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Phytochemical and physicochemical evaluation of Siddha drug Musumuskkai Kudineer

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Abstract

Siddha medicine traditional system of healing that originated in south India, oldest system of medicine. The siddha system is based on a combination of ancient medicinal practices and spiritual disciplines as well as alchemy and mysticism. Plants are important for human health for prevention of several human diseases. The Musumuskkai Kudineer is a herbal formulation used in the management of Pitha Paandu (Iron Deficiency anaemia) in Yugimuni Vaithiya Kaaviyam. **Aim:** The aim of the present of study is to investigate the Phytochemical, Physicochemical and HPTLC fingerprint profile of Musumuskkai Kudineer. **Material and Methods:**The raw drug was purchased from reputed herbal drug shop, Thackkalay, Kanyakumari district and it is purified then made into decoction as per the siddha literature. **Results:** The Phytochemical evaluation showed the presence of Terpenoids, phenols, quinones, Glycosides, alkaloids. The Physico chemical analysis showed the Loss on drying 7.76% total ash 10.25% Acid insoluble ash 1.65%, Water soluble ash 3.19%, Sulphate ash 16.07%, Alcohol soluble extractives 11.42%, Water soluble extractives 17.24%. The HPTLC studies of the alcohol extracts of the plant materials were carried out and the chromatograms and fingerprinting profiles at 254nm, 366nm and 575nm after derivatisation were documented. This information will be used for laying down the pharmacopoeial standards of Musumuskkai Kudineer.

Keywords: Musumuskkai Kudineer, Pitha Paandu, Phytochemical, Physicochemical, HPTLC

1. Introduction

Siddha system is unique among the Indian system of medicine. It is believed to have been developed by the Siddhar's the ancient supernatural spiritual saints of India. In Siddha system of medicine, the drug sources are obtained from plant, mineral, metal and animals [1]. Medicinal plants are richest bio sources of drugs of traditional system of medicine, modern medicine, nutraceuticals, food supplement, folk medicine, pharmaceuticals intermediates and chemical entities for synthetic

drugs [2]. Iron deficiency is a form of micronutrient malnutrition [MNM]. It has high prevalence in developing regions of the world children and pregnant women been mostly affected. Iron deficiency leads to anaemia which manifests as a condition in which the number of red blood cells or their oxygen carrying capacity is not sufficient to meet the physiologic needs it develops when the rate of red cell production fails to keep pace with destruction or loss of cells [3]. As per the world health organization (WHO) approximately 1.62 billion suffer from iron deficiency anaemia worldwide which constitute 47% in preschool children, 25% in school children, 30% in non-pregnant women and 24% in people older than 60 years of age with 12% in adult men which is the least [4]. Iron deficiency anaemia is compared with Pitha Paandu noi. Because its symptoms told in modern aspect may correlated with symptoms referred in "yugivaithiya chinthaamani" [5]. The symptoms are yellowish discoloration of body, pallor of tongue, hand, foot, eye vision diminished, thirst, dyspnoea, giddiness [6]. Standardisation is the process of implementing and developing technical standards. Standardization of herbal formulation is essential in order to assess the quality of drug [7]. The present is to investigate the Phytochemical, Physicochemical and HPTLC analysis of the trial drug Musumuskkai Kudineer.

2. Materials and Methods

2.1.Collection of raw drugs:

The required drugs were purchased from from the herbal drug shop, Thackkalay, Kanyakumari district, Tamilnadu, India

2.2. Authentication:

The drugs will be identified and authenticated by Gunapadam experts at Government Siddha Medical College, Palayamkottai – 627002

2.3. Purification and preparation of the drug:

The adulterant dust and other materials are removed. The drug is purified, dried and grinded into kudineerchooranam .10 gm of kudineer chooranam is added with 400 ml of water and boiled until it reduced into ¼ of its of its quantity and make kashayam to dispense.

Table :1 Ingredient of Musumuskkai Kudineer [8]

S. No	Tamil name	Scientific name	Part used
1.	Musumuskkai	*Mukia maderaspatana* Linn	Whole plant
2.	Kizhanelli	*Phyllanthus amarus* Linn	Whole plant

2.4. Standardisation parameters:

The various standardization parameters used in this studies are preliminary Phytochemical, Physicochemical and HPTLC analysis.

2.4.1. Preliminary phytochemical analysis:

1.Test for Saponins:

To a few mg of extract distilled water is added and shaken well. The formation of foam indicates the presence of saponin.

2.Test for Tannin:

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue colour shows presence of tannin.

