminations Total viable count[10], *Pseudomonas aeruginosa*[11], Total fungal count count[12], *Escheria colii*[13], *Salmonella* sp.[14], *Staphylococcus aureus*[15] and Enterobacteriaceae[16] were determined at CEPC (Cashew Export Promotion Council of India) Laboratory and Technical Division, Kollam, Kerala.

8. Pesticide Residues: The Organophosphorus Pesticides such as Dichlorvos, Ethoprofos, Disulfofon, Parathion Methyl, Fenchlorphos, Prothiofos, Guthion, Fenithrothion, Malathion and Chlorpyrifos; and Organochlorine pesticides such as EndosulfanI, EndosulfanII, Heptachlor, Endrin, 44'DDD, 44'DDE, 44'DDT, Alpha BHC, Beta BHC, Heptachlorepoxide, Aldrin, Dieldrin, Gamma BHCand Delta BHC were determined at CEPC (Cashew Export Promotion Council of India) Laboratory and Technical Division, Kollam, Kerala.[17]

9. Aflatoxins: Aflatoxins G2, G1, B2 and B1were determined at CEPC (Cashew Export Promotion Council of India) Laboratory and Technical Division, Kollam, Kerala.[18]

RESULTS AND DISCUSSION

Nilappanai chooranam and its ingredients are given in Fig 1 and was prepared as mentioned in Materials and methods.

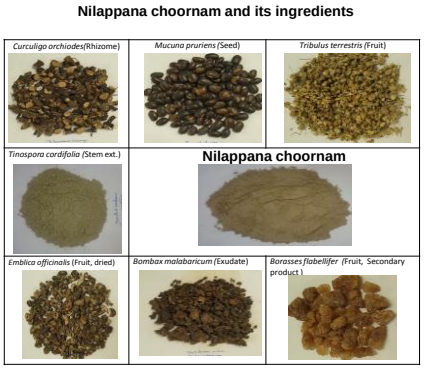


Figure. I: Nilappanai chooranam and its ingredients.

Nilappanai chooranam prepared was a pale brown smooth powder with characteristic odour and astringent taste.

The Physico-chemical parameters of 3 batches of the chooranam were analysed and the average value of results obtained is reported (Table 2). All the experiments were repeated till concordant results were obtained.

Table. 2: Physico-chemical parameters of Nilappanai chooranam.

Sl. No.	Parameters	Results
1.	Loss on Drying at 105^0C %	11.37
2.	Total Ash Content %	6.87
3.	Acid Insoluble Ash %	1.54
4.	Water Soluble Extractive %	25.46
5.	Alcohol Soluble Extractive %	17.20
6.	Volatile oil %	0.5
7.	pH	3.5
8.	Total Sugar	3.07
9.	Reducing Sugar	3.00

Preliminary phytochemical analysis showed the presence of Polyphenols, Steroids, Tannins Alkaloids, Flavonoids, Glycosides and terpenoids. High Performance Thin Layer Chromatographic (HPTLC) studies of Nilappanai chooranam was carried out. The images obtained for the three batches of chooranam are given below (Fig 2).

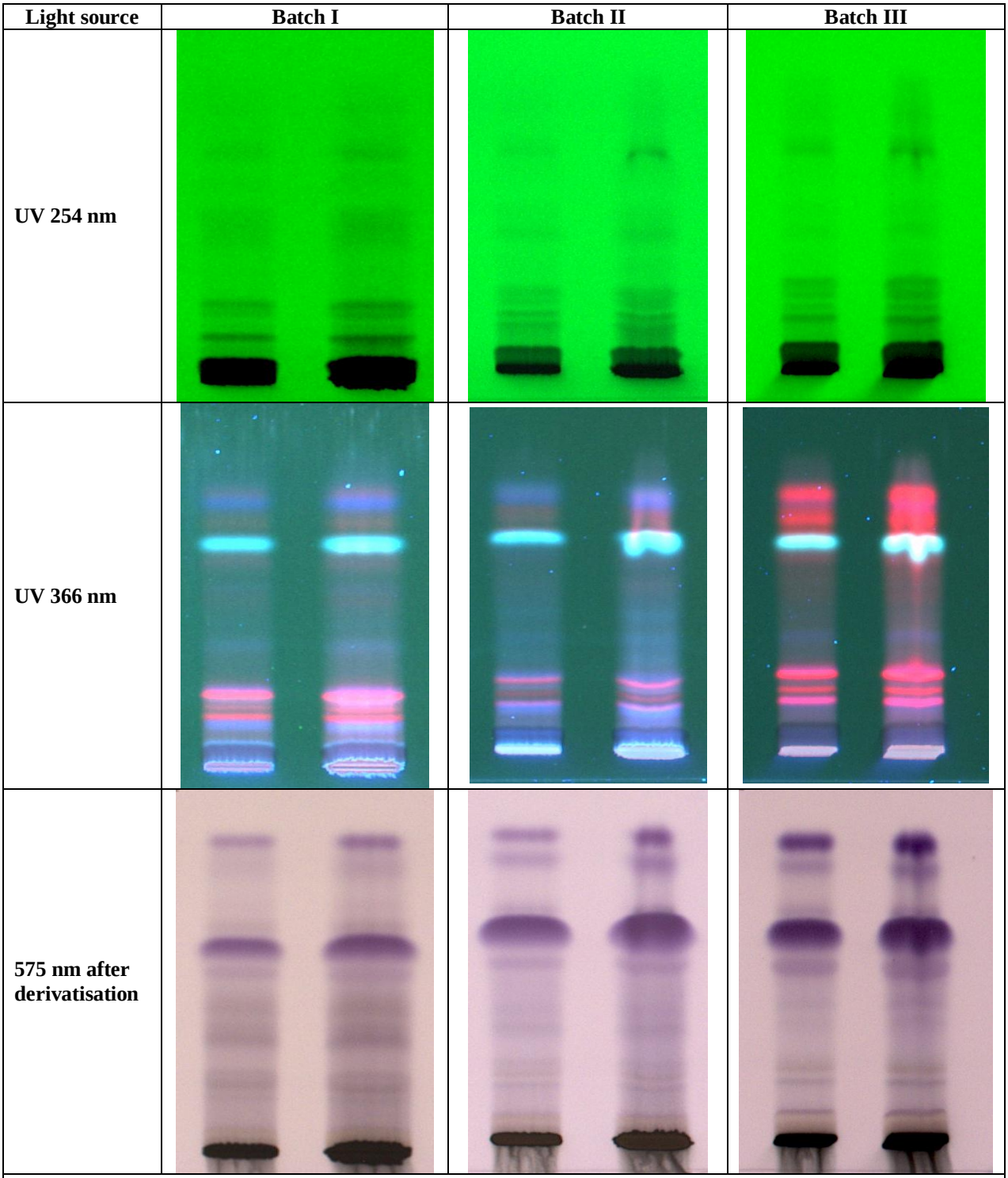
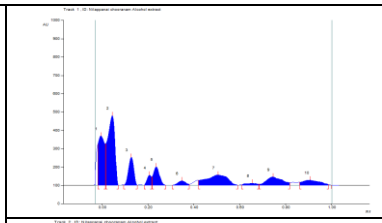


Fig. 2: HPTLC photo documentation of Ethyl alcohol extract of Nilappanai chooranam Solvent system: Toluene: Ethyl acetate: Formic acid (5: 2: 0.1); Volume applied: Track 1- 10 µl; Track 2 – 20 µl.

