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Standardization of Nandhi Mezhugu, a poly herbomineral Siddha formulation

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ABSTRACT

Background: Standardisation is essential for scientific validation of any poly herbo mineral formulation. Nandhi mezhugu is a classical Siddha herbo mineral formulation has indication for many diseases such as all types of arthritis, male and female reproductive tract disorders, different types of cancers eg ovarian cancer, testicular cancer, cancer penis, cancer cervix, oral and cheek cancers, fistula, hydrocele, chronic ulcers, skin diseases eg eczema, leucoderma, diabetic carbuncle, chronic ulcers, Hanson's disease etc. **Aim**: The aim of this study was to standardise Nandi mezhugu. **Materials and Methods:** The drug was prepared as per the procedure mentioned in Siddha Literature and then subjected to the following analysis such as physico chemical, heavy metals, pesticide residue, aflatoxin, qualitative phytochemical, qualitative inorganic analysis, TLC photo documentation and HPTLC finger print profile. **Results and conclusion:** The drug was free of microbial contamination and aflatoxins and pesticide residues. Hence the drug was safe for consumption.

KEYWORDS: Serankottai, Etti kottai, Rasa chendooram, Nervalam, Nandukkal, Pooneeru.

1. INTRODUCTION:

Recent years have witnessed that, there is an exponential growth and demand in traditional medicine due to the new global trend of "Return to Nature". It has been estimated that eighty percent of the world population are using herbal and complementary medicines for their primary healthcare needs, which provides a new sphere of growth for traditional medicine[1].

The Siddha system of medicine is being time tested and still cater to the health needs of the society. For Global acceptance, this system of medicine has to undergo scientific validation through quality control measures of the medicinal raw drugs as well as standard operating procedures for preparing Siddha medicines. Nandi mezhugu is one such drug which is to be standardized. It is enlisted in Siddha Formulary of India, Part I. it is prescribed for various ailments[2][3]. Nandhi Melugu was subjected to the following analysis such as physico chemical, heavy metals, pesticide residue, aflatoxin, qualitative phytochemical, qualitative inorganic analysis, TLC photo documentation and HPTLC finger print profile as per WHO[4] and AYUSH Guidelines[5] .

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2. MATERIALS AND METHODS

2.1. Collection and Authentication of Raw Drugs

All the ingredients of Nandi Mezhugu were purchased from Indian Medical Practitioners Co-operative Pharmacy and Stores Limited Sales (IMPCOPS), Chennai-600041. The metals and mineral raw drugs were identified and authenticated in the chemistry lab; the herbal raw drugs were identified and authenticated by Dr.Sasikala Ethirajalu Research Officer-Scientist II (Pharmacognosy) in Siddha Central research Institute, Arumbakkam, Chennai-106.

2.2 . Preparation of the drug Nandhi Mezhugu

Ingredients:

