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PHYSIOCHEMICAL ANALYSIS AND HPTLC ANALYSIS OF *ETTIKOTTAI MATHIRAI*-SIDDHA POLYHERBAL FORMULATION

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Abstract

Background: Siddha system of medicine is one of the holistic systems widely being practice in Tamil Nādu and the concept pertaining to drug ingredients are from plant (*Mooligai)*, Metals, Minerals (*Thaadhu*) and Animal (*Jeevam*) origin. Traditional medicines require newer standardization, manufacturing, quality control, and scientifically rigorous research to meet standardization. *Ettikottai Mathirai*(EM) is the polyherbal formulation mentioned in *Naam nadu vaithiyam* indicated for 18 types of *soolai*(Pain), Lumbar pain.

Aim: This study aims to develop standardization of *Ettikottai Mathirai (*EM) as per PLIM (Pharmacopeia Laboratory of Indian Medicine) guidelines.

Method: The test drug EM focused on Physico-chemical analysis, Instrumental analysis such as HPTLC Analysis.

Result: Physiochemical analysis report showed loss of drying EM is 10.88%, total ash is 4.69%, acid insoluble ash is 0.89%, water soluble ash is 19.50%,pH of EM is 5.85, Average weight of tablet is 162mg, friability test of EM is 0.19%,Hardness of test drug EM 20.33N. The disintegration test of sample EM is 120 mins.Width and thickness of EM is .78mm and 6.75mm respectively. The drug EM had HPTLC fingerprint analysis revealed the presence of eleven phytocomponents at 254nm, ten phytocomponents at 366nm and ten phytocomponents at 520nm. All the results of this study ensures that *Ettikottai Mathirai* possess therapeutic properties and for further evidence for its safety and efficacy pharmacological study has to be carried out.

Keyword: Standardization, *Ettikottai Mathirai*, Siddha medicine,HPTLC,Physio-chemical analysis, *Strychnos nux-vomicas*.

1. Introduction

The Siddha system of medicine is a prestigious system belonging to South India. According to Siddha system, medicine is a substance that helps to alleviate or eradicate the disease, gives strength to the body, and normalizes the functions of the body [1].The Siddha medicine was split into two groups, internal medicine and external medicine. Among them, *Mathirai*(Pills) is one of the internal medicine having shelf life of 1 year. The raw drugs are triturated with the *kudineer* or juices of plant leaves and fruits. They are made into different sizes, dried and stored[2]. Siddha medicine incorporates wide usage of herbs, metals, minerals and animal products were used to prepare medicine in treating lot of medical aliments. The scientific evaluation is needed to validates is preciousness. It helps to ensure safety to the public and effective traditional treatment for diseases [3]. *Ettikottai Mathirai*(EM) is the polyherbal formulation mentioned in *Naam nadu vaithiyam* indicated for 18 types of *soolai*(Pain), Lumbar pain,Which comprises of four herbal drugs. *Milagu*(Piper nigrum), *Chukku* (*Zingiber officinale*), *Ettikottai* (*Strychnos nux-vomica*) , *Kirampu* (*Syzygium aromaticum*). In recent days, the demand for Siddha formulations has expanded globally due to their rising popularity and long-term stability. As a result, both domestically and globally, the commercialization of Siddha medicine manufacture has drawn attention. For Siddha formulations, determining the necessary standards, quality control, and safety were imperative. Therefore, the processing units of Siddha pharmaceuticals must focus on producing standard, effective, genuine, safe, and high-quality drugs[4]. It is very important to establish a system of standardization for every end product of the medicine since the scope for variation in different batches of medicine is enormous[5]. Here, in the present study, test drug *Ettikottai Mathirai*(EM) was prepared and its physicochemical and HPTLC fingerprint analytical techniques was based on the PLIM guidelines.

2. Materials and Method

2.1 Collection of Raw drug

The raw drug was purchased in Country shop, Chennai and authenticated by botanist and from Department of Gunapadam ,National Institute of Siddha ,Chennai.