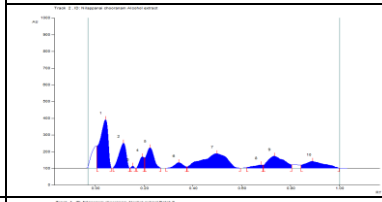
The fingerprint profiles and R_f values of the 3 batches of chooranam at 254 nm, 366 nm and 575 nm are given in Fig 3, 4 and 5 respectively. The R_f values and colour of major bands obtained for the 3 batches at 254 nm, 366 nm and 575 nm are given in Table 3, 4 and 5 respectively.

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and is the simplest separation technique today available to the analyst.[19] HPTLC is a micro analytical separation and determination method which has a wide application in herbal drug analysis.

Batch I

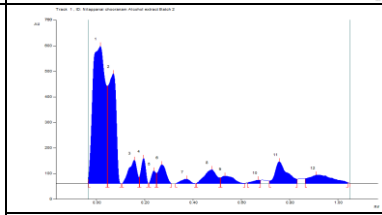


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	222.9 AU	-0.00 Rf	269.6 AU	24.00 %	0.02 Rf	25.0 AU	5195.1 AU	19.56 %
2	0.02 Rf	226.0 AU	0.05 Rf	379.8 AU	33.82 %	0.07 Rf	0.6 AU	8164.9 AU	30.73 %
3	0.09 Rf	1.5 AU	0.13 Rf	152.4 AU	13.57 %	0.16 Rf	0.4 AU	2439.7 AU	9.18 %
4	0.19 Rf	0.6 AU	0.21 Rf	54.9 AU	4.88 %	0.22 Rf	45.5 AU	715.3 AU	2.69 %
5	0.22 Rf	46.1 AU	0.24 Rf	100.2 AU	8.92 %	0.28 Rf	0.1 AU	1801.3 AU	6.78 %
6	0.31 Rf	0.1 AU	0.35 Rf	24.0 AU	2.14 %	0.38 Rf	4.8 AU	570.6 AU	2.15 %
7	0.42 Rf	26.4 AU	0.51 Rf	56.8 AU	5.06 %	0.59 Rf	0.0 AU	3647.8 AU	13.73 %
8	0.61 Rf	2.5 AU	0.66 Rf	11.5 AU	1.02 %	0.68 Rf	5.4 AU	369.6 AU	1.39 %
9	0.69 Rf	5.6 AU	0.75 Rf	45.5 AU	4.05 %	0.82 Rf	14.6 AU	2195.5 AU	8.26 %
10	0.86 Rf	15.6 AU	0.91 Rf	28.3 AU	2.52 %	0.99 Rf	4.7 AU	1466.1 AU	5.52 %



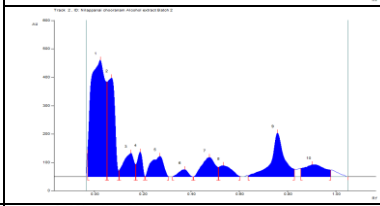
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	132.6 AU	0.05 Rf	290.9 AU	32.45 %	0.07 Rf	0.0 AU	6577.8 AU	24.89 %
2	0.07 Rf	1.4 AU	0.12 Rf	149.8 AU	16.72 %	0.14 Rf	0.2 AU	3024.6 AU	11.44 %
3	0.14 Rf	1.4 AU	0.16 Rf	12.5 AU	1.40 %	0.17 Rf	0.1 AU	95.7 AU	0.36 %
4	0.17 Rf	0.4 AU	0.19 Rf	68.0 AU	7.59 %	0.20 Rf	62.4 AU	914.2 AU	3.46 %
5	0.20 Rf	62.5 AU	0.23 Rf	122.9 AU	13.71 %	0.27 Rf	0.4 AU	2575.7 AU	9.74 %
6	0.29 Rf	0.0 AU	0.34 Rf	33.1 AU	3.69 %	0.38 Rf	8.0 AU	829.1 AU	3.14 %
7	0.38 Rf	8.2 AU	0.50 Rf	86.2 AU	9.62 %	0.60 Rf	0.1 AU	6177.3 AU	23.37 %
8	0.62 Rf	4.8 AU	0.68 Rf	19.7 AU	2.20 %	0.69 Rf	18.2 AU	539.8 AU	2.04 %
9	0.69 Rf	18.4 AU	0.74 Rf	72.2 AU	8.05 %	0.81 Rf	21.2 AU	3368.3 AU	12.74 %
10	0.84 Rf	18.1 AU	0.89 Rf	41.0 AU	4.57 %	1.00 Rf	0.0 AU	2329.0 AU	8.81 %

Batch II



Track 1, ID: Nilappanai chooranam Alcohol extract Batch 2

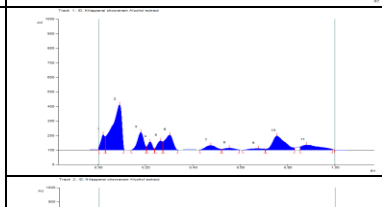
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	16.3 AU	0.02 Rf	537.5 AU	35.51 %	0.05 Rf	81.3 AU	19318.5 AU	44.66 %
2	0.05 Rf	381.4 AU	0.07 Rf	429.2 AU	28.35 %	0.10 Rf	0.2 AU	9260.8 AU	21.41 %
3	0.11 Rf	0.1 AU	0.16 Rf	91.7 AU	6.06 %	0.18 Rf	24.1 AU	2115.0 AU	4.89 %
4	0.18 Rf	26.7 AU	0.20 Rf	98.6 AU	6.51 %	0.22 Rf	2.2 AU	1263.0 AU	2.92 %
5	0.22 Rf	1.4 AU	0.24 Rf	49.3 AU	3.26 %	0.25 Rf	40.7 AU	621.4 AU	1.44 %
6	0.25 Rf	40.9 AU	0.27 Rf	73.8 AU	4.87 %	0.31 Rf	0.3 AU	1616.3 AU	3.74 %
7	0.33 Rf	0.4 AU	0.38 Rf	17.0 AU	1.13 %	0.41 Rf	0.2 AU	460.6 AU	1.06 %
8	0.41 Rf	0.9 AU	0.48 Rf	54.5 AU	3.60 %	0.51 Rf	23.2 AU	1878.1 AU	4.34 %
9	0.51 Rf	23.2 AU	0.53 Rf	29.1 AU	1.92 %	0.62 Rf	1.0 AU	1084.1 AU	2.51 %
10	0.63 Rf	3.2 AU	0.67 Rf	13.9 AU	0.92 %	0.68 Rf	12.7 AU	316.5 AU	0.73 %
11	0.72 Rf	10.6 AU	0.76 Rf	85.7 AU	5.66 %	0.83 Rf	17.4 AU	2968.3 AU	6.86 %
12	0.87 Rf	19.5 AU	0.91 Rf	33.5 AU	2.21 %	1.04 Rf	4.7 AU	2358.5 AU	5.45 %