Table 1: Ingredients of Nandhi mezhugu

Sl.No.	Ingredients	Source	Quantity
1.	Purified Marking Nut (Serankottai) *	*Semecarpus anacardium* L.f. (Fruit)	1Kg
2.	Purified Nux Vomica (Ettikkottai) seeds **	*Strychnos nux-vomica* L. (Seed)	315 gms
3.	Ghee (Nei)	*Bos indicus* – Ghee	1400 gms
4.	Common Alum (Padikaram) *	Aluminium Potassium Sulphate	1120 gms
5.	Palm Jaggery (Panai Vellam)	*Borassus flabellifer* L.(Palm Jaggery)	2240 gms
6.	Honey (Then)	*Apies mellifica* – Honey	1400 gms
7.	Ponnimilai parpam *	Calx of Copper pyrites	53 gms
8.	Kalnar Parpam *	Calx of Magnesium calcium silicate	53 gms
9.	Kalmatha Parpam***	Calx of Hydrous Cobalt Arsenate	53 gms
10.	Nandukkal Parpam *	Calx of Crab's fossil	53 gms
11.	Pachai karpooram*	Borneol	53 gms
12.	Kungumap poo**	*Crocus sativus* L. (Style & Stigma)	53 gms
13.	Gorochan (Korochanam)*	Gall stone of bull	53 gms
14.	Prepared Rasa sindooram	Rasa sindooram	140 gms
15.	Chukku***	*Zingiber officinale* Rosc. (Rhizome)	35 gms
16.	Milaku**	*Piper nigrum* L. (Fruit)	35 gms
17.	Tippili***	*Piper longum* L. (Fruit)	35 gms
18.	Elarisi**	*Elettaria cardamomum* (L.) Maton (Seed)	35 gms
19.	Beetle killer roots (Siruthekku)**	*Premna herbacea* Roxb. (Root)	35 gms
20.	Yew leaves (Thalisa Pathiri) **	*Taxus wallichiana* Zucc. Syn. *T. baccata* L. (Leaf)	35 gms
21.	Henbane niger (Kurosani Omam) **	*Trachyspermum ammi* (L.) Sprague (Seed)	35 gms
22.	Vaividankam**	*Embelia ribes* Burm.f. (Fruit)	35 gms
23.	Athividayam (Atis) **	*Aconitum heterophyllum* Wall. ex Royle (Root)	35 gms
24.	Jathikkai (Nutmeg)**	*Myristica fragrans* Houtt. (Kernel)	35 gms
25.	Jathipattri(Mace)**	*Myristica fragrans* Houtt. (Aril)	35 gms
26.	Karunjeeragam***	*Nigella sativa* L.(Seed)	35 gms
27.	Cirakam***	*Cuminum cyminum* L. (Fruit)	35 gms
28.	Ilavankam**	*Syzygium aromaticum* (L.) Merr. & L.M. Perry Syn. *Eugenia caryophyllata* Thunb. (Flower bud)	35 gms
29.	Chevviyam**	*Piper nigrum* L. (Root)	35 gms
30.	Kattu milaku**	*Piper attenuatum* Buch. –Ham. ex Miq. – (Fruit)	35 gms
31.	Kodiveli ver**	*Plumbago zeylanica* L. – (Root)	35 gms
32.	Tippilik kattai**	*Piper longum* L. (Stem)	35 gms
33.	Kacakaca	*Papaver somniferum* L. (Seed)	35 gms
34.	Perumcirakam**	*Foeniculum vulgare* Mill. (Fruit)	35 gms
35.	Nervalam*	*Croton tiglium* L. (Seed)	35 gms
36.	Cittarathai**	*Alpinia officinarum* Hance (Rhizome)	35 gms
37.	Catamancil**	*Nardostachys jatamansi* (D. Don) DC. Syn. *N. grandiflora* DC. (Rhizome)	35 gms
38.	Akkirakaram**	*Anacyclus pyrethrum* (L.) Lag. (Root)	35 gms
39.	Nattu Amukkara**	*Withania somnifera* (L.) Dunal (Root)	35 gms
40.	Rasa Chendooram (Raw material) *	Red Sulphide of mercury	25gms

Sl.No.	Ingredients	Source	Quantity
41.	Pooneeru *	Fuller's earth	25gms
42.	Mayilthutham*	Copper sulphate	25gms
43.	Palthutham **	Zinc sulphate	25gms
44.	Rasam***	Mercury	25gms
45.	Pooram*	Mercurous chloride	25gms
46.	Lingam*	Mercuric sulphide	25gms
47.	Manosilai*	Arsenic monosulphide	25gms
48.	Ganthagam*	Sulphur	25gms
49.	Thalagam*	Arsenic trisulphide	25gms
50.	Kuthiraipal pashanam***	Potassium Aluminium Silicate	25gms
51.	Vellaipashanam *	Arsenic trioxide	25gms
52.	Gowripashanam *	Arsenic pentasulphide	25gms
53.	Avuri elai	*Indigofera tinctoria* L. (Leaf)	Q.S

Drugs were purified as per the Siddha literatures * Siddha Materia Medica, **Sikitcha Rathina Deepam, ***Sarakku Suththi Muraigal[6],[7],[8].

Preparation:

(a) Fry items 1 & 2 in 3 ingredients .Ground 1 & 2 into a fine paste.
(b) Ground 4, 14-39 in to fine powder separately.

(c) Ground 40-52 in to a paste with juice of drug no 53 for 6 hours and were made cakes and dried. Covered the cakes with the paste of drug no 53 and subjected to calcination process, then the product was taken and powdered.

(d) Syrup of palm jaggery was prepared and the ingredients (a),(b) and (c) and the calxes were added, after that ghee and honey were added. To this mixture powders of 11, 12, 13 were added, thoroughly mixed and stored in Air tight Container.[2],[3]

2.3. Organoleptic properties

The organoleptic characters such as colour, odour, taste, consistency were observed.