Table 1: Ingredients for *Ettikottai Mathirai*

S.NO	INGREDIENS	BOTANICAL NAME	PART USED	QUANTITY
1	*Milagu*	*Piper nigrum*	Seed	1 *palam*
2	*Chukku*	*Zingiber officinale*	Rhizome	1 *palam*
3	*Ettikottai*	*Strychnos nux-vomica*	Seed	1 *palam*
4	*Lavagam*	*Syzygium aromaticum*	Flower bud	1 *palam*
5	*Lemon juice*	*Citrus lemon*	Fruit	1 *palam*

2.2 Perparation of Ettikottai Mathirai

Equal quantity of all the ingredients was roasted and made as a fine powder. Grinding the powder gently by adding lemon juice until to make a pills[6].Ingredients were tabulated (Table 1).

2.3Physiochemical Analysis according to PLIM Guideline

As per AYUSH - PLIM guideline [7, 8], the test drug *Ettikottai Mathirai* (EM) was tested its physiochemical properties and also to assess the active principles and elements present in the drug by HPTLC.

Physicochemical studies like total ash, water-soluble ash, acid soluble ash, acid-insoluble ash, water,and alcohol soluble extract, loss on drying at 105 ^{0}C were done at Siddha Central Research Institute, Anna Govt. Hospital Campus, Arumbakkam, Chennai. Results were tabulated in (Table 2).

2.4 Physicochemical Evaluation

2.4.1 Solubility Test

The sample EM was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like Chloroform, Ethanol, Distilled water, Ethyl acetate, Hexane, Dimethyl sulfoxide (DMSO), and the results are observed individually.

2.4.2 Loss on Drying

An accurately weighed 2gm of EM formulation was taken in a tarred evaporating dish. The crude drug was heated 105ºc for 5 hours in an oven till a constant weight and then weighed. The percentage moisture content of the sample was calculated with reference to the shade dried material.

2.4.3.Determination of Total Ash

EM was accurately weighed 2g in silica dish and incinerated in furnace at a temperature 400 ºC until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

2.4.4 Determination of Acid Insoluble Ash

The ash obtained by Total ash test was boiled with 25ml of hydrochloric acid for 6 minutes and filtered using ash less filter paper. Then the Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

2.4.5.Determination of Water Soluble Extractive

5gms of EM was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaken frequently for six hours and it was allowed to stand for eighteen hours. The solution was filtered rapidly with taking precautions to prevent loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, further dried at 105ºC to constant weight and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

2.4.6 Determination of Alcohol Soluble Extractive

The test drug EM was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaken frequently for six hours and it was allowed to stand for eighteen hours. The solution was filtered rapidly

with taking precautions to prevent loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, further dried at 105ºC to constant weight and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drugs.

2.4.7 pH value

About 5 gram of EM will be dissolved in 25 ml of distilled water and filtered the resultant solution is allowed to stand for 30 minutes and then subjected to pH evaluation.

2.5 Instrumental Analysis of Ettikottai Mathirai (EM)

The test drug *Ettikottai Mathirai* (EM) was analyzed to generate the fingerprint using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC).

2.5.1 Thin layer chromatography-TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60E254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system after the run plates are dried and was observed using visible light Short-wave UV light 254mm and light long-wave UV light 365 nm[9]. TLC of EM was shown in Fig 1 and tabulated in table 3.