Track 2, ID: Nilappanai chooranam Alcohol extract Batch 2

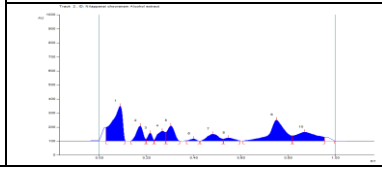
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	93.9 AU	0.03 Rf	410.3 AU	30.89 %	0.05 Rf	33.9 AU	16842.0 AU	39.15 %
2	0.05 Rf	334.0 AU	0.07 Rf	347.9 AU	26.19 %	0.10 Rf	21.0 AU	6981.6 AU	16.23 %
3	0.10 Rf	22.5 AU	0.15 Rf	82.8 AU	6.23 %	0.17 Rf	43.6 AU	2447.4 AU	5.69 %
4	0.17 Rf	43.7 AU	0.19 Rf	86.9 AU	6.54 %	0.21 Rf	1.4 AU	1268.8 AU	2.95 %
5	0.21 Rf	0.8 AU	0.27 Rf	71.9 AU	5.41 %	0.31 Rf	0.4 AU	2494.1 AU	5.80 %
6	0.32 Rf	0.0 AU	0.38 Rf	25.2 AU	1.90 %	0.41 Rf	0.1 AU	669.2 AU	1.56 %
7	0.41 Rf	0.3 AU	0.48 Rf	68.0 AU	5.12 %	0.51 Rf	33.4 AU	2549.8 AU	5.93 %
8	0.51 Rf	33.5 AU	0.54 Rf	39.2 AU	2.95 %	0.61 Rf	1.0 AU	1406.8 AU	3.27 %
9	0.64 Rf	3.7 AU	0.76 Rf	154.2 AU	11.61 %	0.83 Rf	26.8 AU	5773.8 AU	13.42 %
10	0.86 Rf	29.5 AU	0.90 Rf	42.0 AU	3.16 %	0.98 Rf	23.8 AU	2590.9 AU	6.02 %

Batch III



Track 1, ID: Nilappanai chooranam Alcohol extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	8.0 AU	0.02 Rf	105.5 AU	11.12 %	0.03 Rf	93.2 AU	1259.3 AU	5.44 %
2	0.03 Rf	94.1 AU	0.09 Rf	311.8 AU	32.86 %	0.11 Rf	6.5 AU	8401.3 AU	36.31 %
3	0.14 Rf	0.2 AU	0.18 Rf	121.6 AU	12.81 %	0.20 Rf	23.1 AU	2176.8 AU	9.41 %
4	0.21 Rf	23.5 AU	0.22 Rf	56.8 AU	5.99 %	0.24 Rf	9.3 AU	740.1 AU	3.20 %
5	0.24 Rf	10.4 AU	0.26 Rf	63.3 AU	6.67 %	0.27 Rf	57.4 AU	917.2 AU	3.96 %
6	0.28 Rf	57.7 AU	0.30 Rf	103.5 AU	10.90 %	0.34 Rf	0.4 AU	2396.5 AU	10.36 %
7	0.43 Rf	1.1 AU	0.48 Rf	31.3 AU	3.30 %	0.52 Rf	6.1 AU	988.3 AU	4.27 %
8	0.52 Rf	6.2 AU	0.56 Rf	13.7 AU	1.45 %	0.60 Rf	1.8 AU	410.0 AU	1.77 %
9	0.61 Rf	0.4 AU	0.68 Rf	10.5 AU	1.10 %	0.71 Rf	7.2 AU	404.7 AU	1.75 %
10	0.71 Rf	7.3 AU	0.76 Rf	96.5 AU	10.17 %	0.83 Rf	15.2 AU	3546.2 AU	15.33 %
11	0.85 Rf	21.7 AU	0.88 Rf	34.3 AU	3.62 %	0.99 Rf	5.8 AU	1895.9 AU	8.19 %

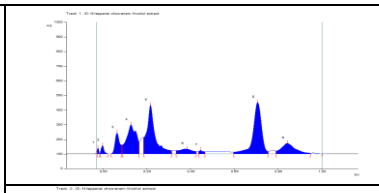


Track 2, ID: Nilappanai chooranam Alcohol extract

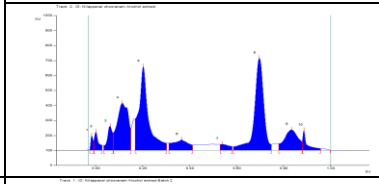
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	96.6 AU	0.09 Rf	247.6 AU	28.13 %	0.11 Rf	2.1 AU	7301.8 AU	27.61 %
2	0.14 Rf	0.2 AU	0.18 Rf	106.5 AU	12.10 %	0.20 Rf	13.0 AU	2007.7 AU	7.59 %
3	0.20 Rf	13.4 AU	0.22 Rf	55.0 AU	6.25 %	0.23 Rf	6.7 AU	691.7 AU	2.62 %
4	0.24 Rf	7.3 AU	0.27 Rf	69.7 AU	7.92 %	0.28 Rf	61.4 AU	1507.5 AU	5.70 %
5	0.28 Rf	61.8 AU	0.31 Rf	107.0 AU	12.16 %	0.34 Rf	0.2 AU	2230.1 AU	8.43 %
6	0.37 Rf	1.9 AU	0.40 Rf	14.2 AU	1.62 %	0.43 Rf	0.0 AU	256.4 AU	0.97 %
7	0.43 Rf	0.1 AU	0.48 Rf	48.3 AU	5.49 %	0.53 Rf	13.8 AU	1627.1 AU	6.15 %
8	0.53 Rf	14.1 AU	0.55 Rf	20.7 AU	2.35 %	0.60 Rf	1.5 AU	597.0 AU	2.26 %
9	0.61 Rf	0.3 AU	0.75 Rf	148.4 AU	16.86 %	0.82 Rf	36.7 AU	6451.9 AU	24.39 %
10	0.82 Rf	36.8 AU	0.87 Rf	62.6 AU	7.12 %	0.96 Rf	26.9 AU	3776.7 AU	14.28 %

Fig. 3: HPTLC finger print profile of 10µl and 20µl of Alcohol extract Nilappanai chooranam at 254 nm.

