

Physicochemical Standardization of Siddha Herbal Drug Formulation - Koraikkizhangu Kudineer

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Abstract: *Siddha systems of medicines have been widely used for thousands of years in India. Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body. The medicine in this system is prepared from raw material of herbs, metals, minerals and animal products. The present study was attempt to evaluate the physicochemical parameters of Koraikkizhangu Kudineer which is a pure herbal Siddha preparation. This medicine is used in traditional Siddha system of medicine to treat 'Alathidu sannai' (Attention Deficit Hyperactivity Disorder). ADHD is one of the most common neurobehavioral disorder of children. But there is no standardization work reported on Koraikkizhangu Kudineer. Physicochemical parameters, Aflatoxin test were carried out. TLC and HPTLC analysis were also determined. There finding will be useful to world establishing quality control parameters for the standardization of Siddha medicine Koraikkizhangu Kudineer and can be used as reference standards for the preparation of a standardized pharmaceutical product and further quality control researches*

Keywords: Siddha herbal drug, Koraikkizhangu Kudineer and Standardization

1. Introduction

Siddha system is originated in southern part of India, which is one of the ancient traditional systems of medicine. Indian systems of medicines have been widely used for thousands of years in India.^[1] Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body.^[2]

As per the estimates of World Health Organization (WHO), more than 80% of global population uses plants or their products as the primary source of medicinal agents.^[3] Most of the medicines are mixture of compounds and because of its synergistic action; toxicity is being diminished, thereby increasing bioavailability through the cells of the body.^[4]

The medicine in this system is prepared from raw material of herbs, metals, minerals and animal products. 'Koraikkizhangu Kudineer' is one of the Siddha drug chosen from the text Pillai Pini Maruthuvam. It is indicated for 'Alathidu sannai' (Attention Deficit Hyperactivity Disorder). ADHD is one of the most common neurobehavioral disorder of childhood, one of the most prevalent chronic health conditions affecting school aged children, and most extensively studied mental disorder of childhood.^[5]

The Siddha system of medicine is one of the options for ADHD treatment. In our Siddha paediatric text the symptoms of ADHD is related to Sanni noi especially in

Alathidu Sanni, which is due to raised or altered function of Vatham, Pitham and Kabam. In Siddha texts therapeutic management of Alathidu Sanni recommended with Koraikkizhangu Kudineer as internal medicine to regulate the affected mukutram and neurobehavioral changes of children to regulate the brain function.

The use of scientific tools is essential to validate the traditional claim. Though Siddha drugs are considered to be safe and effective, it is the utmost duty of the physicians to standardizing the Siddha prepared medicine before trying out in human being.

Aim and Objective

The aim of this study is to do physicochemical analysis, TLC and HPTLC analysis and Aflatoxin detection for the drug 'Koraikkizhangu Kudineer'.

2. Materials and Methods

Collection of raw drugs

The required drugs were purchased from Ramaswamy Chettiyar country drug shop, Kandha swamy Kovil Street, Paris, Chennai.

Authentication

Raw drugs were authenticated by the Medicinal Botanist in National Institute of Siddha, Chennai. The trial drug was prepared in Gunapadam lab, National Institute of Siddha, Chennai-47.

Table 1: Ingredients of Koraikkizhangu Kudineer ^[6]

Drug Name	Botanical Name	English Name	Family	Quantity
Koraikkizhangu	<i>Cyperus rotundus</i>	Nut grass	Cyperaceae	5grams
Seenthil Kodi	<i>Tinospora cordifolia</i>	Heart leaved moonseed	Menispermaceae	5grams
Vasambu	<i>Acorus calamus</i>	Sweet - flag	Acoraceae	5grams
Nannari Ver	<i>Hemidesmus indicus</i>	Indian sarasaparilla	Asclepiadaceae	5grams
Seeragam	<i>Cuminum cyminum</i>	Cumin seeds	Apiaceae	5grams
Peippudal	<i>Trichosanthes cucumerina</i>	The Serpent Cocumber	Cucurbitaceae	5grams
Adathodai Eerku	<i>Justicia adhatoda</i>	Malabar - nut	Acanthaceae	5grams

Purification of the drugs:^[7,8]

- 1) Koraikkizhangu (*Cyperus rotundus*): dry under the moon light
- 2) Seenthilkodi (*Tinospora cordifolia*): The outer epidermal layer is to be peeled off
- 3) Vasambu (*Acorus calamus*): put into the flame directly until it burns completely.
- 4) Nannaariver (*Hemidesmus indicus*): It's should be washed in water.
- 5) Seeragam (*Cuminum cyminum*): It's to be dried in sunlight for 6 hours and then dried.
- 6) Peippudal (*Trichosanthes cucumerina*): It's should be dried in sunlight
- 7) Adatodaieerku (*Justicia adhatoda*): wipe with cotton cloth and dry it.