2.5.2 High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics[10].

Chromatogram Development It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

3. Result

3.1Physicochemical parameters

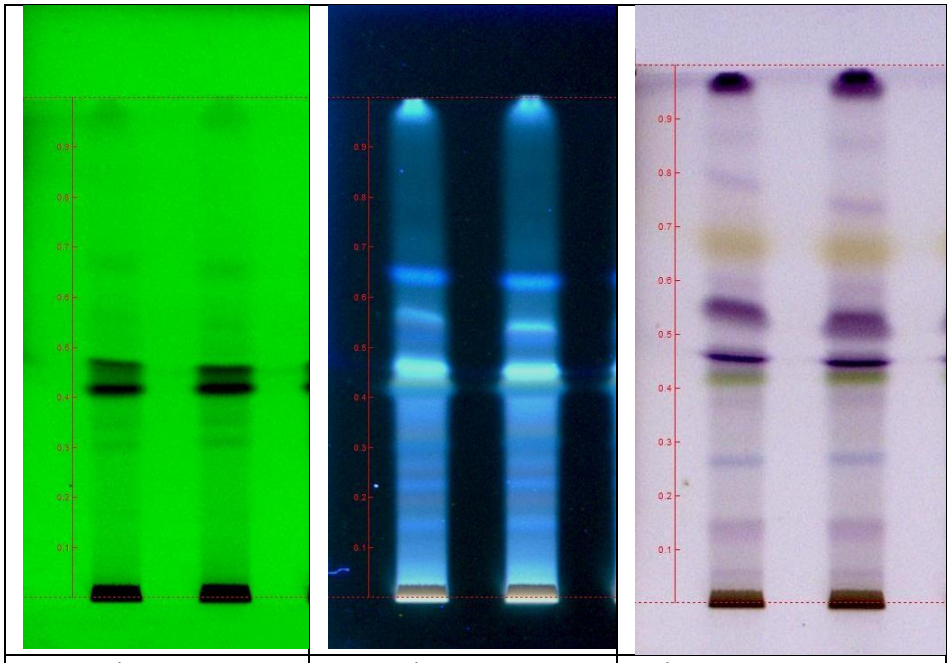
The physicochemical analysis of *Ettikottai Mathirai* was done and tabulated in table 2.

Table 2: Physicochemical Analysis of *Ettikottai Mathirai*

Name of the Experiment	**Mean**
Loss on drying at 105°C, %	10.88
Total ash, %	4.69
Acid insoluble ash, %	0.89
Water soluble extractive, %	19.50
Alcohol soluble extractive, %	19.28
pH value (10% solution)	5.85
Average weight of tablet % Deviation of highest weight tablet % Deviation of lowest weight tablet (Average weight ± 7.5 mg is permissible and 18 tables should pass. 11 tablets deviates from the permissible weight)	162 mg +45.06 -1.23%
Friability test	0.19%
Disintegration test	120 min
Hardness tet	20.33 N
Width of the tablet	6.78 mm
Thickness of the tablet	6.75 mm

3.2 Instrumental Analysis

TLC and HPTLC fingerprinting can be employed for the purpose of creating quality standards for polyherbal compositions. For sample EM fingerprint was mentioned below.



λ=254 nm	λ=366 nm	λ=520 nm (Derivitized)

Fig 1: TLC fingerprint for Ettikottai Mathirai(EM)

Rf and colour of spots

Table 3: TLC Rf and colour of spots of Sample EM

λ=254 nm		λ=366 nm		λ=520 nm (Derivitized)	
Rf	Colour	Rf	Colour	Rf	Colour
0.17	Green	0.14	Blue	0.05	violet
0.31	Green	0.19	Maroon	0.14	violet
0.36	Green	0.22	Blue	0.27	blue
0.42	Green	0.30	Blue	0.38	Light pink
0.46	Green	0.33	Pink	0.42	green
0.55	Green	0.40	Blue	0.44	blue
0.65	Green	0.42	Yellow	0.53	Purple
0.96	Green	0.44	fluorescent	0.58	Light purple
		0.52	Maroon	0.66	Yellow
		0.54	Fluorescent	0.74	Violet
		0.62	Blue	0.86	Light violet
				0.96	Purple

