

## STANDARDISATION OF MURUKKAN VITAI MATHIRAI- A SIDDHA TABLET FORMULATION

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### ABSTRACT

Herbal medicine has been commonly used over the years for treatment and prevention of diseases, health promotion and enhancement of the span and quality of life. There is lack of a systematic approach to assess their safety and effectiveness. Standardization assures the identity, purity and strength of the drug. It helps to avoid adulteration, improper substitution and there by authentication of the samples. Hence an attempt is made to establish the pharmacopoeial standards of Siddha tablet formulation Murukkan Vitai Mathirai. It is one of the most commonly used Siddha medicines for treating the ailments such as intestinal worms, abdominal bloating, distension and indigestion. In this study, the formulation was standardized by determining its organoleptic characters, powder microscopy, physico-chemical properties and HPTLC finger printing. The physico-chemical parameters such as loss on drying at 105°C, total ash, acid insoluble ash, water & alcohol soluble extractives and pH of 10 % water extract and preliminary phytochemicals were analyzed. The microscopic standards could be used to detect the ingredients in the compound formulations and to decide the authenticity of the formulation. The physicochemical characters, HPTLC fingerprinting profile and botanical parameters together may be used for quality evaluation and standardization of Murukkan Vitai Mathirai.

**KEYWORDS:** Murukkan Vitai Mathirai, Physicochemical characters, HPTLC fingerprinting profile, Botanical parameters, Standardisation.

### INTRODUCTION

Traditional medicines are in practice by all over the world, not only for primary health care but also for the betterment of health. The World Health Organization has also recognized the importance of traditional medicines and has been active in creating strategies, guidelines and standards for herbal medicines. Herbal medicine has been commonly used over the years for treatment and prevention of diseases, health promotion and enhancement of the span and quality of life. However, there is lack of a systematic approach to assess their safety and effectiveness.<sup>[1,2]</sup>

Standardization assures that products are reliable in terms of quality, efficacy, performance and safety. It helps to avoid adulteration and improper substitution. Hence an attempt is made to establish pharmacopoeial standards for a Siddha tablet formulation Murukkan Vitai Mathirai (MVM). It is one of the most commonly used Siddha medicines for treating the ailments such as

intestinal worms, abdominal bloating, distension and indigestion.<sup>[3]</sup> In this study, the formulation was standardized by determining its organoleptic characters, powder microscopy, physico-chemical properties and HPTLC finger printing. Recently chromatographic finger printing has become one of the most potent tools for the quality control of herbal medicines because of its simplicity and reliability.

### MATERIALS AND METHODS

Murukkan Vitai Mathirai consists of seven ingredients (Figure 1) and is listed in Table I. The drug was purchased from Indian Medical Practitioners Co-Operative Pharmacy and Stores Ltd., Adyar.

To identify the presence of genuine ingredients in MVM, 5 pills were taken, crushed and made powder. A few mg of powder was cleared in chloral hydrate, washed in water and mounted in glycerin. Cleared another portion in 2% potassium hydroxide solution, washed in water

and mounted in glycerin. Sudan III, phloroglucinol and a drop of hydrochloric acid and IKI solution were also employed and mounted in glycerin to observe the diagnostic characters of the ingredients.

#### Organoleptic characters

Colour, odour and taste of the drug were noted.

#### Physico-chemical parameters

Physico-chemical parameters of MVM were performed according to standard methodology and WHO guidelines.<sup>[4,5]</sup> The physico-chemical investigations include determination of total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives, loss on drying at 105°C, uniformity of weight and pH of the water extract.

#### Successive soxhlet extraction

The prepared drug was subjected to successive soxhlet extraction using hexane, chloroform and alcohol.

#### Preliminary phytochemical investigation

Preliminary phytochemical analysis for various groups of phytochemicals such as phenols, terpenoids, steroids, flavonoids, quinones, coumarins, alkaloids, tannins and acids were carried out by standard procedures.<sup>[6,7]</sup>

#### High Performance Thin Layer Chromatographic Profile

High Performance Thin Layer Chromatography (HPTLC) has the advantage of many fold possibilities of qualitative and quantitative detection in analyzing herbal medicines with high accuracy and precision.