2.4. Physico Chemical Parameters:

Loss on drying at 105°C, total ash, water soluble ash, acid-insoluble ash, water soluble extractive, alcohol soluble extractive, rancidity, acid value, saponification value, iodine value, pH, total solid, fat content , reducing sugar, total sugar were carried out as per the procedures mentioned in standard references (WHO, Protocol for testing)[4][5][9]

2.5 Assays:

Quantitative assays for Calcium, Magnesium, Potassium, Aluminium, Copper, Iron and Zinc, were observed in ICP-OES using standards. Sulphur (as SO_2) was estimated by following AOAC 990.28 method and Chloride (as NaCl) was calculated by following AOAC 950.52 method.

2.6 . Qualitative Phytochemical Analysis:

Various tests for different types of secondary metabolites, viz., Steroids, terpinoids, alkaloids, flavanoids, etc were carried out as per the procedures quoted in standard organic book. (Ref Harborne)[10]

2.7. Qualitative Inorganic Analysis:

Qualitative test for various cations and anions were carried out as per the methods mentioned in standard practical guide. (Ref : Feigl)[11]

2.8 . Heavy metal Analysis:

Tests for heavy metals, viz., lead, cadmium, arsenic and mercury were carried out in ICP-OES instrument (Perkin Elmer Optima 3000 DV).

2.9. Microbial contamination:

Tests for total bacterial /fungal counts *E. coli, Salmonella* spp., *Staphylococcus aureus* and *Entero bacteriacea* were done. [9]

2.10. Pesticide residues:

Various pesticide residues of organo chlorine and organo phosphorous viz., alphaBHC, betaBHC, gam BHC(Lindane), deltaBHC, Aldrin, Dieldrin, trans Chlordane, cis Chlordane, Endrin, Endrinaldehyde, Endrinketone, Endosulfan-I, Endosulfan-II, Endosulfansulfate, Heptachlor, Heptachlorepoxide, Dicofol, Chlorthalonil, Hexachlorobenzene, o,p"DDT, P,P"DDT, o,p"DDD, p,p"DDD, o,p"DDE, P,P"DDE, 4-Bromo,2-Chlorophenol, Acephate, Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos methyl, Diazinon, Dichlorvos, Dimethoate, Ethion, Etrimfos, Fenitrothion, Iprobenphos, Malathion, Methamidophos, Monocrotophos, Omethoate, Oxydemeton-methyl, Parathion ethyl, Parathion methyl, Phorate,

Phosalone, Phosphamidon, Profenophos, Quinalphos, Triazophos, Phorate sulphone , Phorate sulphoxide were checked by following AOAC 2007.01 methods.

2.11 . Tests for Aflatoxins:

Aflatoxins such as B1,B2,G1and G2 were checked using AOAC 2008.02 methods.

2.12 TLC Photodocumentation[12]/HPTLC Finger print profiling[13]

Sample preparation

Four gm of the drug was extracted successively by hexane, chloroform and ethanol using Soxhlet apparatus. The extracts were filtered freed from solvents and made up to 10 ml in standard flasks using the respective solvents.

TLC plate

Aluminium plate precoated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used for the TLC/HPTLC analysis.

Developing chamber

Camag's twin trough chamber was used for the development.

Solvent system

Many solvent systems were tried for a better separation and the same was achieved in Toluene : Ethyl acetate (10 : 0.5, v/v) for hexane extract; Toluene : Ethyl acetate (5:1.5, v/v) for chloroform extract and ethanol extract.

Derivatization reagent

For derivatization vanillin-sulphuric acid reagent was used (1 gram vanillin dissolved in a mixture of ethanol and sulphuric acid with the composition 95 ml : 5 ml).

Instrument

Linomat 5 automatic applicator, CAMAG's visualizer, CAMAG's scanner 030618 attached with WINCATS software were the instruments used for photo documentation and HPTLC finger printing. CAMAG's plate heater was used for derivatization.

Procedure

5 µl, 10 µl and 15 µl of the hexane, chloroform and ethanol extracts were applied on three different plates as 10 mm bands with 8 mm distance in between and developed up to 8 cm in the above mentioned solvent systems. The air dried developed plates were visualized under UV 254 and 366 nm for documenting TLC chromatograms. The plates were scanned in UV 254 nm (all extracts) & 366 nm (hexane and chloroform) and the finger print profiles were recorded. Then the plates were dipped in vanillin-sulphuric acid reagent and heated in an oven at 105ÚC until the development of colored spots. TLC photo documentation in white light after derivatization were recorded and finger print profiles at 575 nm (hexane and chloroform) were also recorded.