Batch I

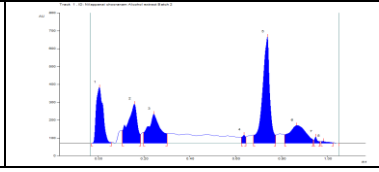


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	25.9 AU	-0.02 Rf	39.5 AU	3.15 %	-0.01 Rf	0.0 AU	180.5 AU	0.56 %
2	-0.01 Rf	4.2 AU	0.00 Rf	58.1 AU	4.64 %	0.02 Rf	0.3 AU	508.1 AU	1.59 %
3	0.04 Rf	0.3 AU	0.06 Rf	144.0 AU	11.50 %	0.09 Rf	60.1 AU	2007.0 AU	6.27 %
4	0.09 Rf	60.4 AU	0.13 Rf	199.3 AU	15.90 %	0.17 Rf	83.2 AU	6189.5 AU	19.32 %
5	0.19 Rf	112.3 AU	0.22 Rf	331.9 AU	26.49 %	0.31 Rf	21.9 AU	8652.3 AU	27.01 %
6	0.34 Rf	21.8 AU	0.38 Rf	32.8 AU	2.62 %	0.43 Rf	16.7 AU	1466.1 AU	4.58 %
7	0.44 Rf	15.6 AU	0.45 Rf	24.5 AU	1.96 %	0.47 Rf	14.9 AU	324.7 AU	1.01 %
8	0.60 Rf	11.4 AU	0.71 Rf	351.0 AU	28.01 %	0.76 Rf	16.5 AU	9525.6 AU	29.74 %
9	0.79 Rf	19.6 AU	0.84 Rf	71.8 AU	5.73 %	0.95 Rf	4.4 AU	3175.8 AU	9.92 %



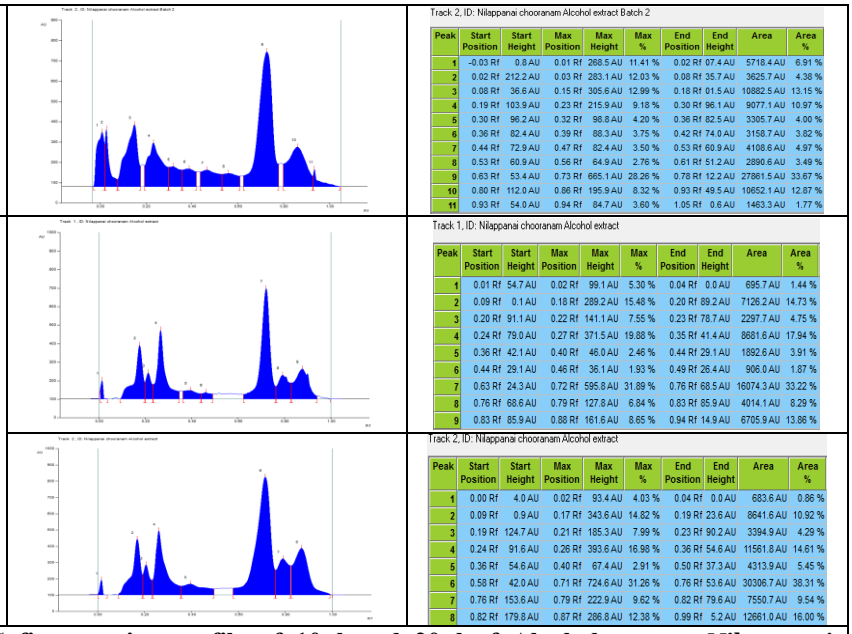
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.0 AU	-0.01 Rf	96.8 AU	4.32 %	-0.01 Rf	62.6 AU	671.0 AU	1.13 %
2	-0.00 Rf	68.7 AU	0.00 Rf	122.0 AU	5.44 %	0.03 Rf	33.5 AU	1460.0 AU	2.46 %
3	0.04 Rf	30.6 AU	0.06 Rf	159.9 AU	7.13 %	0.08 Rf	16.1 AU	2256.3 AU	3.80 %
4	0.08 Rf	116.5 AU	0.12 Rf	313.9 AU	14.00 %	0.15 Rf	46.2 AU	10582.7 AU	17.84 %
5	0.17 Rf	209.5 AU	0.20 Rf	558.0 AU	24.89 %	0.30 Rf	48.2 AU	15967.1 AU	26.92 %
6	0.31 Rf	46.5 AU	0.37 Rf	71.0 AU	3.17 %	0.41 Rf	35.0 AU	3254.8 AU	5.49 %
7	0.53 Rf	38.2 AU	0.54 Rf	39.2 AU	1.75 %	0.58 Rf	24.8 AU	1068.8 AU	1.80 %
8	0.59 Rf	23.5 AU	0.70 Rf	613.0 AU	27.34 %	0.75 Rf	39.0 AU	16949.8 AU	28.58 %
9	0.78 Rf	43.4 AU	0.83 Rf	136.1 AU	6.07 %	0.88 Rf	57.0 AU	5422.1 AU	9.14 %
10	0.88 Rf	64.9 AU	0.89 Rf	131.9 AU	5.88 %	0.96 Rf	10.3 AU	1683.6 AU	2.84 %

Batch II



Track 1, ID: Nilappanai chooranam Alcohol extract Batch 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	0.1 AU	0.01 Rf	312.3 AU	21.05 %	0.06 Rf	4.7 AU	6424.0 AU	17.14 %
2	0.11 Rf	71.2 AU	0.16 Rf	219.9 AU	14.82 %	0.19 Rf	50.2 AU	6218.3 AU	16.59 %
3	0.20 Rf	61.3 AU	0.25 Rf	162.8 AU	10.97 %	0.30 Rf	54.7 AU	5822.4 AU	15.54 %
4	0.63 Rf	33.9 AU	0.64 Rf	46.7 AU	3.14 %	0.64 Rf	36.1 AU	469.1 AU	1.25 %
5	0.68 Rf	45.3 AU	0.74 Rf	595.5 AU	40.13 %	0.77 Rf	46.0 AU	12981.5 AU	34.64 %
6	0.81 Rf	48.2 AU	0.87 Rf	99.8 AU	6.73 %	0.94 Rf	20.2 AU	5010.1 AU	13.37 %
7	0.94 Rf	17.2 AU	0.95 Rf	35.6 AU	2.40 %	0.97 Rf	9.1 AU	318.7 AU	0.85 %
8	0.97 Rf	9.4 AU	0.98 Rf	11.2 AU	0.76 %	1.02 Rf	3.6 AU	232.7 AU	0.62 %