#### Sample preparation

1 gm powdered defatted drug was extracted with 10 ml of chloroform, kept the solution overnight and boiled for 10 minutes. The extract was filtered and concentrated to 1 ml.<sup>[8]</sup>

#### Sample application

The defatted chloroform extract of MVM was applied as two tracks on silica gel 60 F<sub>254</sub> pre-coated aluminium sheets in the form of bands of length 10 mm, volume 5 µl and 10 µl and with distance 10 mm between two bands through CAMAG microlitre syringe using Automatic TLC Sampler 4 (ATS4).

## RESULTS AND DISCUSSION

**Table1. Ingredients of MurukkanVitai Mathirai**

Sl.No.	Ingredients	Botanical Name	Parts used	Quantity
1	Cukku	<i>Zingiber officinale</i> Rosc.	D. Rz.	1 part
2	Milaku	<i>Piper nigrum</i> L.	Fr.	1 part
3	Tippili	<i>Piper longum</i> L.	Fr.	1 part
4	Cirakam	<i>Cuminum cyminum</i> L.	Fr.	1 part
5	Katukarokini	<i>Picrorhiza kurroa</i> Royle ex Benth	Rt.	1 part
6	Murukkanvittu (Palacuvittu)	<i>Butea monosperma</i> (Lam.) Taub.	Sd.	1 part
7	Nervalam (purified)	<i>Croton tiglium</i> L.	Sd.	6 parts

#### Mobile phase and development of chromatogram

The plate was developed using the mobile phase comprising of Toluene: Ethyl acetate: Formic acid (5:1:2 drops), which was found to give a good separation and resolution of components of extract. Linear ascending development was carried out in 10 cm x 10 cm twin trough glass chamber saturated with mobile phase. The mobile phase was allowed to migrate a distance of 70 mm. After the development, the TLC plate was dried completely.

#### Documentation

The plate was kept in CAMAG visualizer and the images were captured under UV light at 254 nm and 366 nm. The possibility of visual evaluation of separated samples on the plate is one of the most valuable aspects of TLC. It reaches a completely new dimension in HPTLC through the use of state-of-the-art techniques for generating and evaluating digital images.

#### Densitometric Analysis

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<sub>f</sub> values and finger print profile were recorded. Concentrations of compound chromatographed were evaluated as peak areas.

#### Derivatisation

By derivatization, substances that do not respond to visible or UV light can be rendered detectable. The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105°C 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.

The fingerprinting profiles were recorded.



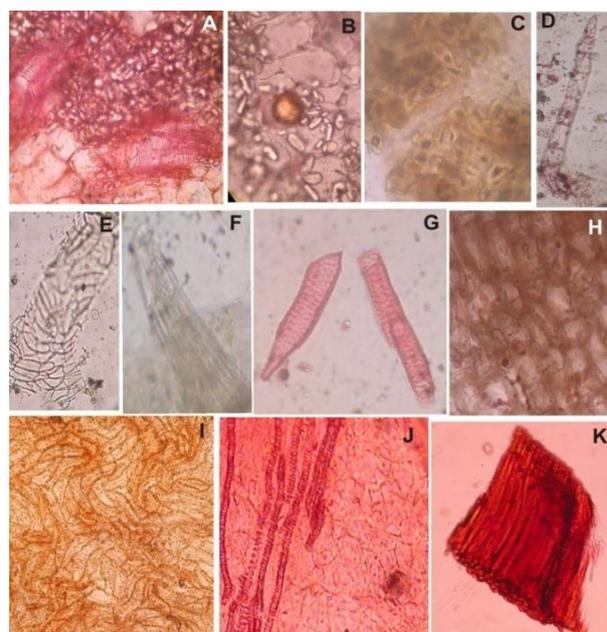
**Figure 1: Murukkan Vitai Mathirai and its ingredients.**

#### Organoleptic characters

Black coloured pills with a pleasant odour, taste slightly bitter.

