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Standardization of Milagathi Choornam

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Abstract

Milagathi chooranam is a poly herbal formulation originated from Siddha system of Indian medicine. It is mainly used for ulcer (Gunmam). Though the individual herbs used in the formulation have the previous record of standardization, there is no record on the formulation hence the same is aimed. All the ingredients were procured and botanically authenticated. Ingredients were purified individually as per the classical literature after which the formulation was prepared. The prepared drug was subjected to analyses. The derived physico-chemical parameters, TLC profiling, HPTLC fingerprint profiles serve as diagnostic parameters to identify this polyherbal formulation.

The achieved results of physico-chemical, TLC profiling, HPTLC finger print profiling will be useful as tools for authentication and standardization profile of the poly herbal formulation.

Keywords: *Piper nigrum*, *Piper longum*, Sodium chloride, Ulcer, HPTLC.

1. Introduction

Siddha is the indigenous system of Indian Medicine practiced in South India especially in Tamil Nadu. As there is an overall shift towards herbal medicines from modern medicine, the standardization part of herbal medicine became mandatory for the acceptance of the drug by modern scientific community and the pharmacopoeial standards are prerequisites for the quality control of the drug. In the present investigation, Milagathi chooranam, one of the Siddha formulations mentioned in the classical texts is taken up for the standardization study. The formulation is composed of seven herbal drugs and a salt. The drug is used in the ailment of ulcer. Route of administration the drug is Enteral. The dose of the drug is 500 mg twice a day with food [1]. There is a popular saying in Siddha system of medicine that food is medicine and medicine is food. The drug under investigation is an example for this saying, it is taken along with food and not as medicine. The prepared drugs was investigated for physicochemical, preliminary phytochemical, qualitative test for cations/anions, thin layer chromatographic documentation and high performance thin layer chromatographic finger printing to achieve standardization protocol.

2. Materials and methods

2.1. Plant Materials

All the ingredients were procured from Tampcol Raw Drug Store at Chennai and were identified by the Head, Department of Pharmacognosy, SCRI, Chennai-106. The list of the ingredients is presented in Table 1.

2.2. Purification of the raw materials

Purification of the raw material was done in accordance with the Siddha Formulary of India [2].

2.3. Preparation of *Milagathi chooranam*

After purification process, all the materials were completely dried, then powdered separately and sieved by white cloth which is mentioned as *Vasthirakayam* in classical Siddha text. The sieved ingredient powders were mixed thoroughly to get the *Chooranam* and stored in a clean and air tight glass container.

2.4 Physicochemical/Qualitative analyses

The particle size, loss on drying, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractive and pH were carried as per the methods described in pharmacopoeial texts [2, 3]. Preliminary phytochemical analysis for steroid, triterpene, flavonoid, coumarin, alkaloid, phenol, tannin, acid, glycoside and saponin were carried out as per the methods mentioned in standard organic books [4, 5]. The qualitative tests for anions/cations were done as per the methods followed by academicians.

2.5 TLC/HPTLC analysis

Four gm of the chooranam was soaked overnight in chloroform. Boiled on a water bath for 10 mins, filtered and concentrated to 10 ml. Aluminium plate precoated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used for the TLC/HPTLC analysis. Camag's twin trough chamber was used for the development. Many solvent systems were tried for a better separation and the same was achieved in Toluene: Ethyl acetate (6:1.5, v/v). All the solvents used were of AR grade. For derivatization vanillin-sulphuric acid reagent was used. Linomat 5 TLC applicator, CAMAG visualizer, CAMAG TLC scanner 030618 attached with WINCATS software were the instruments used for TLC photo documentation and HPTLC finger printing. 5 µl, 10 µl and 15 µl of the extract were applied as 8 mm band with 7 mm distance in between and developed up to 8 cm in the above mentioned solvent system. The air dried developed plate was visualized under UV 254/366 nm and TLC chromatograms were documented. The plate was scanned at UV 254 nm & 366 nm and the finger print profiles were recorded. Then the plate was derivatized with vanillin-sulphuric acid reagent and heated in an oven at 105 ^{0}C until the development of colored spots. TLC photo documentation was recorded and finger print profile at 540 nm was also recorded [6, 7].

3. Results & Discussion

The physicochemical values are shown in Table 2. The preliminary phytochemical analysis and the qualitative inorganic analysis results are shown in Table 3. The TLC photo documentations are shown in Figure 1A-C, the R_f values and colour of spots visible under UV 254 nm, 366 nm and after derivatization with vanillin-sulphuric acid are presented in Table 4. The HPTLC finger print profiles are shown in Figure 2, 4, 6. The Tables 5-7 show the R_f value and their relative peak areas. Figures 3, 5, 7 represent the 3D chromatograms of all three tracks before and after derivatization.