3. RESULTS AND DISCUSSION:

3.1. Organoleptic properties

Colour: Dark brown colour; **Odour:** Resinous odour; **Taste:** Metallic taste; **Consistency**: Semisolid.

3.2. Physicochemical Properties

All the results of physico chemical parameters are presented in table 2. The loss on drying was observed as 19.156% and fat content was observed as 20.683%. It is understood that the high value of loss on drying due to the fat content. The total ash was calculated as 6.607% which indicates the content of total inorganics. The water soluble ash value of 2.95 % shows the content of water soluble inorganic salts like sodium chloride, etc. The acid insoluble ash value was calculated as 0.93%. The water soluble and alcohol soluble extractives were estimated as 39.056 % and 23.558 % respectively indicating the presence of high polar secondary metabolites like glycosides, sugars, tannins, saponins, alkaloids, etc. The calculated acid value, saponification value and iodine value were indicative of purity of the ghee used for the preparation and showing the number of milligrams of free acids and saponifiable acid and number of unsaturation in the drug. Though the reducing sugar (3.69 %) and total sugar (7.54%) values are indicative of promoting the growth of organisms, the drug was free from rancidity and the pH value (10 % solution) of 3.35 indicates the acidic nature of the drug. Hence the susceptibility of microbial growth due to presence of sugar may be decreased by the acidity and the shelf life of the drug would be increased.

Table 2. Physicochemical results of Nandhi mezhugu

Sl.No	Parameter	Mean (n=2)
2	Loss on Drying at 105° C	19.16%
3	Total Ash	6.61%
4	Water soluble ash	2.95%
5	Acid-insoluble ash	0.93%
6	Water soluble extractive	39.06%
7	Alcohol soluble extractive	23.56%
8	Rancidity	Nil
9	Acid value	10.592
10	Saponification value	262.62
11	Iodine value	16.864
12	Ph	3.35
13	Total solid	80.84%
14	Fat content	20.68%
15	Reducing Sugar	3.69%
16	Total Sugar	7.54%

3.3. Qualitative Phytochemical Analysis

The extract of Nandi mezhugu was subjected to various phytochemical tests as per the standard procedure (Ref. Harborne book). All the tested secondary metabolites were present in the drug which would improve the therapeutic efficacy of the drug.

3.4. Qualitative Inorganic Analysis

The qualitative inorganic analysis of the drug revealed the presence of mercury, magnesium, aluminium, calcium, sodium, potassium, copper, zinc, iron, cobalt, chloride, carbonate, nitrate, sulphate, sulphide, arsenate, acetate, silicate and arsenate which are all biologically important radicles.

3.5. Heavy metal analysis

The content of lead and cadmium are within the admissible limit of WHO standards. While the content of arsenic and mercury are high due to the reasons they are added in the drug in the form of rasam, lingam, pooram, thalagam, manosilai, vellai pashanam, gowri pashanam, kalmatham and rasa chenduram/sinduram. But they are not present in the elemental form and hence non toxic. In continuation of the standardisation of trail drug we had studied the safety of the trail drug as per OECD guidelines(acute, sub-acute sub-chronic toxicity studies) showed non-toxic effect in rodents(unpublished). The result (table 3) from the safety study provided was encouraging and opened a venue in the management of auto immune disease like Rheumatoid arthritis that needs long term treatment with Nandhi mezhugu. Physico-chemical forms of heavy metals in the indigenous medicine is totally different from the known Physico-chemical forms of that metal.[14]

Table 3. Heavy metals present in Nandhi mezhugu

Heavy metal (in ppm)	Quantity (in ppm)	WHO limit
Lead (as Pb)	2.95	10
Arsenic (as As)	7233.42	3
Cadmium (as Cd)	0.01	0.3
Mercury (as Hg)	9336.61	1

3.6 . Microbial contamination

In the microbial study, the drug was found free from *E. coli, Salmonella* spp., *Staphlococcus aureus* and *Enterobacteriacea*. The results are shown in the table 4. The total bacterial count and the total fungal count were within the permissible limits of WHO standards. Hence the drug is safe for consumption.

Table 4. Microbial contamination results of Nandhi mezhugu

S. No	Parameter	Value	WHO Limit (CFU/g)
1.	*E. coli*	Absent	10
2.	*Salmonella* spp.	Absent	None
3.	*Staphylococcus aureus*	Absent	Absent
4.	*Enterobacteriacea*	Absent	103
5.	Total Bacterial count	2 x 10^3	105
6.	Total Fungal count	Less than 10	103

3.7 Pesticide residue

All the tested organochlorine pesticides organophosphorus pesticides were found to be lower than the limit of quantification, ie., 0.01 ppm and hence safe as internal medicine.