#### Microscopy

Microscopy of Murukkan Vidai Mattirai was studied to identify the ingredients (Figure 2). Yellowish to reddish brown oleo – resin cells, vessels with annular, reticulate or spiral thickenings indicated the presence of dried rhizome of *Zingiber officinale* Rosc.; characteristic groups of isodiametric stone cells interspersed with polygonal parenchyma cells must be from the fruit of *Piper nigrum* L.; spindle shaped elongated stone cells with wide lumen, multicellular, uniseriate trichome were observed from the fruit of *Piper longum* L.; parquetry tissue, pleuricellular, pleuriseriate trichomes from the fruit of *Cuminum cyminum* L.; cork cells, pitted vessels, long thick walled tracheids, cylindrical with blunt tapering ends must be from the root of *Picrorhiza kurroa* Royle ex Benth.; fragments of orange to brown colour testa, osteosclereids observed are from the seed of *Butea monosperma* (Lam.) Taub.; elongated cells containing reddish – brown contents, nucellus in surface view embedded with vascular strands must be from the seed of *Croton tiglium* L.



**Figure 2: Microscopy of Murukkan Vitai Mathirai.**

- A. Vessels with spiral thickenings
- B. Yellowish – reddish brown oleo resin cells
- C. Isodiametric stone cells interspersed with polygonal parenchyma cells
- D. Multicellular uniseriate trichome
- E. Parquetry tissue
- F. Pleuricellular, pleuriseriate trichome
- G. Pitted vessels
- H. Fragment of cork cells
- I. Fragment of testa
- J. Nucellus in surface view embedded with vascular strands
- K. Elongated cells containing reddish brown contents.

#### Physico-chemical parameters

The physico-chemical parameters and successive extractive values of the drug with solvents of increasing order of polarity are given in Table 2. These parameters are useful in establishing the profile quality of the drug and are important for its evaluation.

**Table 2. Physico-chemical parameters of Murukkan Vitai Mathirai.**

Sl.No	Parameters	Result
1	Loss on drying at 105° C %	8.03
2	Total ash content %	6.42
3	Acid insoluble ash %	1.00
4	Water soluble extractive %	12.49
5	Alcohol soluble extractive %	33.56
6	pH of water extract	6.54
7	Uniformity of weight, (%) variation	± 0.5031
8	Successive Extraction %	
	Hexane	24.33
	Chloroform	3.47
	Alcohol	7.24

The loss on drying of the drug was found to be 8.03 % which may be due to the presence of volatile oil and/or moisture content in the ingredients. The total ash was found to be 6.42 % and was within the permissible limit. Total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash—which is derived from the plant tissue itself and non physiological ash—which is the residue of sand and soil adhering to the plant surface.<sup>[9]</sup> Acid insoluble ash particularly indicates contamination with siliceous material. Water soluble extractive and alcohol soluble extractive were found to be 12.49 % and 33.56 % respectively. Most of the highly polar secondary metabolites are extracted with water and alcohol. The pH value, 6.54 showed that the drug is slightly acidic in nature. Regarding Uniformity of weight, % variation observed was  $\pm 0.5031$ .

#### Successive soxhlet extraction

Extractable matters obtained for soxhlet extraction comprised of constituents that are extracted with solvents in increasing order of polarity.

#### Preliminary phytochemical investigation

The preliminary phytochemical investigations of drugs show the presence of major secondary metabolites which reveal the potent therapeutic activity. The preliminary phytochemical investigations of the extracts of MVM

showed the presence of terpenoids, flavonoids, phenols, alkaloids and tannins.

#### High Performance Thin Layer Chromatographic Profile

TLC photo documentation profiles of the defatted chloroform extract of MVM at 254 nm, 366 nm and after derivatisation under white light are given in Figure 3. The solvent system,

Toluene: Ethyl acetate: Formic acid (5:1:2 drops) efficiently resolved the components present in the crude extract.  $R_f$  values and colour of major spots of the extract are shown in Table 3. 11 visible spots at 254 nm with  $R_f$  values 0.03, 0.08, 0.10, 0.14, 0.21, 0.25, 0.32, 0.57, 0.65, 0.83 and 0.90; 11 spots with  $R_f$  values 0.03, 0.07, 0.13, 0.16, 0.29, 0.39, 0.48, 0.56, 0.64, 0.70 and 0.82 at 366 nm; and 6 spots with  $R_f$  values 0.07, 0.20, 0.47, 0.57, 0.66 and 0.94 after derivatisation under white light were observed. The  $R_f$  values reflect the phytoconstituents of the plant which may establish the identification of the genuine source. HPTLC fingerprinting pattern of the defatted chloroform extract of MVM at 254 nm are shown in Figure 4 (1) and Figure 4 (2), at 366 nm in Figure 5 (1) and Figure 5 (2) and at 575 nm after derivatisation in Figure 6 (1) and Figure 6 (2).