The loss on drying of the drug was found to be 7.475% which may attribute to essential oil rich ingredients. The volatile compounds may be protecting the drug from microbial growth. The total ash was found to be 11.775% which is due to the presence of the inorganic ingredient present in the drug. The acid insoluble ash shows that the drug contains only negligible amount of acid insoluble ash content. The extractive values, viz., water and alcohol were found to be 28.8% and 31.9% which shows that most secondary metabolites are extractable with the above solvents and also it shows the high polar secondary metabolites such as glycosides, tannins, proteins, etc. in the drug. The pH value of 6.5 shows that the drug is slightly acidic in nature. The particle size analysis shows that the drug is fine in nature. The qualitative phytochemical test results show that the drug is present with almost all type of secondary metabolites except anthraquinone and saponins. The qualitative inorganic test results show that the drug is free from all four heavy metals and present with sodium, potassium, magnesium, citrate, chloride ions which have essential role in the metabolism.

The TLC photo documentation of the drug under UV 254 nm shows three major spots at R_f 0.40, 0.47 and 0.57 and other spots are minor. The TLC photo documentation of the drug under UV 366 nm also shows three major spots at R_f 0.30, 0.40 and 0.67 and the same TLC plate after derivatization with vanillin-sulphuric acid shows the spot at R_f 0.67 which is the only major spot and all other are minor compared to that spot. The pink colour spot at R_f 0.82 may be thymol, a volatile component from *Trachyspermum ammi* [5].

Table 1: Details of Ingredients of Milagathi Choornam

S. No.	Tamil Name	Botanical Name	Part Used	Quantity
1.	Milagu	*Piper nigrum* Linn.	Fruit	2 part
2.	Kadukkai	*Terminalia chebula* (Retz.)	Fruit rind	2 part
3.	Chukku	*Zingiber officinale* Rosc	Rhizome	2 part
4.	Karunjeeragam	*Nigella sativa* Linn.	Fruit	2 part
5.	Perungayam	*Ferula asafoetida*	Oleo resin	2 part
6.	Thippili	*Piper longum* Linn.	Fruit	1part
7.	Omam	*Trachyspermum ammi* (L.) Sprague ex Turrill	Fruit	1 part
8.	Induppu	Sodium chloride	-	1part

Table 2: Physicochemical values of *Milagathi chooranam*

S. No	Parameter	Mean (n=2)±SD
1.	Loss on Drying at 105 °C (%)	7.475±0.216
2.	Total Ash (%)	11.775±0.408
3.	Acid insoluble Ash (%)	0.175±0.012
4.	Water Soluble Extractive (%)	28.8±0.180
5.	Alcohol Soluble Extractive (%)	31.9±0.250
6.	pH	6.5±0.050
7.	Particle size	Completely passes through sieve no.44

Table 3: Qualitative Phytochemical/Inorganic Results of *Milagathi chooranam*

S. No	Phytochemical Tests	Inference
1.	Acid	+ ve
2.	Proteins	+ ve
3.	Albumin	+ ve
4.	Alkaloids	+ ve
5.	Anthraquinones	- ve
6.	Coumarin	+ ve
7.	Steroid	+ ve
8.	Flavonoid	+ ve
9.	Triterpene	+ ve
10.	Glycoside	+ ve
11.	Saponin	- ve
12.	Tannins	+ ve
13.	Starch	+ ve
14.	Volatile oil	+ ve
15.	Sodium	+ ve
16.	Potassium	+ ve
17.	Magnesium	+ ve
18.	Lead	- ve
19.	Cadmium	- ve
20.	Arsenic	- ve
21.	Mercury	- ve
22.	Chloride	+ ve
23.	Citrate	+ ve

Table 4: R_f and colour of spots of TLC of chloroform extract of *Milagathi chooranam*

UV 254 nm		UV 366 nm		After derivatization with Vanillin-Sulphuric acid	
R_f	Colour	R_f	Colour	R_f	Colour
0.12	Green	0.02	Pink	0.08	Purple
0.18	Green	0.06	Blue	0.14	Purple
0.31	Green	0.10	Blue	0.21	Purple
0.40	Green	0.16	Blue	0.28	Yellow
0.47	Green	0.20	Blue	0.31	Grey
0.57	Green	0.30	Blue	0.56	Purple
0.67	Green	0.34	Greenish Yellow	0.67	Purple
-	-	0.40	Greenish Yellow	0.77	Purple
-	-	0.67	Blue	0.82	Pink
-	-	0.70	Pink	0.94	Purple
-	-	0.81	Pink	-	-