Track 2, ID: Nilappanai chooranam Alcohol extract Batch 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	0.8 AU	0.01 Rf	268.5 AU	11.41 %	0.02 Rf	07.4 AU	5718.4 AU	6.91 %
2	0.02 Rf	212.2 AU	0.03 Rf	283.1 AU	12.03 %	0.08 Rf	35.7 AU	3625.7 AU	4.38 %
3	0.08 Rf	36.6 AU	0.15 Rf	305.6 AU	12.99 %	0.18 Rf	01.5 AU	10882.5 AU	13.15 %
4	0.19 Rf	103.9 AU	0.23 Rf	215.9 AU	9.18 %	0.30 Rf	96.1 AU	9077.1 AU	10.97 %
5	0.30 Rf	96.2 AU	0.32 Rf	98.8 AU	4.20 %	0.36 Rf	82.5 AU	3305.7 AU	4.00 %
6	0.36 Rf	82.4 AU	0.39 Rf	88.3 AU	3.75 %	0.42 Rf	74.0 AU	3158.7 AU	3.82 %
7	0.44 Rf	72.9 AU	0.47 Rf	82.4 AU	3.50 %	0.53 Rf	60.9 AU	4108.6 AU	4.97 %
8	0.53 Rf	60.9 AU	0.56 Rf	64.9 AU	2.76 %	0.61 Rf	51.2 AU	2890.6 AU	3.49 %
9	0.63 Rf	53.4 AU	0.73 Rf	665.1 AU	28.26 %	0.78 Rf	12.2 AU	27861.5 AU	33.67 %
10	0.80 Rf	112.0 AU	0.86 Rf	195.9 AU	8.32 %	0.93 Rf	49.5 AU	10652.1 AU	12.87 %
11	0.93 Rf	54.0 AU	0.94 Rf	84.7 AU	3.60 %	1.05 Rf	0.6 AU	1463.3 AU	1.77 %

Batch III

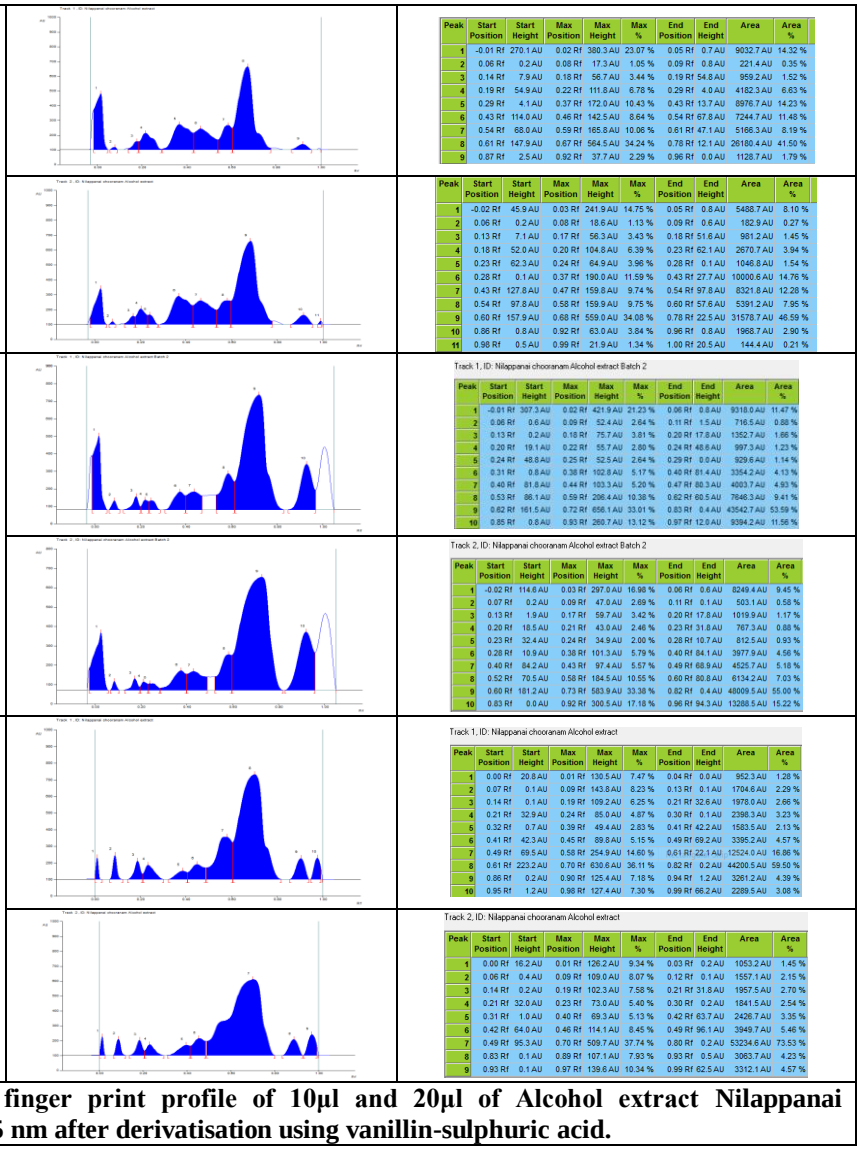
Track 1, ID: Nilappanai chooranam Alcohol extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	54.7 AU	0.02 Rf	99.1 AU	5.30 %	0.04 Rf	0.0 AU	695.7 AU	1.44 %
2	0.09 Rf	0.1 AU	0.18 Rf	289.2 AU	15.48 %	0.20 Rf	89.2 AU	7126.2 AU	14.73 %
3	0.20 Rf	91.1 AU	0.22 Rf	141.1 AU	7.55 %	0.23 Rf	78.7 AU	2297.7 AU	4.75 %
4	0.24 Rf	79.0 AU	0.27 Rf	371.5 AU	19.88 %	0.35 Rf	41.4 AU	8681.6 AU	17.94 %
5	0.36 Rf	42.1 AU	0.40 Rf	46.0 AU	2.46 %	0.44 Rf	29.1 AU	1892.6 AU	3.91 %
6	0.44 Rf	29.1 AU	0.46 Rf	36.1 AU	1.93 %	0.49 Rf	26.4 AU	906.0 AU	1.87 %
7	0.63 Rf	24.3 AU	0.72 Rf	595.8 AU	31.89 %	0.76 Rf	68.5 AU	16074.3 AU	33.22 %
8	0.76 Rf	68.6 AU	0.79 Rf	127.8 AU	6.84 %	0.83 Rf	85.9 AU	4014.1 AU	8.29 %
9	0.83 Rf	85.9 AU	0.88 Rf	161.6 AU	8.65 %	0.94 Rf	14.9 AU	6705.9 AU	13.86 %