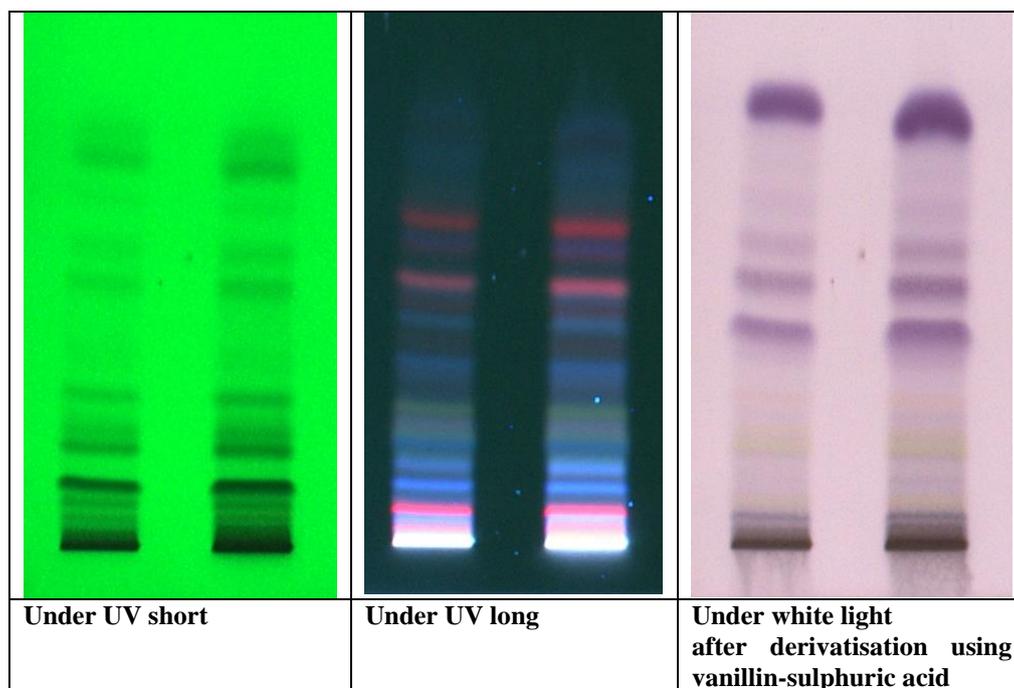


Figure 3: TLC photo documentation of defatted chloroform extract of MVM.

Solvent system–Toluene: Ethyl acetate: Formic acid (5:1:2 drops).

Table 3:  $R_f$  values and colour of major bands of defatted chloroform extract of MVM.

Under UV 254 nm		Under 366 nm		After derivatisation under White light	
$R_f$ Values	Colour	$R_f$ Values	Colour	$R_f$ Values	Colour
0.03	Dark Green	0.03	Pink	0.07	Grey
0.08	Green	0.07	Pink	0.20	Yellow
0.10	Green	0.13	Blue	0.47	Dark Purple
0.14	Dark Green	0.16	Blue	0.57	Dark Purple
0.21	Green	0.29	Light Yellow	0.66	Purple
0.25	Green	0.39	Blue	0.94	Dark Purple
0.32	Green	0.48	Blue		
0.57	Green	0.56	Pink		
0.65	Green	0.64	Light Pink		
0.83	Dark Green	0.70	Pink		
0.90	Green	0.82	Blue		