3.Test for Terpenoids:

To a few mg of extract in chloroform, add conc. H_2SO_4. Presence of dark brown precipitate indicates the presence of terpenoids.

4.Test for Phenol:

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue or green colour shows presence of phenol.

5.Test for Steroids (Lieberman Burchard Test):

To a few mg of the extract 2 ml of chloroform is added in a dry test tube. Few drops of acetic acid

are added, heated and few drops of acetic anhydride and 2 drops of concentrated sulphuric acid are added. The green colour indicates the presence of steroid.

6.Test for Quinones:

To a few mg of extract, add few drops of concentrated sulphuric acid. Appearance of red colour shows the presence of quinone.

7. Test for Glycosides:

Substance is treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of glycoside.

8. Test for Carbohydrates:

To the sample solution, added few drops of α-naphthol and 2-3 ml conc. H2SO4. The appearance of reddish violet or purple ring at the junction of two liquids indicates the presence of Carbohydrates.

9. Test for Alkaloids (Dragendorff's Test):

Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorff's reagent were added. The presence of orange red precipitates indicates the presence of alkaloids.

10.Test for Flavonoids:

To the substance in alcohol add 10% NaOH or ammonia. A dark yellow color indicates the presence of flavanoid

11.TestforProteins(Biuret test):

To the sample solution in a test tube, add sodium hydroxide solution and then add a few drops of very dilute (1 %) copper II sulphate solution and mix gently. Appearance of purple color indicates the presence of protein.

2.4.2. Physicochemical analysis:

The physicochemical analysis such as determination of loss on drying at 105^0c, total ash value, acid insoluble ash, water soluble ash, PH value, alcohol soluble extractives, water soluble extractives were carried out by standard methods.[9]

2.4.3. 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. In addition, it is a reliable method for the quantification at nanograms level of samples hence it is conveniently adopted for routine quality control analysis providing a chromatographic fingerprint to identify the purity of the phytocompounds in the raw materials.[10]

Procedure:

Developing solvent system

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the solvent system: Toluene: Ethyl acetate: (5:2)

Sample application

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm.

Densitometry

The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation

The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105^0 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. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

3. Results and Discussion

Preliminary phytochemical analysis:

Table: 2 Phyto - chemical analysis of Musumuskkai Kudineer

Phytochemicals	Results
Saponins	-
Tannins	-
Phenols	+
Terpenoids	+
Alkaloids	+
Flavanoids	-
Steroids	-
Glycosides	+
Carbohydrates	-
Quinones	+
Proteins	-

The qualitative analysis of the Musumuskkai Kudineer showed the presence of alkaloid, terpenoid, glycoside, phenol and quinones.

Alkaloid:

Alkaloid the most revered of all phytochemicals are said to be pharmacologically active and their action is felt in blood vessels. They inhibit cyclic adenosine monophosphate (CAMO) phosphodiesterase leading to accumulation of CAMP. This effect stimulates phosphorylation of proteins and synthesis of proteins which improve erythropoiesis [11].

Terpenoid:

Terpenoid have antioxidant power promote of tissue reduce the permeability of blood capillaries and increase their resistance to hemolysis [12].

Glycoside:

Glycoside may be responsible for the acclaimed anti anaemic potential of plants used in traditional medicine [13].

Phenols:

The antioxidant activity of phenol mainly attributed to their redox properties because of which they act as reducing agents, electron / hydrogen donator and singlet oxygen quenchers and have metal chelating potential [14].

Physicochemical analysis:

The physicochemical constants are important parameters for detecting adulteration or improper handling of drugs [15]. Various physicochemical parameters viz loss on drying, ash values and extractive values were determined. The results were summarized in Table 3.

Table :3 Physicochemical analysis of Musumuskkai Kudineer

Sl. No	Parameters	Result
1	LOD at 105^0C	7.76%
2	Total Ash	10.25%
3	Acid insoluble ash	1.65%
4	Water soluble ash	3.19%
5	Sulphated ash	16.07%
6	PH of water extract	5.98
7	Alcohol soluble extractives	11.42%
8	Water soluble extractives	17.24%

Loss on drying:

The moisture content of the drug is determined by loss on drying. These will also indicate stability and shelf life of the drug. Loss on drying of Musumuskkai Kudineer 7.76%

Total Ash value:

The amount of minerals and earthy material present in the drug is represented by total Ash value. The value of Musumuskkai Kudineer 10.25%

Acid insoluble Ash:

The amount of siliceous material in the drug is represented by acid insoluble Ash. The value Musumuskkai Kudineer 1.65%

Water soluble Ash:

Water soluble Ash represent easy facilitation of diffusion and osmosis mechanism [16]. Here the value of Musumuskkai Kudineer 3.19%

pH:

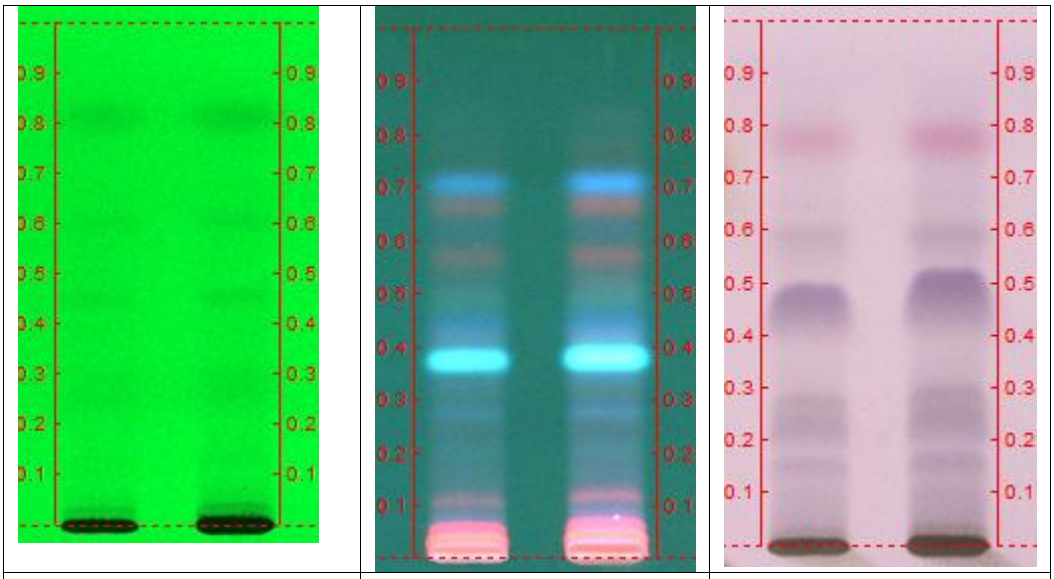
pH is a measure of hydrogen ion concentration whether it is acidic or alkaline nature. 7.0 is neutral, above 7.0 is alkaline and below 7.0 is acidic.

The pH of the drug Musumuskkai Kudineer is 5.98 which is acidic in nature. Acid drug is essential for its bioavailability and effectiveness. Acid drugs better absorbed in stomach [17].

Extractive value:

Alcohol soluble extractives and water-soluble extractives of the sample drug is 11.42% and 17.24% respectively.

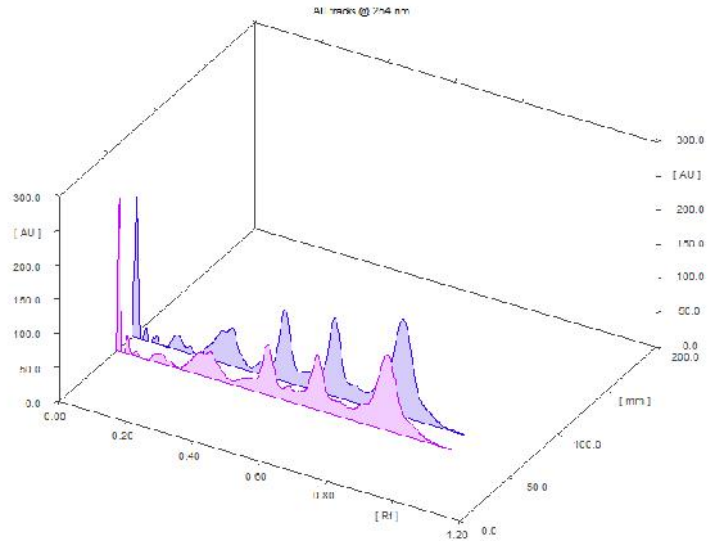
High performance thin layer chromatography:

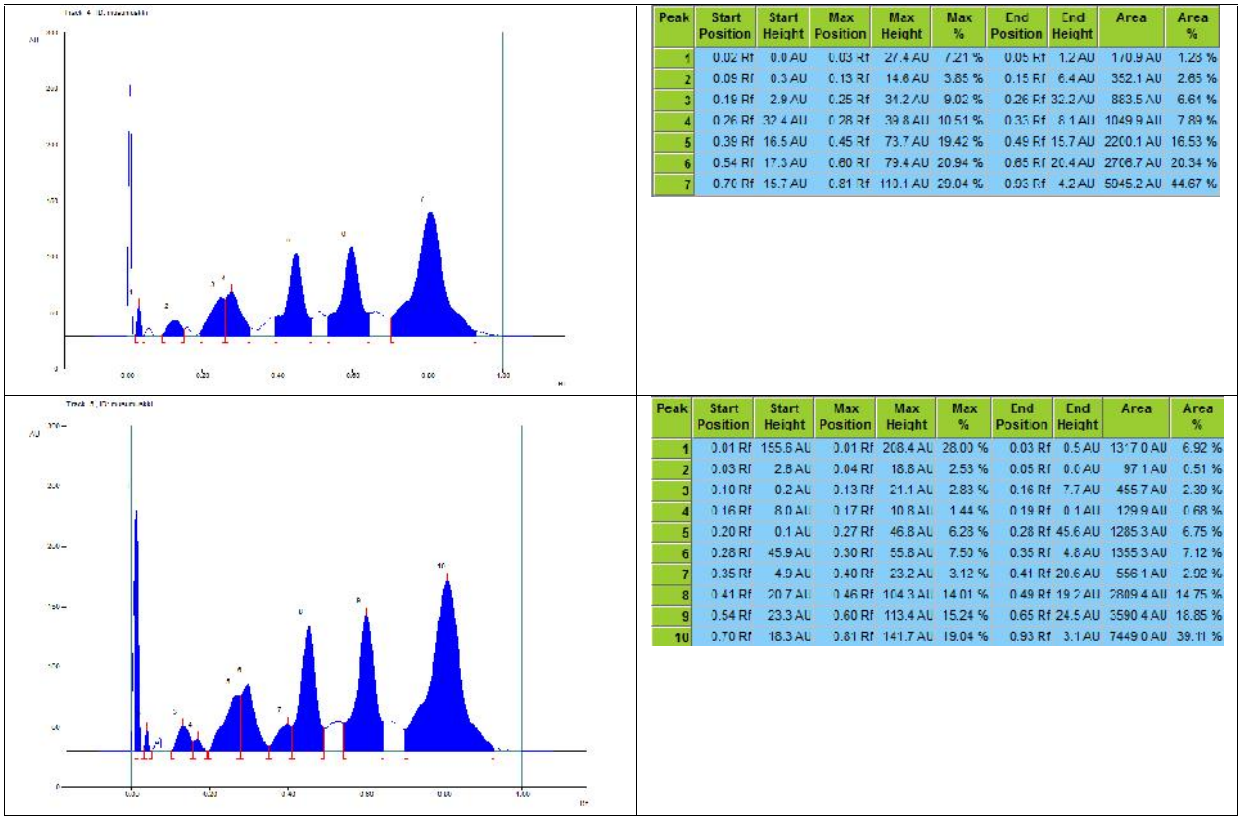


Under UV short	Under UV long	Under white light

Fig 1: HPTLC profile of alcohol extract of Musumuskkai Kudineer viewed in UV short; UV long; White light after derivatisation using vanillin-sulphuric acid; Solvent system –Toluene: Ethyl acetate – (5:2); volume applied; Track 1-5 µl: Track 2-10 µl

254nm:





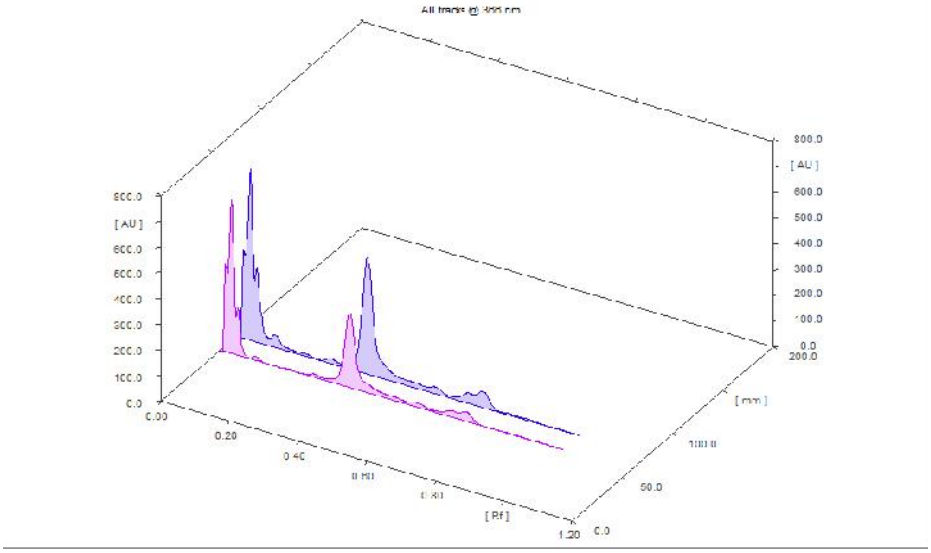
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.03 Rf	27.4 AU	7.21 %	0.05 Rf	1.2 AU	170.9 AU	1.23 %
2	0.09 Rf	0.3 AU	0.13 Rf	14.6 AU	3.85 %	0.15 Rf	6.4 AU	352.1 AU	2.65 %
3	0.19 Rf	2.9 AU	0.25 Rf	34.2 AU	9.02 %	0.26 Rf	32.2 AU	883.5 AU	6.64 %
4	0.26 Rf	32.4 AU	0.28 Rf	39.8 AU	10.51 %	0.33 Rf	8.1 AU	1049.9 AU	7.89 %
5	0.39 Rf	16.5 AU	0.45 Rf	73.7 AU	19.42 %	0.49 Rf	15.7 AU	2200.1 AU	16.53 %
6	0.54 Rf	17.3 AU	0.60 Rf	79.4 AU	20.94 %	0.65 Rf	20.4 AU	2706.7 AU	20.34 %
7	0.70 Rf	15.7 AU	0.81 Rf	110.1 AU	29.04 %	0.93 Rf	4.2 AU	5945.2 AU	44.67 %

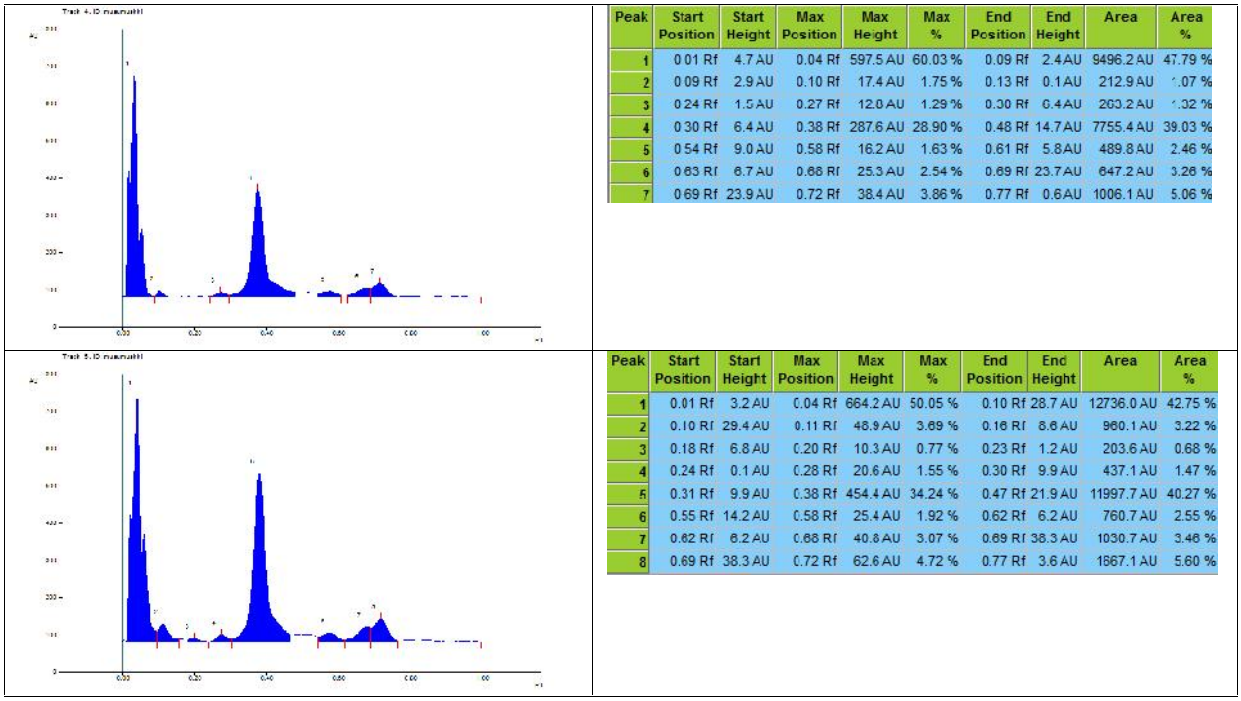
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	155.8 AU	0.01 Rf	208.4 AU	28.00 %	0.03 Rf	0.5 AU	1317.0 AU	6.92 %
2	0.03 Rf	2.8 AU	0.04 Rf	18.8 AU	2.53 %	0.05 Rf	0.0 AU	97.1 AU	0.51 %
3	0.10 Rf	0.2 AU	0.13 Rf	21.1 AU	2.83 %	0.16 Rf	7.7 AU	455.7 AU	2.39 %
4	0.16 Rf	8.0 AU	0.17 Rf	10.8 AU	1.44 %	0.19 Rf	0.1 AU	129.9 AU	0.68 %
5	0.20 Rf	0.1 AU	0.27 Rf	46.8 AU	6.28 %	0.28 Rf	45.6 AU	1285.3 AU	6.75 %
6	0.28 Rf	45.9 AU	0.30 Rf	55.8 AU	7.50 %	0.35 Rf	4.8 AU	1355.3 AU	7.12 %
7	0.35 Rf	4.9 AU	0.40 Rf	23.2 AU	3.12 %	0.41 Rf	20.6 AU	556.1 AU	2.92 %
8	0.41 Rf	20.7 AU	0.46 Rf	104.3 AU	14.01 %	0.49 Rf	19.2 AU	2809.4 AU	14.75 %
9	0.54 Rf	23.3 AU	0.60 Rf	113.4 AU	15.24 %	0.65 Rf	24.5 AU	3590.4 AU	18.85 %
10	0.70 Rf	18.3 AU	0.81 Rf	141.7 AU	19.04 %	0.93 Rf	3.1 AU	7449.0 AU	39.11 %