3.2.1HPTLC Finger Printing Analysis of EM

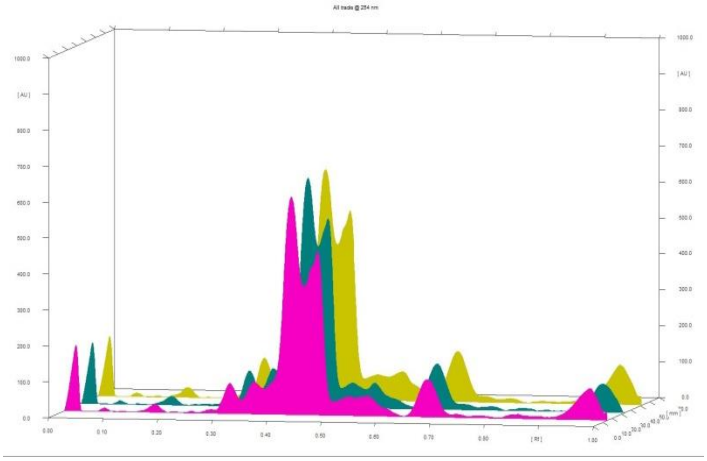


Fig 2:3D Chromatogram of 254 nm

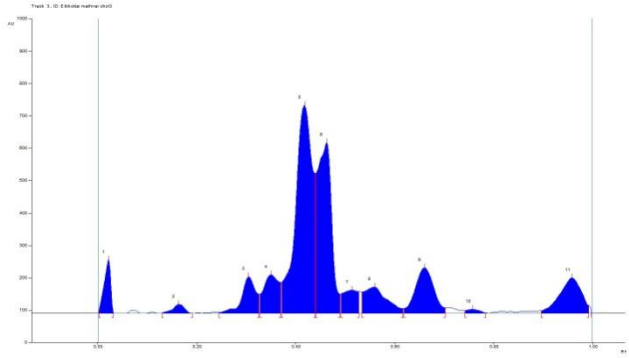


Fig 3: HPTLC finger print profile of Chloroform extract scanning 254 nm

Table 4: Peak Table @ 254 nm

Track 3, ID: Ettikottai mathirai chcl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	8.9 AU	0.02 Rf	164.4 AU	8.20 %	0.03 Rf	1.5 AU	1902.8 AU	3.46 %
2	0.13 Rf	0.3 AU	0.16 Rf	27.2 AU	1.36 %	0.19 Rf	0.0 AU	555.8 AU	1.01 %
3	0.25 Rf	2.0 AU	0.31 Rf	112.7 AU	5.62 %	0.33 Rf	58.5 AU	2768.5 AU	5.04 %
4	0.33 Rf	58.7 AU	0.35 Rf	119.0 AU	5.93 %	0.37 Rf	95.6 AU	3217.0 AU	5.86 %
5	0.37 Rf	96.2 AU	0.42 Rf	641.1 AU	31.96 %	0.44 Rf	31.5 AU	19300.1 AU	35.13 %
6	0.44 Rf	432.1 AU	0.46 Rf	526.8 AU	26.27 %	0.49 Rf	59.6 AU	13041.0 AU	23.74 %
7	0.49 Rf	60.0 AU	0.51 Rf	72.1 AU	3.59 %	0.53 Rf	67.0 AU	1890.3 AU	3.44 %
8	0.53 Rf	66.4 AU	0.56 Rf	81.1 AU	4.04 %	0.62 Rf	14.3 AU	3219.1 AU	5.86 %
9	0.62 Rf	14.5 AU	0.66 Rf	140.0 AU	6.98 %	0.70 Rf	17.0 AU	4573.1 AU	8.33 %
10	0.74 Rf	8.4 AU	0.76 Rf	11.8 AU	0.59 %	0.79 Rf	1.4 AU	272.8 AU	0.50 %
11	0.90 Rf	7.6 AU	0.96 Rf	109.6 AU	5.46 %	0.99 Rf	23.9 AU	4191.2 AU	7.63 %