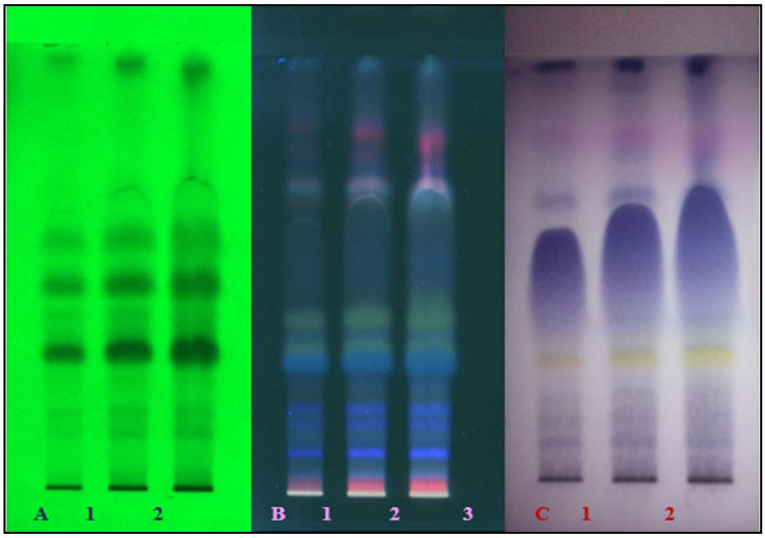

Fig 1: TLC photodocumentation of chloroform extract of *Milagathi chooranam*
A.UV 254 nm; B.UV 366 nm; C. After derivatization; Track 1.5 µl; Track 2.10 µl; Track 3.15 µl

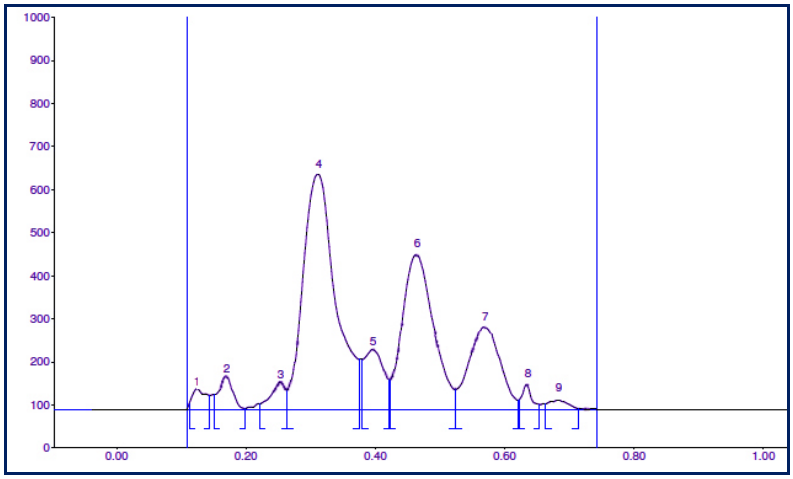

Fig 2: HPTLC finger print profile of chloroform extract of *Milagathi chooranam* at UV 254 nm