3.8 Assays

Presence of calcium was detected which may due to added nandukkal in the drug; presence of potassium and aluminium may be due to padikaram, kuthiraipal padanam, vediuppu. Occurrence of copper is due to mayilthutham, ponnimilai; presence of iron and zinc is due to added of palm jaggery, palthutham respectively. Sulphur and chloride occurrence is due to added of lingam, thalagam, gowripasanam, gandhagam, padigaram, rasa chendooram, mayilthutham, palthutham and pooram, kariuppu respectively (table 5).

Table 5. Assays results of Nandhi mezhugu

Calcium (as Ca)	3165.72	mg/kg
Magnesium(as Mg)	358.72	mg/kg
Potassium (as K)	1950.25	mg/kg
Aluminum (as Al)	4943.37	mg/kg
Copper (as Cu)	501.35	mg/kg
Iron (as Fe)	497.3	mg/kg
Zinc (as Zn)	26.29	mg/kg
Sulphur (as SO_2)	BLQ (LOQ : 10.0)	mg/kg
Chloride (as NaCl)	0.03	g/100g

3.9 . Test for Aflatoxins (B1,B2,G1,G2)

All the four aflatoxin were not detected in the drug. As the total fungal count was within the permissible limit, txins were not promoted in the drug and is free from these aflatoxins.

3.10. TLC/HPTLC

The TLC photodocumentation of hexane extract of Nandi mezhugu under UV 254 nm showed 5 visible spots at R_f value 0.25, 0.30, 0.38, 0.50 and 0.71 (all green); under UV 366 nm showed three visible spots at R_f value 0.30 (blue), 0.38 (fluorescent blue) and 0.71 (pale

blue). After derivatization with vanillin-sulphuric acid, showed 8 spots at 0.20, 0.25, 0.30, 0.35 (all purple), 0.38 (brown), 0.46, 0.57 and 0.71 (all purple) (table 6, fig 1 and 2-10).

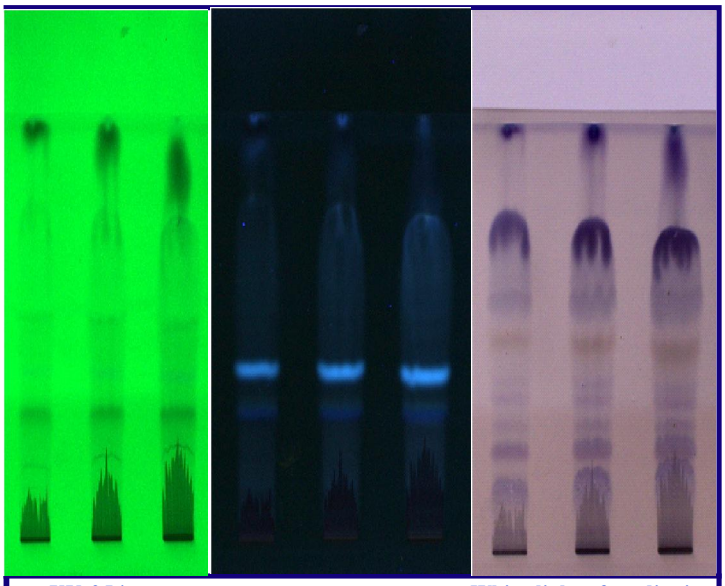

Fig.1.TLC photo documentation of hexane extract of Nandi mezhugu

Table 6. TLC results of Nandhi mezhugu

Under UV 254 nm		Under UV 366 nm		White light after derivatization	
R_f	Colour	R_f	Colour	R_f	Colour
-		-	-	0.2	Purple
0.25	All green	-	-	0.25	Purple
0.3		0.3	Blue	0.3	Purple
0.38		-	-	0.35	Purple
-		0.38	Fluorescent blue	0.38	Brown
0.5		-	-	0.46	Purple
-		-	-	0.57	Purple
0.71		0.71	Pale blue	0.71	Purple