Fig 2: HPTLC fingerprint profile of 5µl and 10 µl of alcohol extract of Musumuskkai Kudineer of 254 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 254 nm, the sample reveals the presence of 10 prominent peaks corresponds to the presence of 10versatile phytocomponents present within it. Rf value of the peaks ranges from 0.01Rf – 0. 70Rf.Further the peak 9 and 10 occupies the major percentage of area of 18.85% and 39.11% which denotes the abundant existence of such compound.

366nm





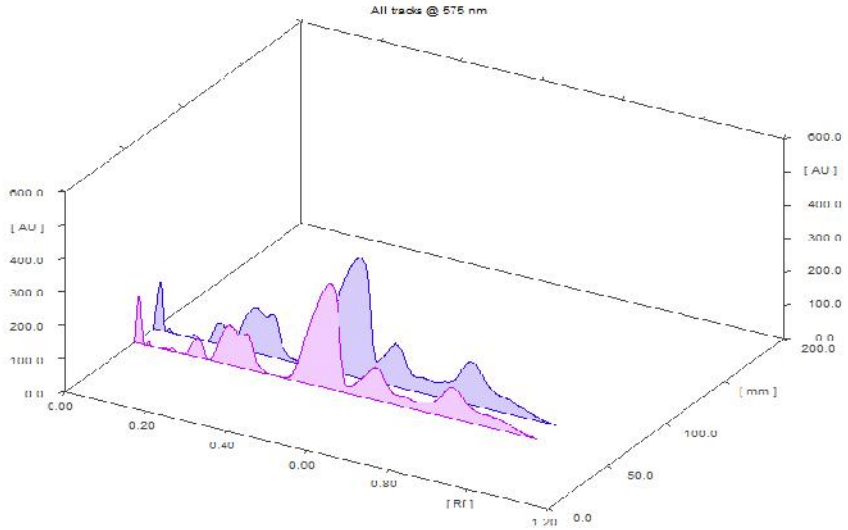
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.7 AU	0.04 Rf	597.5 AU	60.03 %	0.09 Rf	2.4 AU	9496.2 AU	47.79 %
2	0.09 Rf	2.9 AU	0.10 Rf	17.4 AU	1.75 %	0.13 Rf	0.1 AU	212.9 AU	1.07 %
3	0.24 Rf	1.5 AU	0.27 Rf	12.0 AU	1.29 %	0.30 Rf	0.4 AU	263.2 AU	1.32 %
4	0.30 Rf	6.4 AU	0.38 Rf	287.6 AU	28.90 %	0.48 Rf	14.7 AU	7755.4 AU	39.03 %
5	0.54 Rf	9.0 AU	0.58 Rf	16.2 AU	1.63 %	0.61 Rf	5.8 AU	489.8 AU	2.46 %
6	0.63 Rf	6.7 AU	0.66 Rf	25.3 AU	2.54 %	0.69 Rf	23.7 AU	647.2 AU	3.26 %
7	0.69 Rf	23.9 AU	0.72 Rf	38.4 AU	3.86 %	0.77 Rf	0.6 AU	1006.1 AU	5.06 %