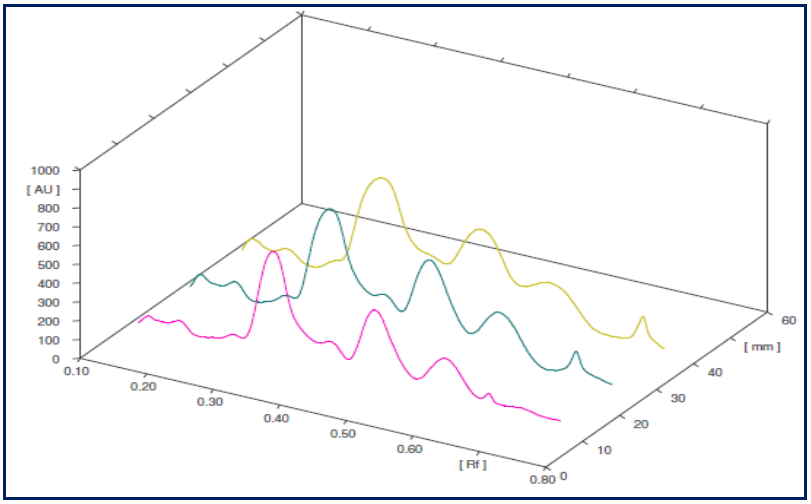

Fig 3: 3D chromatograms of all tracks at UV 254 nm

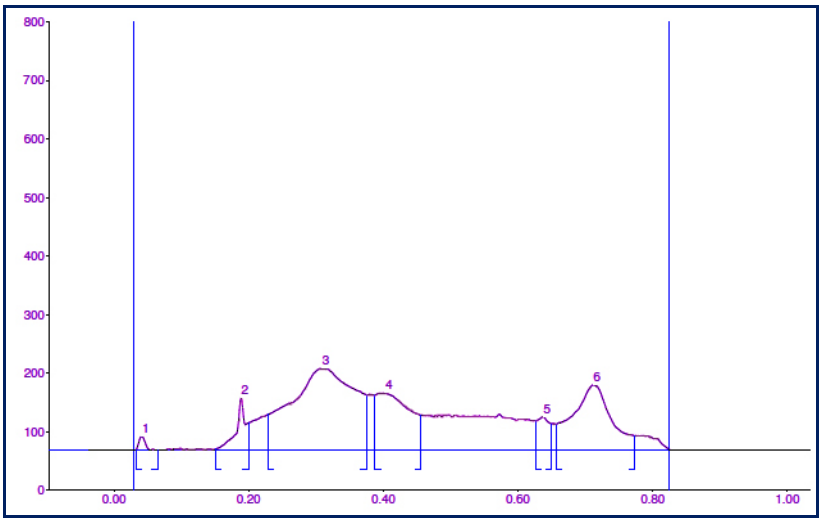

Fig 4: HPTLC finger print profile of chloroform extract of *Milagathi chooranam* at UV 366 nm

Table 5: R_f values and the Peak Areas at UV 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.11	6.0	0.12	77.7	4.24	0.14	44.0	1496.6	1.77
2	0.15	42.3	0.18	80.7	4.41	0.20	0.1	1974.0	2.34
3	0.21	1.6	0.26	71.6	3.91	0.26	65.6	1765.6	2.09
4	0.27	66.3	0.32	587.1	32.07	0.38	180.8	33993.2	40.24
5	0.38	181.3	0.40	198.3	10.83	0.42	129.1	5975.0	7.07
6	0.43	129.6	0.47	436.8	23.86	0.53	96.9	23328.2	27.61
7	0.53	97.0	0.57	245.1	13.39	0.64	3.4	13124.8	15.54
8	0.66	5.9	0.69	133.4	7.29	0.74	3.8	2824.0	3.34

Table 6: R_f values and the Peak Areas at UV 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	0.4	0.04	22.7	4.42	0.06	0.0	190.6	0.75
2	0.15	1.1	0.19	88.2	17.19	0.20	45.6	1147.2	4.50
3	0.23	60.1	0.31	138.6	27.01	0.38	93.6	12447.6	48.86
4	0.39	93.2	0.40	97.0	18.89	0.46	58.8	4647.2	18.24
5	0.63	49.1	0.64	56.4	10.98	0.65	43.8	1060.7	4.16
6	0.66	43.8	0.71	110.4	21.51	0.77	23.7	5981.6	23.48