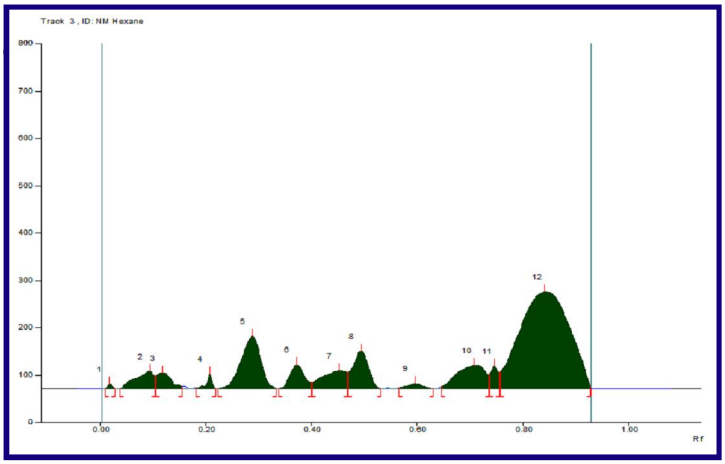

Fig. 2. HPTLC finger print profile of hexane extract of Nandi mezhugu at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.6 AU	0.02 Rf	10.4 AU	1.48 %	0.03 Rf	0.4 AU	76.3 AU	0.26 %
2	0.04 Rf	0.1 AU	0.10 Rf	36.7 AU	5.20 %	0.11 Rf	27.3 AU	1071.6 AU	3.65 %
3	0.11 Rf	27.7 AU	0.12 Rf	33.8 AU	4.78 %	0.16 Rf	5.4 AU	781.4 AU	2.66 %
4	0.18 Rf	1.7 AU	0.21 Rf	30.7 AU	4.34 %	0.22 Rf	0.1 AU	263.5 AU	0.90 %
5	0.22 Rf	0.3 AU	0.29 Rf	111.4 AU	15.77 %	0.33 Rf	0.2 AU	3421.0 AU	11.66 %
6	0.34 Rf	0.4 AU	0.37 Rf	50.5 AU	7.15 %	0.40 Rf	12.8 AU	1146.2 AU	3.90 %
7	0.40 Rf	13.0 AU	0.45 Rf	38.7 AU	5.48 %	0.47 Rf	35.1 AU	1443.4 AU	4.92 %
8	0.47 Rf	35.2 AU	0.50 Rf	80.1 AU	11.34 %	0.53 Rf	0.4 AU	1964.0 AU	6.69 %
9	0.57 Rf	1.3 AU	0.60 Rf	10.8 AU	1.52 %	0.63 Rf	0.1 AU	264.7 AU	0.90 %
10	0.65 Rf	4.2 AU	0.71 Rf	50.4 AU	7.13 %	0.74 Rf	29.5 AU	2243.2 AU	7.64 %
11	0.74 Rf	30.4 AU	0.75 Rf	48.5 AU	6.87 %	0.76 Rf	34.3 AU	562.2 AU	1.92 %
12	0.76 Rf	34.9 AU	0.84 Rf	204.6 AU	28.95 %	0.93 Rf	4.7 AU	16115.0 AU	54.90 %

Fig. 3. R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 254 nm

The HPTLC finger print profile of hexane extract at UV 254 nm showed 12 peaks in which the peak at R_f 0.84 was the major peak with an area of 54.90 % followed by a peak at R_f 0.29 with an area of 11.66 %. All other peaks are minor with an individual area less than 10 %.