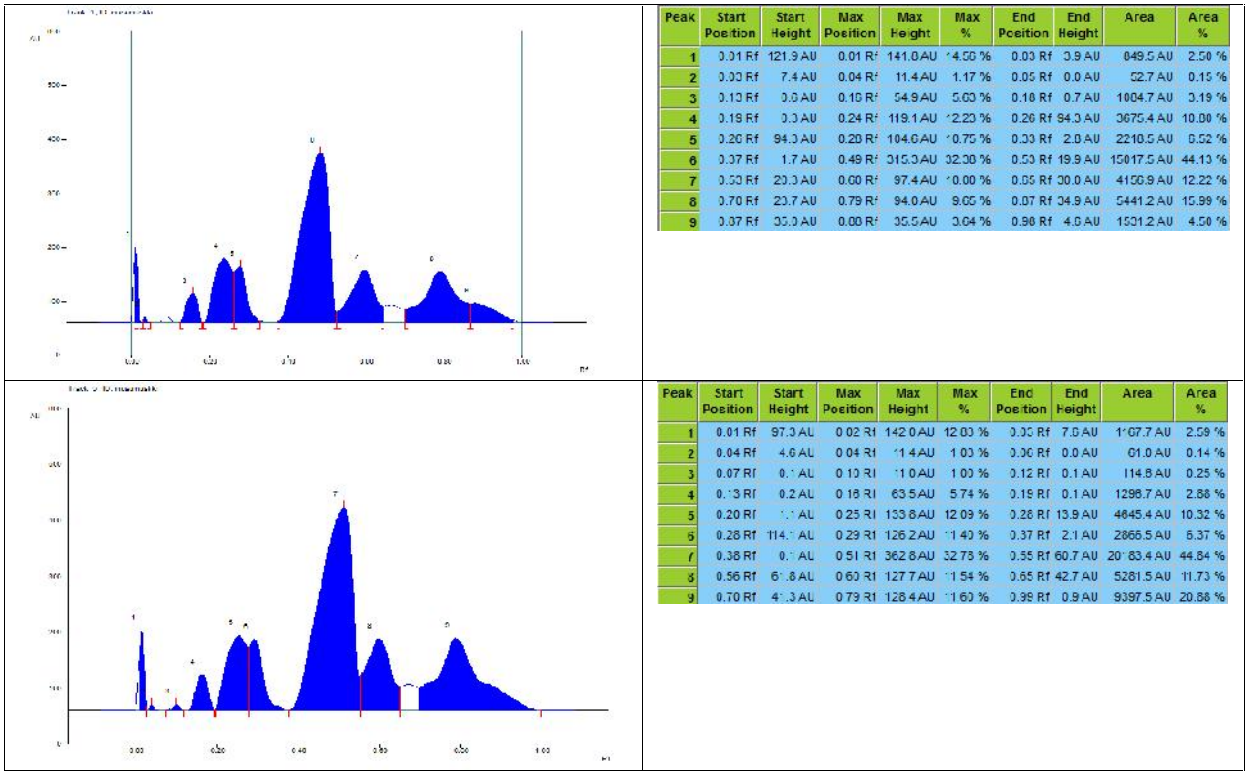
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	3.2 AU	0.04 Rf	664.2 AU	50.05 %	0.10 Rf	28.7 AU	12736.0 AU	42.75 %
2	0.10 Rf	29.4 AU	0.11 Rf	48.9 AU	3.69 %	0.16 Rf	6.6 AU	960.1 AU	3.22 %
3	0.18 Rf	6.8 AU	0.20 Rf	10.3 AU	0.77 %	0.23 Rf	1.2 AU	203.6 AU	0.68 %
4	0.24 Rf	0.1 AU	0.28 Rf	20.6 AU	1.55 %	0.30 Rf	9.9 AU	437.1 AU	1.47 %
5	0.31 Rf	9.9 AU	0.38 Rf	454.4 AU	34.24 %	0.47 Rf	21.9 AU	11997.7 AU	40.27 %
6	0.55 Rf	14.2 AU	0.58 Rf	25.4 AU	1.92 %	0.62 Rf	6.2 AU	760.7 AU	2.55 %
7	0.62 Rf	6.2 AU	0.66 Rf	40.8 AU	3.07 %	0.69 Rf	38.3 AU	1030.7 AU	3.46 %
8	0.69 Rf	38.3 AU	0.72 Rf	62.6 AU	4.72 %	0.77 Rf	3.6 AU	1667.1 AU	5.60 %

Fig 3: HPTLC fingerprint profile of 5 µl and 10 µl of alcohol extract of Musumuskkai Kudineer at 366 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 366 nm,the sample reveals the presence of 8 prominent peaks corresponds to presence of 8 versatile phytocomponents present within it.Rf value of the peak ranges from 0.01Rf – 0. 69Rf.Further the peak 1 and 5 occupies the major percentage of area of 42.75% and 40.27%, which denotes the abundant existence of such compound.