Preparation of Koraikkizhangu Kudineer:

Ingredients mentioned above are made as a coarse powder and then soaked it in a vessel containing of water 1.3 liter and heat till it comes to 1/8 th of its volume and then filtered the decoction.

Standardization Parameters:

The various standardization parameters Physicochemical analysis, TLC and HPTLC analysis and Heavy metal analysis were studied.

I. Physico-chemical evaluation:^[9 to 11]

The physicochemical analysis of the test drug Koraikkizhangu kudineer was carried out as per WHO guidelines (Anonymous 1998). The test procedures were done at Regional Research Institute of Unani Medicine in Chennai-13.

Percentage Loss on Drying:

Test drug was accurately weighed in evaporating dish .The sample was dried at 105°C for 5 hours and then weighed.

$\text{Percentage loss in drying} = \frac{\text{Loss of weight of sample}}{\text{Wt of the sample}} \times 100$

Determination of Total Ash:

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 °C 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.

$\text{Total Ash} = \frac{\text{Weight of Ash}}{\text{Wt of the Crude drug taken}} \times 100$

Determination of Acid Insoluble Ash:

The ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter was collected in crucible and was washed with hot water and

ignited to constant weight. Percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

$\text{Acid insoluble Ash} = \frac{\text{Weight of Ash}}{\text{Wt of the Crude drug taken}} \times 100$

Determination of Alcohol Soluble Extractive:

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

$\text{Alcohol sol extract} = \frac{\text{Weight of Extract}}{\text{Wt of the Sample taken}} \times 100$

Determination of pH:

Test sample was dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 minutes and the subjected to pH evaluation.

II. TLC and HPTLC Analysis:**TLC Analysis**

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 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 Shortwave UV light 254nm and light long-wave UV light 365 nm.^[12]

HPTLC Analysis

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 singlestep 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.^[13]

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 R_f values were tabulated.

III. Aflatoxin detection:

Standard:

Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

Solvent:

Chloroform: Acetone: Water (14: 2: 0.02)

Test sample:

Koraikkizhangu Kudineer 15µl.

Procedure:

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly the test sample was placed and allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of Chloroform: Acetone: Water (14: 2: 0.02) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

3. Results and Discussion

1) Physico chemical analysis of Koraikkizhangu Kudineer:

Koraikkizhangu Kudineer was analyzed to obtain parameters, such as, Loss on drying (105°C), Total ash, Acid Insoluble ash, Alcohol soluble extractives and pH (5%).

Chloroform extract at 254 nm:

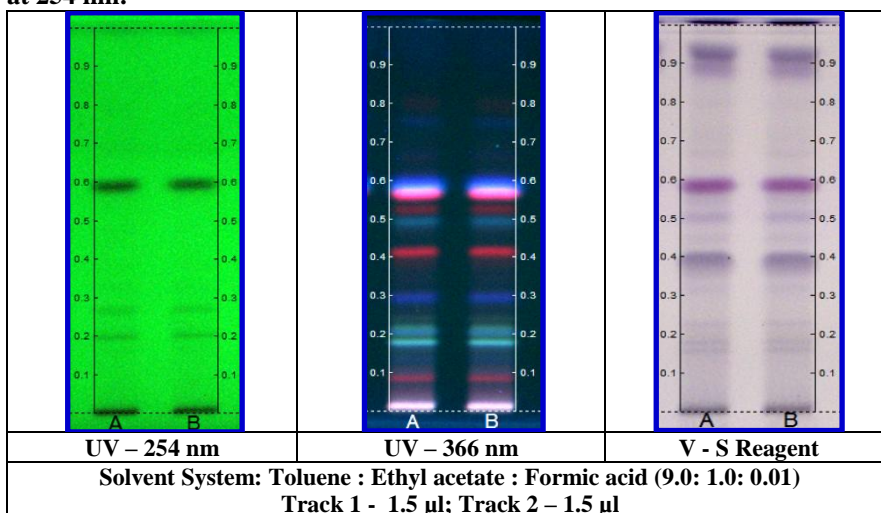


Figure 1: Chloroform extract

Table 2: Physicochemical parameters

S. No	Parameters	Results
1.	Loss on drying (105°C)	2.53%
2.	Total ash	9.89 %
3.	Acid Insoluble ash	8.43%
Extractive values		
4.	Alcohol soluble extractives	74.27%
5.	pH (5%)	6.48