Track 2, ID: Nilappanai chooranam Alcohol extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	4.0 AU	0.02 Rf	93.4 AU	4.03 %	0.04 Rf	0.0 AU	683.6 AU	0.86 %
2	0.09 Rf	0.9 AU	0.17 Rf	343.6 AU	14.82 %	0.19 Rf	23.6 AU	8641.6 AU	10.92 %
3	0.19 Rf	124.7 AU	0.21 Rf	185.3 AU	7.99 %	0.23 Rf	90.2 AU	3394.9 AU	4.29 %
4	0.24 Rf	91.6 AU	0.26 Rf	393.6 AU	16.98 %	0.36 Rf	54.6 AU	11561.8 AU	14.61 %
5	0.36 Rf	54.6 AU	0.40 Rf	67.4 AU	2.91 %	0.50 Rf	37.3 AU	4313.9 AU	5.45 %
6	0.58 Rf	42.0 AU	0.71 Rf	724.6 AU	31.26 %	0.76 Rf	53.6 AU	30306.7 AU	38.31 %
7	0.76 Rf	153.6 AU	0.79 Rf	222.9 AU	9.62 %	0.82 Rf	79.6 AU	7550.7 AU	9.54 %
8	0.82 Rf	179.8 AU	0.87 Rf	286.8 AU	12.38 %	0.99 Rf	5.2 AU	12661.0 AU	16.00 %

Fig. 4: HPTLC finger print profile of 10µl and 20µl of Alcohol extract Nilappanai chooranam at 366 nm.



Batch I

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	270.1 AU	0.02 Rf	380.3 AU	23.07 %	0.05 Rf	0.7 AU	9032.7 AU	14.32 %
2	0.06 Rf	0.2 AU	0.08 Rf	17.3 AU	1.05 %	0.09 Rf	0.8 AU	221.4 AU	0.35 %
3	0.14 Rf	7.9 AU	0.18 Rf	56.7 AU	3.44 %	0.19 Rf	54.8 AU	959.2 AU	1.52 %
4	0.19 Rf	54.9 AU	0.22 Rf	111.8 AU	6.78 %	0.29 Rf	4.0 AU	4182.3 AU	6.63 %
5	0.29 Rf	4.1 AU	0.37 Rf	172.0 AU	10.43 %	0.43 Rf	13.7 AU	8976.7 AU	14.23 %
6	0.43 Rf	114.0 AU	0.46 Rf	142.5 AU	8.64 %	0.54 Rf	67.8 AU	7244.7 AU	11.48 %
7	0.54 Rf	68.0 AU	0.59 Rf	165.8 AU	10.06 %	0.61 Rf	47.1 AU	5166.3 AU	8.19 %
8	0.61 Rf	147.9 AU	0.67 Rf	564.5 AU	34.24 %	0.78 Rf	12.1 AU	26180.4 AU	41.50 %
9	0.87 Rf	2.5 AU	0.92 Rf	37.7 AU	2.29 %	0.96 Rf	0.0 AU	1128.7 AU	1.79 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	45.9 AU	0.03 Rf	241.9 AU	14.75 %	0.05 Rf	0.8 AU	5488.7 AU	8.10 %
2	0.06 Rf	0.2 AU	0.08 Rf	18.6 AU	1.13 %	0.09 Rf	0.6 AU	182.9 AU	0.27 %
3	0.13 Rf	7.1 AU	0.17 Rf	56.3 AU	3.43 %	0.18 Rf	51.6 AU	981.2 AU	1.45 %
4	0.18 Rf	52.0 AU	0.20 Rf	104.8 AU	6.39 %	0.23 Rf	62.1 AU	2670.7 AU	3.94 %
5	0.23 Rf	62.3 AU	0.24 Rf	64.9 AU	3.96 %	0.28 Rf	0.1 AU	1046.8 AU	1.54 %
6	0.28 Rf	0.1 AU	0.37 Rf	190.0 AU	11.59 %	0.43 Rf	27.7 AU	10000.6 AU	14.76 %
7	0.43 Rf	127.8 AU	0.47 Rf	159.8 AU	9.74 %	0.54 Rf	97.8 AU	8321.8 AU	12.28 %
8	0.54 Rf	97.8 AU	0.58 Rf	159.9 AU	9.75 %	0.60 Rf	57.6 AU	5391.2 AU	7.95 %
9	0.60 Rf	157.9 AU	0.68 Rf	559.0 AU	34.08 %	0.78 Rf	22.5 AU	31578.7 AU	46.59 %
10	0.86 Rf	0.8 AU	0.92 Rf	63.0 AU	3.84 %	0.96 Rf	0.8 AU	1968.7 AU	2.90 %
11	0.98 Rf	0.5 AU	0.99 Rf	21.9 AU	1.34 %	1.00 Rf	20.5 AU	144.4 AU	0.21 %

Batch II

Track 1, ID: Nilappanai chooranam Alcohol extract Batch 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	307.3 AU	0.02 Rf	421.9 AU	21.23 %	0.06 Rf	0.8 AU	9318.0 AU	11.47 %
2	0.06 Rf	0.6 AU	0.09 Rf	52.4 AU	2.64 %	0.11 Rf	1.5 AU	716.5 AU	0.88 %
3	0.13 Rf	0.2 AU	0.18 Rf	75.7 AU	3.81 %	0.20 Rf	17.8 AU	1352.7 AU	1.66 %
4	0.20 Rf	19.1 AU	0.22 Rf	55.7 AU	2.80 %	0.24 Rf	48.6 AU	997.3 AU	1.23 %
5	0.24 Rf	48.8 AU	0.25 Rf	52.5 AU	2.64 %	0.29 Rf	0.0 AU	929.6 AU	1.14 %
6	0.31 Rf	0.8 AU	0.38 Rf	102.8 AU	5.17 %	0.40 Rf	81.4 AU	3354.2 AU	4.13 %
7	0.40 Rf	81.8 AU	0.44 Rf	103.3 AU	5.20 %	0.47 Rf	80.3 AU	4003.7 AU	4.93 %
8	0.53 Rf	86.1 AU	0.59 Rf	296.4 AU	10.38 %	0.62 Rf	60.5 AU	7646.3 AU	9.41 %
9	0.62 Rf	161.5 AU	0.72 Rf	656.1 AU	33.01 %	0.83 Rf	8.4 AU	43542.7 AU	53.59 %
10	0.85 Rf	0.6 AU	0.93 Rf	260.7 AU	13.12 %	0.97 Rf	12.6 AU	9394.2 AU	11.56 %