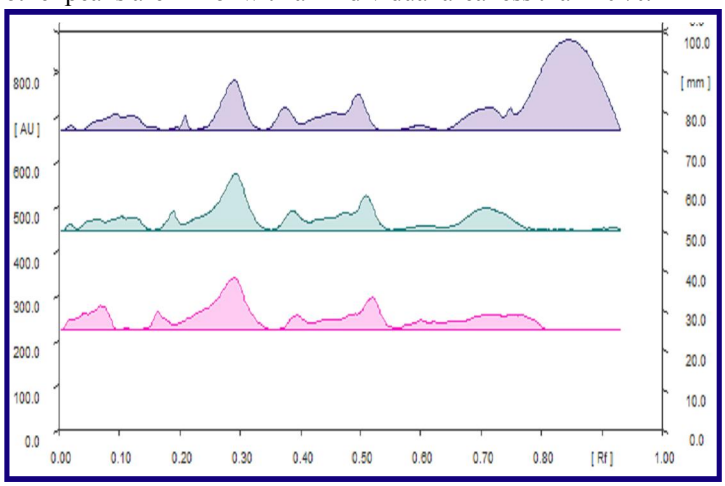

Fig. 4. 3D chromatogram of hexane extract of Nandi mezhugu at 254 nm

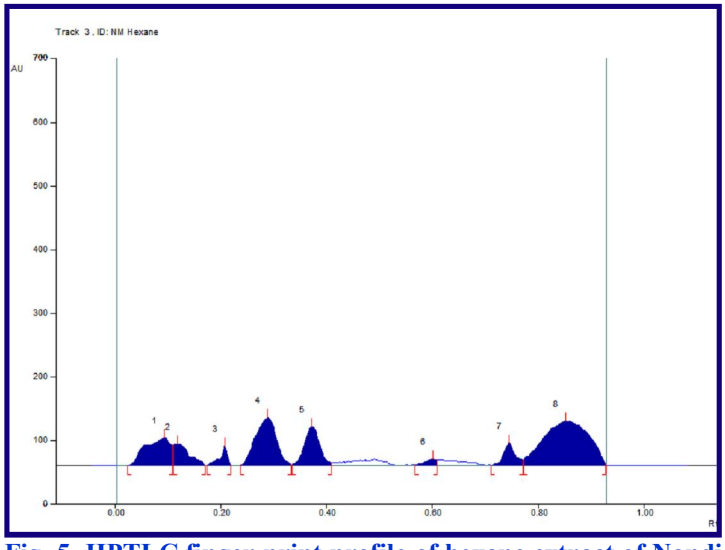

Fig. 5. HPTLC finger print profile of hexane extract of Nandi mezhugu at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.3 AU	0.09 Rf	43.8 AU	12.15 %	0.11 Rf	31.7 AU	1707.3 AU	13.40 %
2	0.11 Rf	31.8 AU	0.12 Rf	34.0 AU	9.43 %	0.17 Rf	0.4 AU	859.7 AU	6.75 %
3	0.17 Rf	0.3 AU	0.21 Rf	31.1 AU	8.62 %	0.22 Rf	0.1 AU	358.4 AU	2.81 %
4	0.24 Rf	0.7 AU	0.29 Rf	75.1 AU	20.84 %	0.33 Rf	1.9 AU	2476.5 AU	19.43 %
5	0.34 Rf	2.0 AU	0.37 Rf	61.3 AU	17.01 %	0.41 Rf	4.3 AU	1477.3 AU	11.59 %
6	0.57 Rf	0.8 AU	0.60 Rf	10.3 AU	2.87 %	0.61 Rf	8.6 AU	177.1 AU	1.39 %
7	0.71 Rf	0.5 AU	0.75 Rf	35.1 AU	9.74 %	0.77 Rf	8.5 AU	644.6 AU	5.06 %
8	0.77 Rf	8.9 AU	0.85 Rf	69.7 AU	19.34 %	0.93 Rf	2.4 AU	5043.3 AU	39.57 %

Fig. 6. R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 366 nm.

The HPTLC finger print profile of hexane extract at UV 366 nm showed 8 peaks in which the peak at R_f 0.85 was the major peak with an area of 39.57 % followed by a peak at R_f 0.29 (19.43 %), 0.09 (13.40 %) and 0.37 (11.59 %). All other peaks are minor with an individual area less than 10 %.