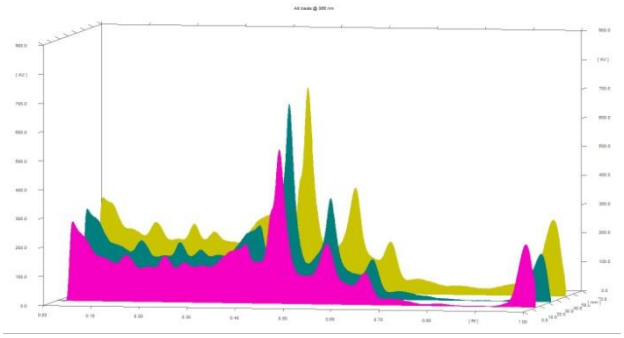


Fig 4: 3D Chromatogram of 366 nm

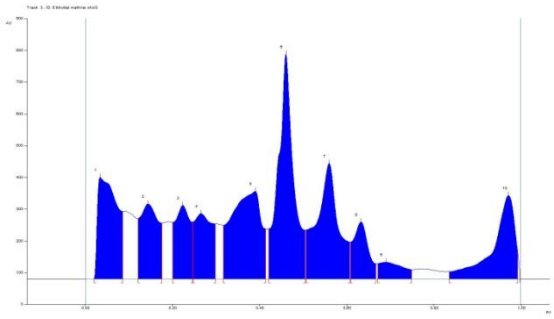


Fig 5: HPTLC finger print profile of Chloroform extract scanning 366 nm

Table 5: Peak Table @ 366 nm

Track 3, ID: Ettikottai mathirai chcl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	2.2 AU	0.03 Rf	320.1 AU	11.28 %	0.09 Rf	12.6 AU	12514.5 AU	11.55 %
2	0.12 Rf	190.3 AU	0.14 Rf	236.1 AU	8.32 %	0.18 Rf	76.1 AU	8692.3 AU	8.02 %
3	0.20 Rf	177.9 AU	0.22 Rf	232.2 AU	8.18 %	0.25 Rf	79.2 AU	7043.4 AU	6.50 %
4	0.25 Rf	179.7 AU	0.27 Rf	206.0 AU	7.26 %	0.30 Rf	72.9 AU	7333.3 AU	6.77 %
5	0.32 Rf	168.9 AU	0.39 Rf	276.3 AU	9.74 %	0.41 Rf	57.7 AU	16500.3 AU	15.23 %
6	0.42 Rf	158.0 AU	0.46 Rf	708.3 AU	24.96 %	0.51 Rf	53.6 AU	21438.4 AU	19.78 %
7	0.51 Rf	153.7 AU	0.56 Rf	363.7 AU	12.82 %	0.61 Rf	15.9 AU	15585.9 AU	14.38 %
8	0.61 Rf	116.2 AU	0.63 Rf	179.6 AU	6.33 %	0.67 Rf	47.9 AU	5402.3 AU	4.99 %
9	0.67 Rf	48.7 AU	0.69 Rf	52.9 AU	1.86 %	0.75 Rf	29.6 AU	2576.6 AU	2.38 %
10	0.84 Rf	22.5 AU	0.97 Rf	262.7 AU	9.26 %	0.99 Rf	84.9 AU	11271.4 AU	10.40 %