Track 2, ID: Nilappanai chooranam Alcohol extract Batch 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	114.6 AU	0.03 Rf	297.0 AU	16.98 %	0.06 Rf	0.6 AU	8249.4 AU	9.45 %
2	0.07 Rf	0.2 AU	0.09 Rf	47.0 AU	2.69 %	0.11 Rf	0.1 AU	503.1 AU	0.58 %
3	0.13 Rf	1.9 AU	0.17 Rf	59.7 AU	3.42 %	0.20 Rf	17.8 AU	1019.9 AU	1.17 %
4	0.20 Rf	18.5 AU	0.21 Rf	43.0 AU	2.46 %	0.23 Rf	31.8 AU	767.3 AU	0.88 %
5	0.23 Rf	32.4 AU	0.24 Rf	34.9 AU	2.00 %	0.28 Rf	10.7 AU	812.5 AU	0.93 %
6	0.28 Rf	10.9 AU	0.38 Rf	101.3 AU	5.79 %	0.40 Rf	84.1 AU	3977.9 AU	4.56 %
7	0.40 Rf	84.2 AU	0.43 Rf	97.4 AU	5.57 %	0.49 Rf	68.9 AU	4525.7 AU	5.18 %
8	0.52 Rf	70.5 AU	0.58 Rf	184.5 AU	10.55 %	0.60 Rf	80.8 AU	6134.2 AU	7.03 %
9	0.60 Rf	181.2 AU	0.73 Rf	583.9 AU	33.38 %	0.82 Rf	8.4 AU	48009.5 AU	55.00 %
10	0.83 Rf	0.0 AU	0.92 Rf	300.5 AU	17.18 %	0.96 Rf	94.3 AU	13288.5 AU	15.22 %

Batch III

Track 1, ID: Nilappanai chooranam Alcohol extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	20.8 AU	0.01 Rf	130.5 AU	7.47 %	0.04 Rf	0.0 AU	952.3 AU	1.28 %
2	0.07 Rf	0.1 AU	0.09 Rf	143.8 AU	8.23 %	0.13 Rf	0.1 AU	1704.6 AU	2.29 %
3	0.14 Rf	0.1 AU	0.19 Rf	109.2 AU	6.25 %	0.21 Rf	32.6 AU	1978.0 AU	2.66 %
4	0.21 Rf	32.9 AU	0.24 Rf	85.0 AU	4.87 %	0.30 Rf	0.1 AU	2398.3 AU	3.23 %
5	0.32 Rf	0.7 AU	0.39 Rf	49.4 AU	2.83 %	0.41 Rf	42.2 AU	1583.5 AU	2.13 %
6	0.41 Rf	42.3 AU	0.45 Rf	89.8 AU	5.15 %	0.49 Rf	69.2 AU	3395.2 AU	4.57 %
7	0.49 Rf	69.5 AU	0.58 Rf	254.9 AU	14.60 %	0.61 Rf	22.1 AU	12524.0 AU	16.86 %
8	0.61 Rf	223.2 AU	0.70 Rf	630.6 AU	36.11 %	0.82 Rf	0.2 AU	44200.5 AU	59.50 %
9	0.86 Rf	0.2 AU	0.90 Rf	125.4 AU	7.18 %	0.94 Rf	1.2 AU	3261.2 AU	4.39 %
10	0.95 Rf	1.2 AU	0.98 Rf	127.4 AU	7.30 %	0.99 Rf	66.2 AU	2289.5 AU	3.08 %

Track 2, ID: Nilappanai chooranam Alcohol extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	16.2 AU	0.01 Rf	126.2 AU	9.34 %	0.03 Rf	0.2 AU	1053.2 AU	1.45 %
2	0.06 Rf	0.4 AU	0.09 Rf	109.0 AU	8.07 %	0.12 Rf	0.1 AU	1557.1 AU	2.15 %
3	0.14 Rf	0.2 AU	0.19 Rf	102.3 AU	7.58 %	0.21 Rf	31.8 AU	1957.5 AU	2.70 %
4	0.21 Rf	32.0 AU	0.23 Rf	73.0 AU	5.40 %	0.30 Rf	0.2 AU	1841.5 AU	2.54 %
5	0.31 Rf	1.0 AU	0.40 Rf	69.3 AU	5.13 %	0.42 Rf	63.7 AU	2426.7 AU	3.35 %
6	0.42 Rf	64.0 AU	0.46 Rf	114.1 AU	8.45 %	0.49 Rf	96.1 AU	3949.7 AU	5.46 %
7	0.49 Rf	95.3 AU	0.70 Rf	509.7 AU	37.74 %	0.80 Rf	0.2 AU	53234.6 AU	73.53 %
8	0.83 Rf	0.1 AU	0.89 Rf	107.1 AU	7.93 %	0.93 Rf	0.5 AU	3065.7 AU	4.23 %
9	0.93 Rf	0.1 AU	0.97 Rf	139.8 AU	10.34 %	0.99 Rf	62.5 AU	3312.1 AU	4.57 %

Fig. 5: HPTLC finger print profile of 10µl and 20µl of Alcohol extract Nilappanai chooranam at 575 nm after derivatisation using vanillin-sulphuric acid.

Table. 3: R_f values and colour of spots viewed under UV 254 nm.

Batch I		Batch II		Batch III	
R_f values	**Colour**	**R_f values**	**Colour**	**R_f values**	**Colour**
0.11	Dark green	0.07	Dark green	0.11	Dark green
0.18	Green	0.15	Green	0.18	Green
0.22	Green	0.19	Green	0.22	Green
0.27	Green	0.27	Green	0.27	Green
0.31	Green	0.48	Green	0.31	Green
0.48	Green	0.76	Green	0.48	Green
0.55	Green			0.55	Green
0.75	Green			0.75	Green

Table. 4: R_f values and colour of spots viewed under UV 366 nm.

Batch I		Batch II		Batch III	
R_f values	**Colour**	**R_f values**	**Colour**	**R_f values**	**Colour**
0.06	Dark blue	0.08	Dark blue	0.07	Dark blue
0.10	Light blue	0.15	Light blue	0.10	Brown
0.12	Brown	0.16	Pink	0.17	Pink
0.14	Light blue	0.20	Red	0.21	Red
0.16	Pink	0.23	Violet	0.26	F.Pink
0.20	Pink	0.25	Pink	0.40	Violet
0.27	Pink	0.32	Violet	0.65	Red
0.37	Blue	0.39	Blue	0.71	F. Blue
0.54	Red	0.47	Blue	0.79	Red
0.64	Red	0.56	Red	0.87	Red
0.70	Light green	0.73	Light green		
0.77	Red	0.81	Red		
0.83	Violet	0.86	Red		
0.89	Red				

Table. 5: R_f values and colour of spots viewed under at 575 nm after derivatisation.