Koraikkizhangu Kudineer was analyzed to obtain parameters, such as Loss on drying (105°C) – 2.53%, Total ash – 9.89%, Acid Insoluble ash 8.43%, Alcohol soluble extractives – 74.27% and pH (5%) – 6.48 was present in trial medicine. In Koraikkizhangu kudineer, the loss on drying at 105°C was found to be 2.53%, it falls in between the limit range (1-20%). So the determination of moisture content shows the good stability of the drug Koraikkizhangu kudineer. The ash values represent the purity of the drugs. The total ash includes both "physiological ash", which is derived from the organic matter, and "non-physiological"

Ash, which is the residue of the extraneous matters like sand/soil, inorganic materials. The non-physiological ash is represented by acid insoluble ash. The total ash in Koraikkizhangu kudineer found to be 9.89%, and the acid insoluble ash to be 8.43%. The both ash value were under limits. The minimal level of acid insoluble ash shows the less inorganic residue and purity of the drug Koraikkizhangu kudineer. The extractive values helps to indicate the nature of chemical constituents present in the drug. The Alcohol soluble extractives of Koraikkizhangu Kudineer is 74.27%, it shows the possibility of alcohol soluble substance such as tannins, resins and alkaloids to be present in the drug.

2. TLC and HPTLC Analysis of Koraikkizhangu Kudineer:

TLC plate was developed using Toluene: Ethyl acetate: Formic acid (9.0: 1.0: 0.01) as mobile phase. After development allow the plate to dry in air, record the finger print and densitometric chromatogram of the two batch samples of the single compound scanned at 254 and 366 nm.

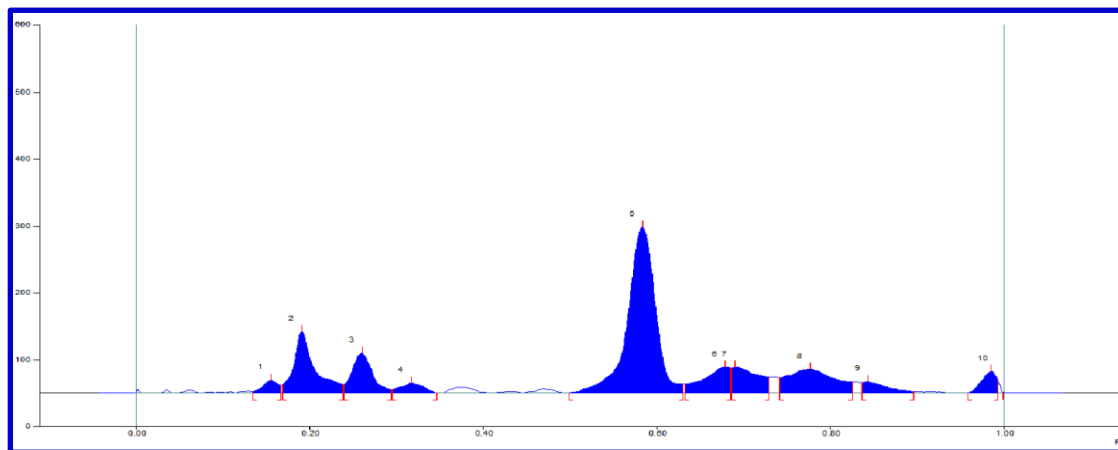


Figure 2: HPTLC finger print of Chloroform extract at 254 nm (Absorbance mode)