575nm





Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	121.9 AU	0.01 Rf	141.0 AU	14.60 %	0.03 Rf	3.9 AU	849.5 AU	2.59 %
2	0.03 Rf	7.4 AU	0.04 Rf	11.4 AU	1.17 %	0.05 Rf	0.0 AU	52.7 AU	0.15 %
3	0.10 Rf	0.0 AU	0.10 Rf	54.9 AU	5.63 %	0.16 Rf	0.7 AU	1004.7 AU	3.19 %
4	0.18 Rf	0.0 AU	0.24 Rf	119.1 AU	12.20 %	0.26 Rf	94.0 AU	3675.4 AU	10.06 %
5	0.26 Rf	94.0 AU	0.28 Rf	104.6 AU	10.75 %	0.30 Rf	2.0 AU	2210.5 AU	6.52 %
6	0.37 Rf	1.7 AU	0.49 Rf	315.0 AU	32.30 %	0.53 Rf	19.9 AU	15017.5 AU	44.13 %
7	0.53 Rf	20.3 AU	0.60 Rf	97.4 AU	10.00 %	0.65 Rf	30.0 AU	4150.9 AU	12.22 %
8	0.70 Rf	23.7 AU	0.79 Rf	94.0 AU	9.65 %	0.87 Rf	34.9 AU	5441.2 AU	15.99 %
9	0.87 Rf	35.0 AU	0.88 Rf	35.5 AU	3.64 %	0.96 Rf	4.0 AU	1531.2 AU	4.50 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	97.3 AU	0.02 Rf	142.0 AU	12.00 %	0.03 Rf	7.0 AU	1107.7 AU	2.59 %
2	0.04 Rf	4.6 AU	0.04 Rf	11.4 AU	1.00 %	0.06 Rf	0.0 AU	61.0 AU	0.14 %
3	0.07 Rf	0.1 AU	0.10 Rf	11.0 AU	1.00 %	0.12 Rf	0.1 AU	114.8 AU	0.25 %
4	0.13 Rf	0.2 AU	0.18 Rf	63.5 AU	5.74 %	0.19 Rf	0.1 AU	1298.7 AU	2.88 %
5	0.20 Rf	1.1 AU	0.25 Rf	133.8 AU	12.09 %	0.28 Rf	13.9 AU	4645.4 AU	10.32 %
6	0.28 Rf	114.1 AU	0.29 Rf	126.2 AU	11.40 %	0.37 Rf	2.1 AU	2865.5 AU	6.37 %
7	0.38 Rf	0.1 AU	0.51 Rf	362.8 AU	32.78 %	0.55 Rf	60.7 AU	20163.4 AU	44.84 %
8	0.56 Rf	61.8 AU	0.60 Rf	127.7 AU	11.54 %	0.65 Rf	42.7 AU	5281.5 AU	11.73 %
9	0.70 Rf	41.3 AU	0.79 Rf	128.4 AU	11.60 %	0.99 Rf	0.9 AU	9397.5 AU	20.88 %

Fig 3: HPTLC fingerprint profile of 5 µl and 10 µl of alcohol extract of Musumuskkai Kudineer at 575 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 575 nm,the sample reveals the presence of 9 prominent peaks corresponds to the presence of 9 versatile phytocomponents present within it.Rf value of the peak ranges from 0.01Rf – 0.70Rf. Further the peak 7 and 9 occupies the major percentage of area of 44.84% and 20.88%, which denotes the abundant existence of such compound

4. Conclusion

In the present investigations the phytochemical and physicochemical characteristics of Musumuskkai Kudineer were studied. Preliminary Phytochemical analysis of Musumuskkai Kudineer showed the presence of alkaloid, glycoside, phenol, terpenoid, and quinones.The HPTLC profiles can be used for the identification and evaluation of the quality of the herbal formulation. Standardization of drug is essential to exhibit conformation of its identify and determination of its purity, quality and quantity. The present study on phytochemical, physicochemical and HPTLC analysis of drug will give fingerprints to the clinical studies.

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