Batch I		Batch II		Batch III	
R_f values	**Colour**	**R_f values**	**Colour**	**R_f values**	**Colour**
0.08	Grey	0.09	Grey	0.09	Grey
0.17	Grey	0.17	Grey	0.19	Grey
0.20	Grey	0.21	Grey	0.23	Grey
0.24	Grey	0.24	Grey	0.40	Purple
0.37	Purple	0.38	Purple	0.46	Purple
0.47	Purple	0.43	Purple	0.54	Purple
0.58	Purple	0.58	Purple	0.70	Dark purple
0.68	Purple	0.73	Dark purple	0.73	Purple
0.72	Light purple	0.89	Purple	0.85	Purple
0.78	Light purple	0.96	Dark purple	0.92	Dark purple
0.92	Light purple				
0.99	Purple				

The metals such as manganese, potassium, magnesium, zinc, aluminium, iron, calcium and sodium were estimated and the results are given in Table 6. Aluminium was found to be absent in the chooranam.

Table. 6: Metallic components of Nilappanai chooranam.

Sl. No.	Name of Metal	Results		
		Batch I (ppm)	**Batch II (ppm)**	**Batch III (ppm)**
1.	Manganese (as Mn)	900	320	33
2.	Potassium (as K)	2000	1360	5970
3.	Magnesium (as Mg)	1000	1200	196
4.	Zinc (as Zn)	100	28	38
5.	Aluminium (as Al)	Not detected	Not detected	Not detected
6.	Iron (as Fe)	70	6610	204
7.	Calcium (as Ca)	3000	7270	828
8.	Sodium (as Na)	900	428	387

Arsenic and the heavy metals cadmium and mercury were not detected in the chooranam. Lead was found to be 3 ppm, 2 ppm and 2 ppm for batch I, II and III respectively (Table 7). The permissible limit for Lead is 10 ppm.

Table. 7: Heavy metalsof Nilappanai Chooranam.

Sl. No.	Name of Metal	Results		
		Batch I	Batch II	Batch III
1.	Arsenic (as As)	Not detected	Not detected	Not detected
2.	Lead (as Pb)	3 ppm	2 ppm	2 ppm
3.	Cadmium (as Cd)	0.1 ppm	Not detected	Not detected
4.	Mercury (as Hg)	Not detected	Not detected	Not detected

Tests for microbial contamination (Table 8) reveals that *Pseudomonas aeruginosa* and *Salmonella* sp. were absent. All other parameters fall within the permissible limits.

Table. 8: Microbial contamination of Nilappanai chooranam.

Sl. No.	Parameter	Results		
		Batch Icfu/g	Batch II cfu/g	Batch III cfu/g
1.	Total viable count	$62x10^4$	$82x10^4$	$52\ x10^4$
2.	*Pseudomonas aeruginosa*	Absent	Absent	Absent
3.	Total fungal count	300	$14x10^3$	$43x10^2$
4.	*Escheria colii*	<10	<10	<10
5.	*Salmonella* sp.	Absent	Absent	Absent
6.	*Staphylococcus aureus*	<10	<10	<10
7.	Enterobacteriaceae	<10	180	<10

Tests for common Organophosphorus Pesticides and Organochlorine pesticides were conducted and found to be absent (Table 9).

Table. 9: Pesticide residues of Nilappanai chooranam.

Sl.No.	Parameter	Results		
		Batch I	Batch II	Batch III
Organophosphorus Pesticides				
1.	Dichlorvos	Not Detected	Not Detected	Not Detected
2.	Ethoprofos	Not Detected	Not Detected	Not Detected
3.	Disulfofon	Not Detected	Not Detected	Not Detected
4.	Parathion Methyl	Not Detected	Not Detected	Not Detected
5.	Fenchlorphos	Not Detected	Not Detected	Not Detected
6.	Prothiofos	Not Detected	Not Detected	Not Detected
7.	Guthion	Not Detected	Not Detected	Not Detected
8.	Fenithrothion	Not Detected	Not Detected	Not Detected
9.	Malathion	Not Detected	Not Detected	Not Detected
10.	Chlorpyrifos	Not Detected	Not Detected	Not Detected
Organochlorine pesticides				
11.	EndosulfanI	Not Detected	Not Detected	Not Detected
12.	EndosulfanII	Not Detected	Not Detected	Not Detected
13.	Heptachlor	Not Detected	Not Detected	Not Detected
14.	Endrin	Not Detected	Not Detected	Not Detected
15.	44'DDD	Not Detected	Not Detected	Not Detected
16.	44'DDE	Not Detected	Not Detected	Not Detected
17.	44'DDT	Not Detected	Not Detected	Not Detected
18.	Alpha BHC	Not Detected	Not Detected	Not Detected
19.	Beta BHC	Not Detected	Not Detected	Not Detected
20.	Heptachlorepoxide	Not Detected	Not Detected	Not Detected
21.	Aldrin	Not Detected	Not Detected	Not Detected
22.	Dieldrin	Not Detected	Not Detected	Not Detected
23.	Gamma BHC	Not Detected	Not Detected	Not Detected
24.	Delta BHC	Not Detected	Not Detected	Not Detected

The presence of aflatoxins in plant material can be hazardous to health if absorbed even in very small amounts. The aflatoxins observed in Nilappanai chooranam (Table 10) is very much less than the permissible limit which is 5 ppm for G2 and G1, and 1 ppm for B2 and B1.

Table. 10: Aflatoxins of Nilappanai chooranam.

Sl. No.	Parameter	Results		
		Batch I Ppm	Batch II ppm	Batch III ppm
1.	Aflatoxin G2	<0.005	< 0.005	< 0.005
2.	Aflatoxin G1	< 0.005	< 0.005	< 0.005
3.	Aflatoxin B2	< 0.005	< 0.005	< 0.005
4.	Aflatoxin B1	< 0.005	< 0.005	< 0.005

CONCLUSION

The need of standardization of ISM drugs demands a lot in towards era for identification and authentication of the drug. The lack of standardization technique fails to determine the quality and efficacy of the drug from its originality which thereby exploits the use of drug from its traditional system of medicine. Thus a definite protocol is followed for its authentication and identification. The present work embodies the investigations carried out to establish methods for quality control of drugs as per WHO guidelines which include botanical features and physico-chemical parameters thereby exploring this formulation on the basis of these standardisation parameters. HPTLC evaluation of Nilappanai chooranam provided specific parameters that will be useful in scientific evaluation, identification and authentication of the drug. The metallic quantity of metallic components observed in the chooranam serves as an additional parameter for standardisation. Moreover the results obtained for toxicological studies such as determination of arsenic and heavy metals, pesticide residues, microbial contamination and aflatoxins ensure the quality of the medicine. The results of the different analysis would help in future for proper identification of Nilappana chooranam. Hence the result of the present study is significant and encouraging towards the goal for standardization.

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