Table 3: R_f values of Chloroform extract at 254 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.13 Rf	2.4 AU	0.16 Rf	18.8 AU	3.15 %	0.17 Rf	12.0 AU	288.1 AU	1.95 %
2	0.17 Rf	12.8 AU	0.19 Rf	92.0 AU	15.45 %	0.24 Rf	13.1 AU	1858.1 AU	12.60 %
3	0.24 Rf	13.1 AU	0.26 Rf	59.5 AU	9.98 %	0.29 Rf	5.3 AU	1162.7 AU	7.88 %
4	0.30 Rf	5.4 AU	0.32 Rf	15.2 AU	2.55 %	0.35 Rf	0.0 AU	334.9 AU	2.27 %
5	0.50 Rf	0.2 AU	0.58 Rf	248.0 AU	41.64 %	0.63 Rf	13.3 AU	6592.2 AU	44.69 %
6	0.63 Rf	13.4 AU	0.68 Rf	39.0 AU	6.54 %	0.69 Rf	37.0 AU	1001.0 AU	6.79 %
7	0.69 Rf	37.3 AU	0.69 Rf	38.7 AU	6.50 %	0.73 Rf	24.4 AU	1006.6 AU	6.82 %
8	0.74 Rf	23.3 AU	0.78 Rf	35.6 AU	5.98 %	0.83 Rf	16.8 AU	1634.0 AU	11.08 %
9	0.84 Rf	16.2 AU	0.84 Rf	16.9 AU	2.84 %	0.90 Rf	1.9 AU	415.4 AU	2.82 %
10	0.96 Rf	0.0 AU	0.99 Rf	31.9 AU	5.35 %	0.99 Rf	19.8 AU	457.3 AU	3.10 %

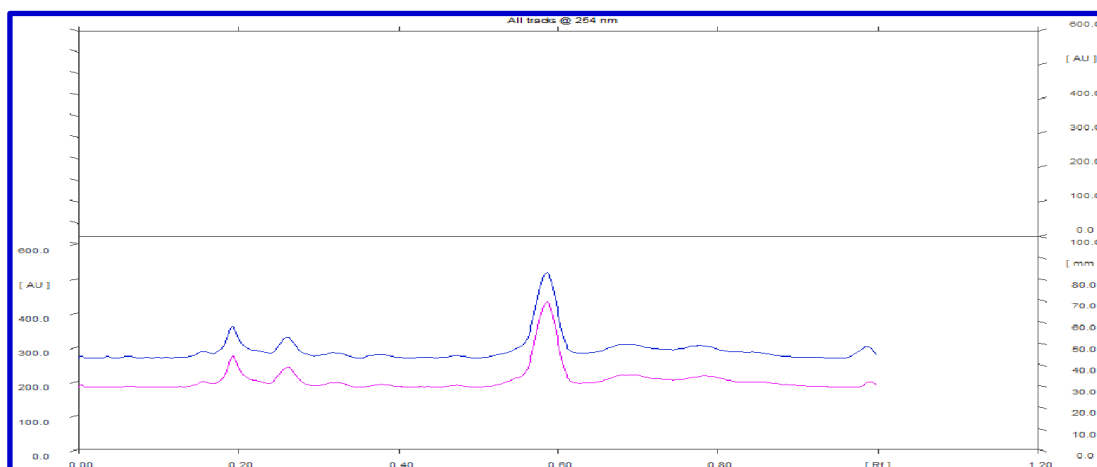
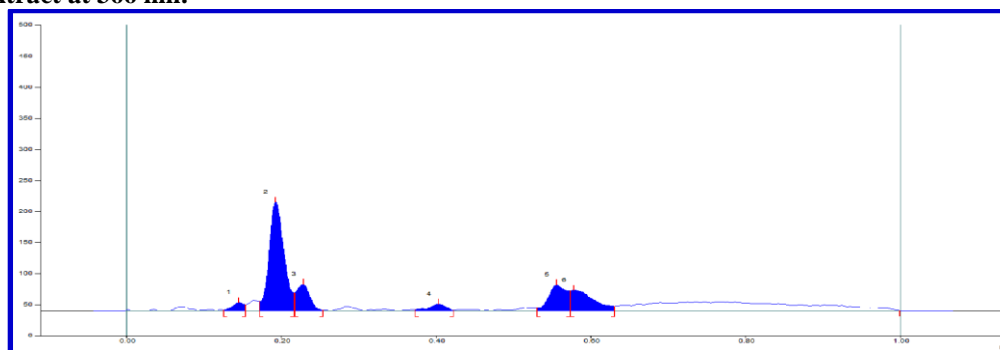


Figure 3: Densitometric chromatogram of Chloroform extract at 254 nm (Absorbance mode)