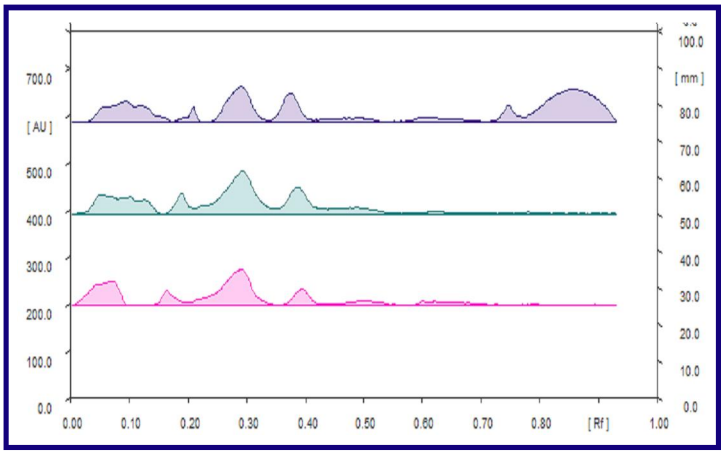

Fig. 7. 3D chromatogram of hexane extract of Nandi mezhugu at 366 nm

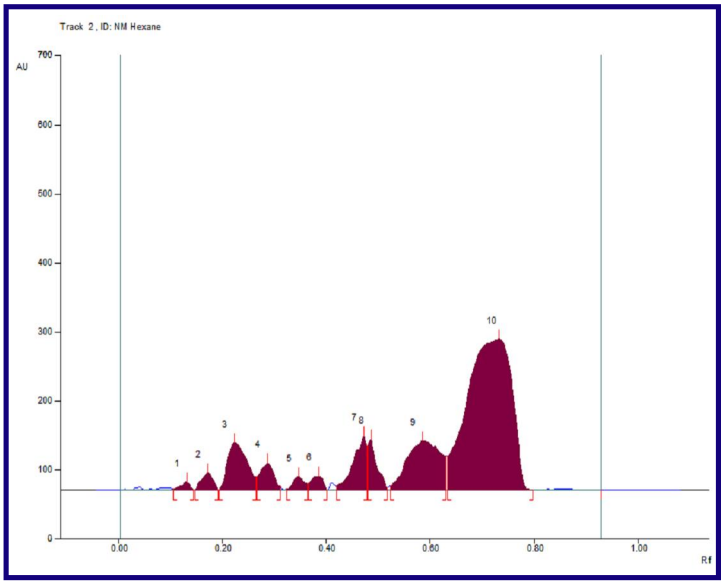

Fig. 8. HPTLC finger print profile of hexane extract of Nandi mezhugu at 575 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	0.9 AU	0.13 Rf	11.1 AU	1.79 %	0.15 Rf	0.5 AU	166.2 AU	0.63 %
2	0.15 Rf	0.0 AU	0.17 Rf	25.1 AU	4.03 %	0.19 Rf	0.5 AU	466.4 AU	1.76 %
3	0.20 Rf	0.5 AU	0.22 Rf	68.0 AU	10.94 %	0.27 Rf	18.9 AU	2126.5 AU	8.02 %
4	0.27 Rf	19.2 AU	0.29 Rf	38.6 AU	6.21 %	0.31 Rf	5.8 AU	844.5 AU	3.18 %
5	0.32 Rf	1.3 AU	0.35 Rf	19.0 AU	3.05 %	0.37 Rf	9.4 AU	342.0 AU	1.29 %
6	0.37 Rf	9.4 AU	0.39 Rf	19.6 AU	3.14 %	0.40 Rf	0.3 AU	388.5 AU	1.47 %
7	0.42 Rf	5.9 AU	0.47 Rf	77.9 AU	12.52 %	0.48 Rf	63.7 AU	1656.2 AU	6.25 %
8	0.48 Rf	64.4 AU	0.49 Rf	73.5 AU	11.81 %	0.52 Rf	3.3 AU	1030.4 AU	3.89 %
9	0.52 Rf	5.8 AU	0.59 Rf	71.3 AU	11.46 %	0.63 Rf	49.2 AU	3806.7 AU	14.35 %
10	0.63 Rf	49.4 AU	0.73 Rf	218.1 AU	35.05 %	0.80 Rf	0.0 AU	15692.3 AU	59.17 %

Fig. 9. R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 575 nm

The HPTLC finger print profile of hexane extract at 575 nm showed 10 peaks in which the peak at R_f 0.73 was the major peak with an area of 59.17 % followed by a peak at R_f 0.59 (14.35 %), 0.22 (8.02 %) and 0.47 (6.25 %). All other peaks are minor with an individual area less than 10 %.

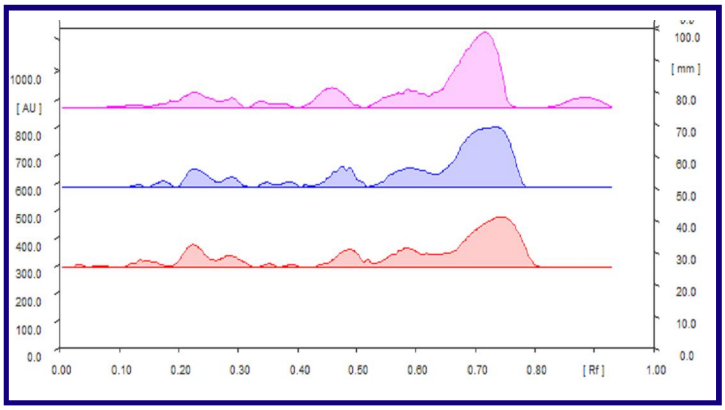

Fig. 10. 3D chromatogram of hexane extract of Nandi mezhugu at 575 nm

4. CONCLUSION:

Based on the above results, it is known that the drug Nandhi mezhugu has validated the traditional claim. The result from the physico chemical and safety study is encouraging and pave the way in the management of auto immune disease like Rheumatoid arthritis .

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