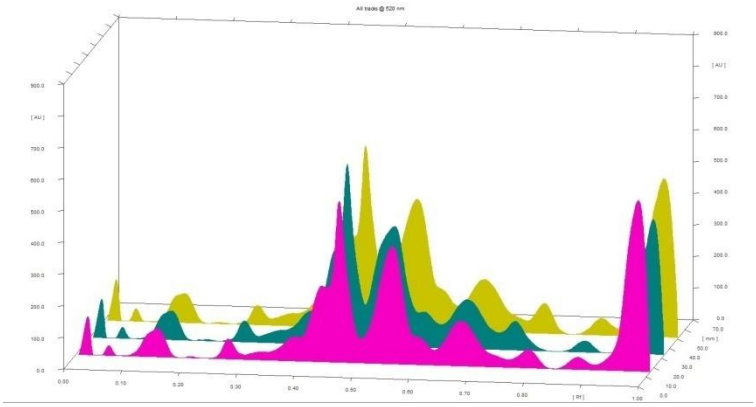


Fig 6: 3D Chromatogram of 520 nm

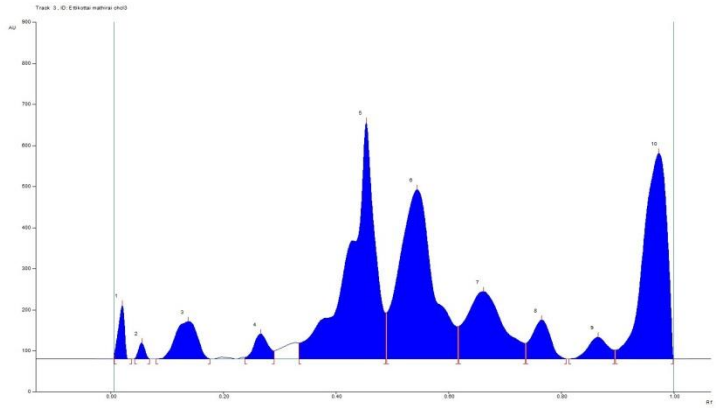


Fig 7: HPTLC finger print profile of Chloroform extract scanning 520 nm

Table 6: Peak Table @ 520 nm

Track 3, ID: Ettikottai mathirai chcl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	9.5 AU	0.02 Rf	130.4 AU	6.15 %	0.04 Rf	0.0 AU	1231.0 AU	1.51 %
2	0.04 Rf	0.7 AU	0.06 Rf	38.4 AU	1.81 %	0.07 Rf	0.4 AU	383.5 AU	0.47 %
3	0.08 Rf	0.0 AU	0.14 Rf	91.4 AU	4.31 %	0.18 Rf	0.1 AU	3073.8 AU	3.76 %
4	0.24 Rf	3.7 AU	0.27 Rf	60.7 AU	2.86 %	0.29 Rf	19.8 AU	1310.3 AU	1.60 %
5	0.33 Rf	38.8 AU	0.45 Rf	575.5 AU	27.14 %	0.49 Rf	11.3 AU	22910.9 AU	28.03 %
6	0.49 Rf	111.8 AU	0.55 Rf	412.1 AU	19.43 %	0.62 Rf	78.6 AU	21412.0 AU	26.20 %
7	0.62 Rf	79.0 AU	0.66 Rf	164.2 AU	7.74 %	0.74 Rf	37.8 AU	9173.6 AU	11.23 %
8	0.74 Rf	38.3 AU	0.77 Rf	94.6 AU	4.46 %	0.81 Rf	0.1 AU	2660.4 AU	3.26 %
9	0.81 Rf	0.0 AU	0.87 Rf	52.8 AU	2.49 %	0.90 Rf	21.8 AU	1615.4 AU	1.98 %
10	0.90 Rf	21.7 AU	0.97 Rf	500.8 AU	23.61 %	1.00 Rf	0.0 AU	17953.6 AU	21.97 %

4. Discussion

Standardisation of traditional medicine is the process of prescribing a set of standards and constant parameters that carry an assurance of quality, efficacy, safety and reproducibility of the drugs [11].The drug *Ettikottai Mathirai* is one of the polyherbal formulation indicates for *soolai,* lumbar pain and severe pain. The loss on drying of sample EM is 10.88% indicates the stability and shelf life of the drug EM is good. The total ash value of KL is 4.69% shows that the drug EM has less contamination and adulteration and it is safe. When acid insoluble ash value is low indicates the quality of the drug. Here, acid insoluble ash value of EM is 0.89%. Hence, it represents the superior quality of the drug EM. Water-soluble extractive value plays an important role in evaluation of crude Drugs and it is 19.50%. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value and it is 1.28% for Ettikottai Mathirai. The pH of the drug EM is 5.85 which is acidic in nature and it is essential for its bioavailability and effectiveness. The result revealed that the drug has good quality and purity and it indicates no adulteration in the raw drug EM.To assess the quality of tablets, Average weight of tablet of EM is 162mg,Friabilty of EM tablet is 0.19%. Disintegration time for test drug EM is 120min.The Hardness of EM is 20.33N.The width and thickness of tablet EM is 6.78mm and 6.75mm respectively.

At 254nm, TLC finger printing analysis of the sample EM reveals the presence of 8 prominent peaks corresponds to presence of 8 versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.17 to 0.96, where at 366nm, sample EM reveals the presence of 11 prominent peaks corresponds to presence of 12 versatile phytocomponents of Rf value peaks ranges from0.14 to 0.62. At 520 nm sample EM reveals the presence of 12 prominent peaks corresponds to presence of 11 versatile phytocomponents of Rf value peaks ranges from0.05 to 0.96.

The sample *Ettikottai Mathirai*(EM) has HPTC finger printing analysis revealed the presence of 11 versatile phytocomponents present at 254nm. Rf value of the peaks ranges from 0.13 to 0.90.At 366nm, presence of 10 versatile phytocomponents present with Rf value of the peaks ranges from 0.02 to 0.84. At 520nm, presence of 10 versatile phytocomponents present with Rf value of the peaks ranges from 0.01 to 0.90.

6.Conclusion

These results obtained in this study suggests that *Ettikottai Mathirai* have quality specifications to develop using tests described in Siddha along with analytical tools available today. EM have a maximal efficacy and reduced adverse effect which helps in development of drug. Hence, the standardization of the drug is the first step for further assessing toxicological and validating pharmacological activities.

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