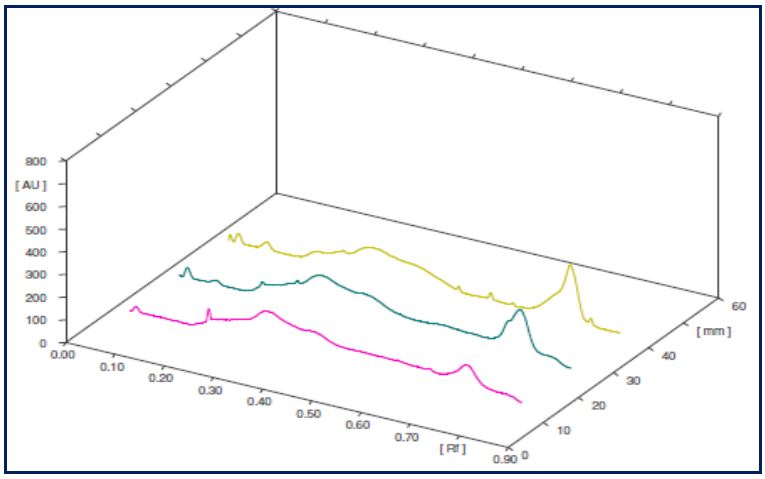

Fig 5: 3D chromatograms of all tracks at UV 366 nm

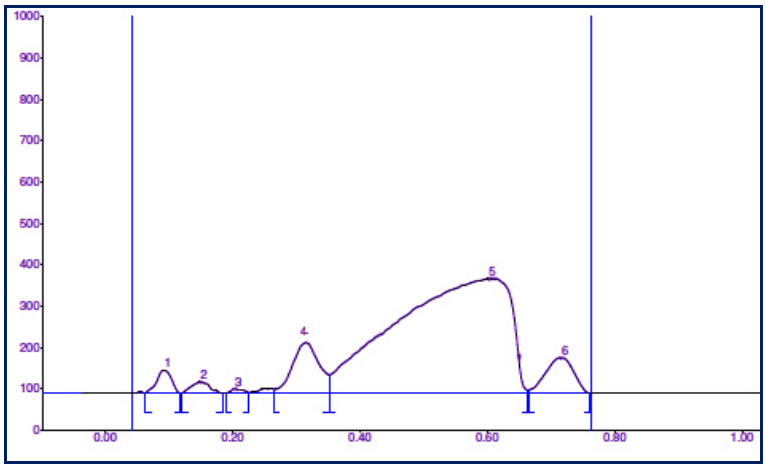

Fig 6: HPTLC finger print profile of chloroform extract of *Milagathi chooranam* at 540 nm

Table 7: R_f values and the Peak Areas at UV 540 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.06	0.4	0.09	55.7	9.54	0.12	0.4	1229.8	2.13
2	0.12	0.4	0.15	28.3	4.84	0.18	0.1	749.8	1.30
3	0.19	0.1	0.20	11.4	1.96	0.23	1.7	202.5	0.35
4	0.27	9.7	0.32	123.7	21.21	0.35	44.9	4901.5	8.50
5	0.35	45.1	0.60	277.4	47.55	0.66	8.0	46990.7	81.45
6	0.67	8.1	0.72	86.9	14.90	0.76	0.0	3621.7	6.28

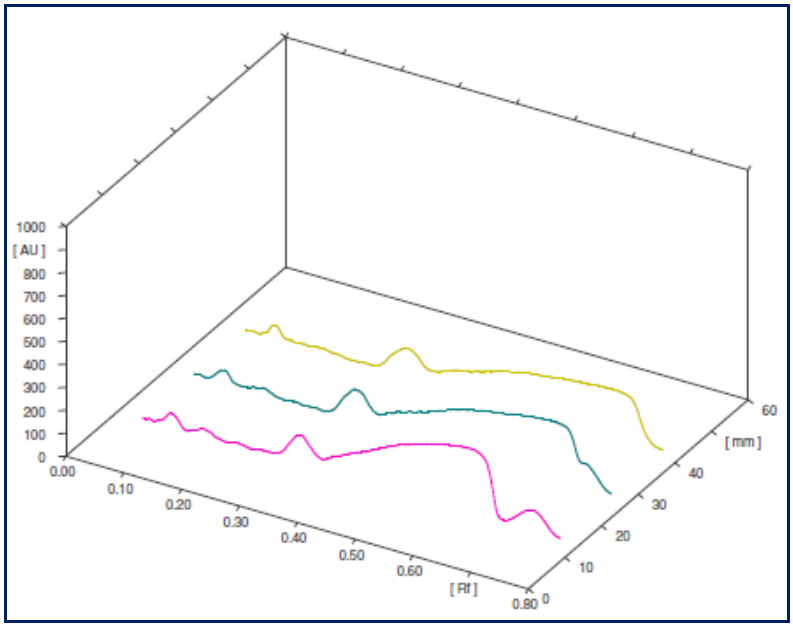

Fig 7: 3D chromatograms of all tracks at 540 nm after derivatization

The HPTLC finger print of the drug at UV 254 nm shows that the peak at R_f 0.32 constitutes 40.24% of the total area of the separated peaks and peaks at R_f 0.47 and 0.57 constitute 27.61% and 15.54% respectively. The 3D superimposable chromatogram shows that the peaks of all tracts are similar to each other.

The HPTLC finger print of the drug at UV 366 nm shows that the peak at R_f 0.31constitutes 48.86% of the total area of the separated peaks and peaks at R_f 0.71 and 0.64 constitute 23.48% and 18.24% respectively. The 3D superimposable chromatogram shows that all three concentrations are similar to each other. The HPTLC finger print of the drug at UV 540 nm shows that the peak at R_f 0.72 constitutes 81.45% of the total area of the separated peaks and all other peaks individually contribute less than ten percentage to the total area. The TLC photo and finger print profile show that 10 µl concentration of the extract is optimum for the better separation and analysis.

4. Conclusions

The achieved results of physico-chemical, preliminary phytochemical tests, qualitative inorganic tests, TLC profiling, HPTLC finger print profiling will be useful as tool for authentication, standardization profile and quality control assessment of the poly herbal formulation.

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6. References

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