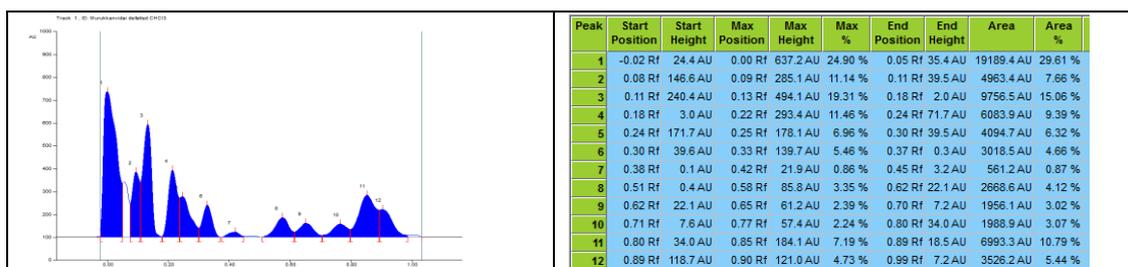


Figure 4 (1): HPTLC fingerprint profile of 5 µl defatted chloroform extract of MVM at 254nm.

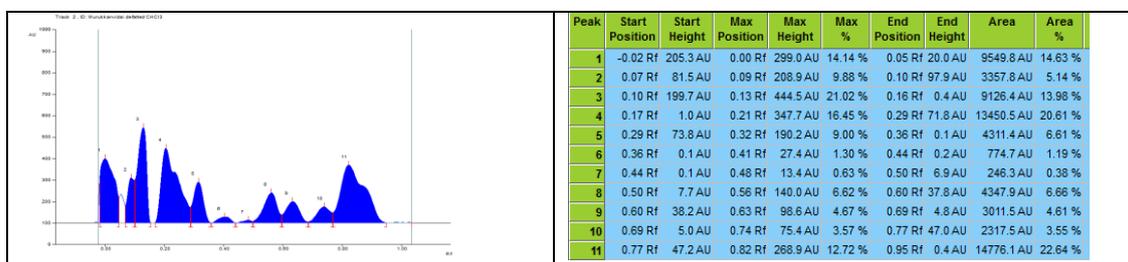


Figure 4 (2): HPTLC fingerprint profile of 10 µl defatted chloroform extract of MVM at 254nm.

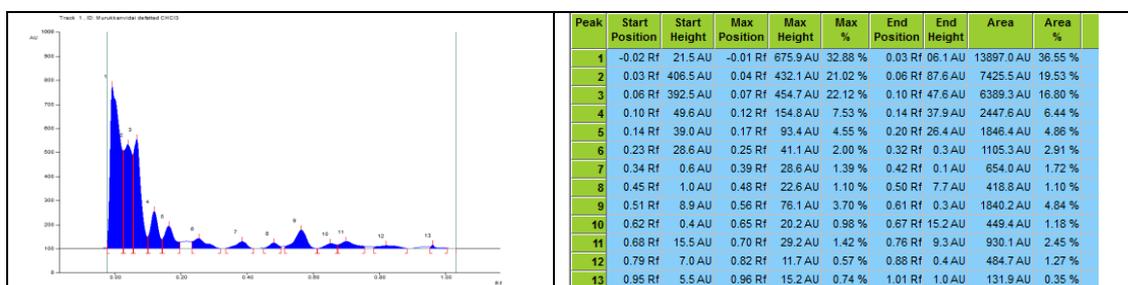


Figure 5 (1): HPTLC fingerprint profile of 5 µl defatted chloroform extract of MVM at 366 nm.

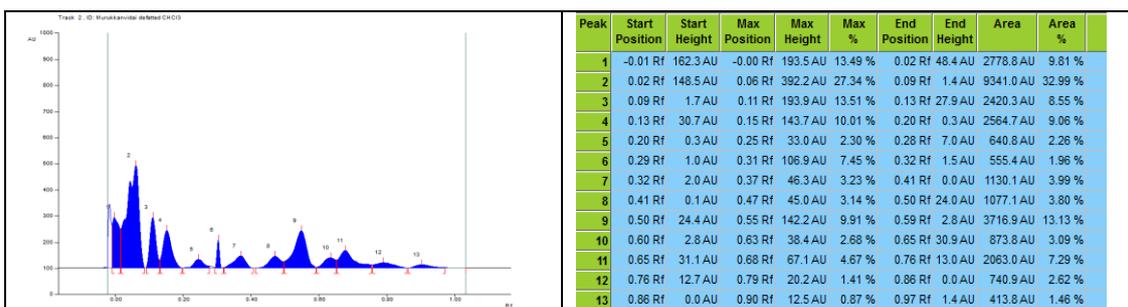


Figure 5(2): HPTLC fingerprint profile of 10 µl defatted chloroform extract of MVM at 366nm.

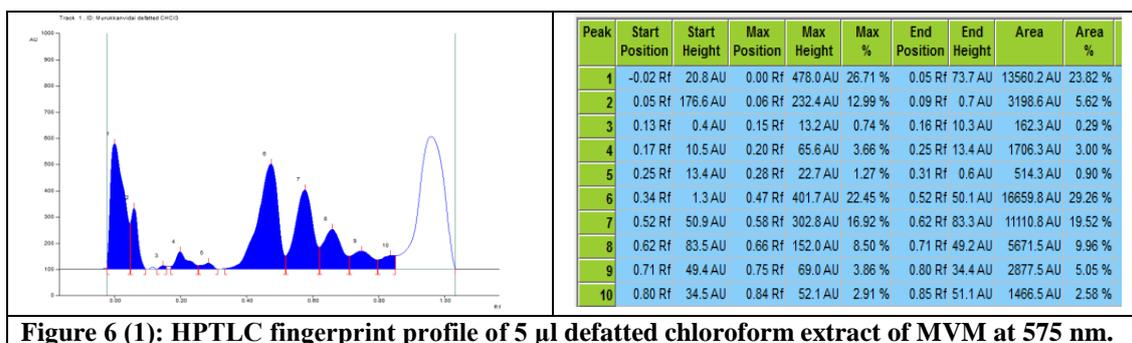


Figure 6 (1): HPTLC fingerprint profile of 5 µl defatted chloroform extract of MVM at 575 nm.

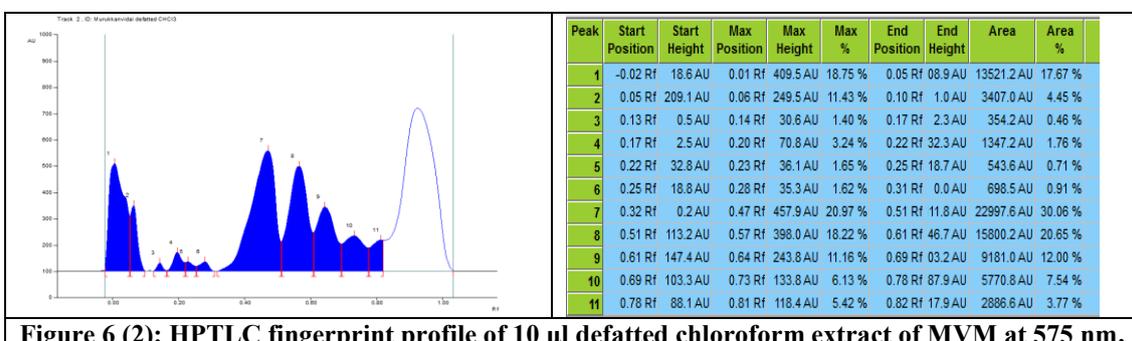


Figure 6 (2): HPTLC fingerprint profile of 10 µl defatted chloroform extract of MVM at 575 nm.

HPTLC fingerprinting is proved to be a reliable, accurate and precise method for herbal drug identification and authentication. Thus the developed chromatograms and  $R_f$  values are specific with selected solvent system under the same experimental conditions and serve the better tool for standardization of the test drug.

## CONCLUSION

It is concluded that the microscopic standards could be used to detect the ingredients in the compound formulations and to decide the authenticity of the formulation. Apart from this, the physicochemical characters and HPTLC fingerprinting profile are very important for monitoring the quality of Murukkan Vitai Mathirai. Hence the data obtained together may be used for quality evaluation and standardization of the compound formulation and thereby the study can certainly help to explore and propagate the strength of traditional Siddha medicine and related activities to the Global reach.

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