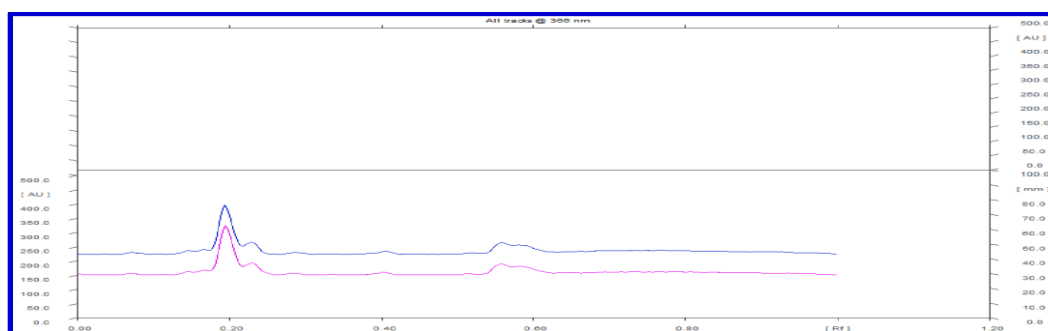
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HPTLC finger printing analysis of Chloroform extract at 254 nm (Absorbance mode), the sample reveals the presence of 10 prominent peaks corresponds to presence of 10

versatile phytochemicals present within it. R_f value of the peaks ranges from 0.13 to 0.96. Further the peak 2 and 5 occupies the major percentage of area of 12.60% and 44.69 % which denotes the abundant existence of such compound.

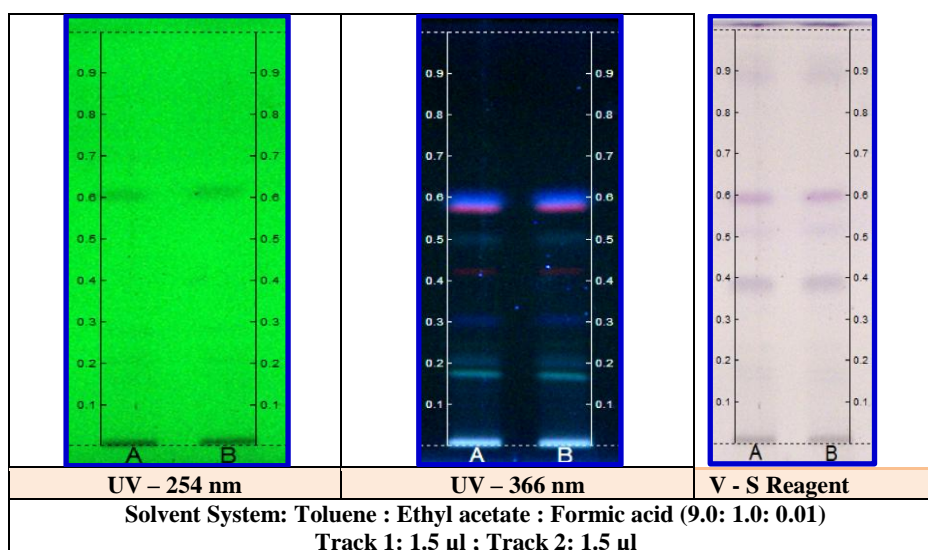
Chloroform extract at 366 nm:**Figure 4:** HPTLC finger print of Chloroform extract at 366 nm (Absorbance mode)**Table 4:** R_f values of Chloroform extract at 366 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.13 Rf	1.1 AU	0.15 Rf	13.2 AU	4.19 %	0.15 Rf	10.1 AU	167.5 AU	3.10 %
2	0.17 Rf	14.8 AU	0.19 Rf	174.5 AU	55.29 %	0.22 Rf	28.6 AU	2784.0 AU	51.55 %
3	0.22 Rf	28.8 AU	0.23 Rf	42.4 AU	13.44 %	0.25 Rf	1.1 AU	617.3 AU	11.43 %
4	0.37 Rf	2.1 AU	0.40 Rf	10.7 AU	3.38 %	0.42 Rf	0.5 AU	196.2 AU	3.63 %
5	0.53 Rf	4.0 AU	0.56 Rf	41.5 AU	13.15 %	0.57 Rf	31.8 AU	804.9 AU	14.90 %
6	0.57 Rf	32.0 AU	0.58 Rf	33.3 AU	10.55 %	0.63 Rf	7.6 AU	830.9 AU	15.39 %

**Figure 5:** Densitometric chromatogram of Chloroform extract at 366 nm (Absorbance mode)**Report**

HPTLC finger printing analysis of Chloroform extract at 366 nm (Absorbance mode), the sample reveals the presence of 6 prominent peaks corresponds to presence of 6 versatile phytocomponents present with in it. R_f value of the peaks

ranges from 0.13 to 0.57. Further the peak 2 and 6 occupies the major percentage of area of 51.55% and 15.39% which denotes the abundant existence of such compound.

Alcohol extract at 254 nm:**Figure 6:** Alcohol extract

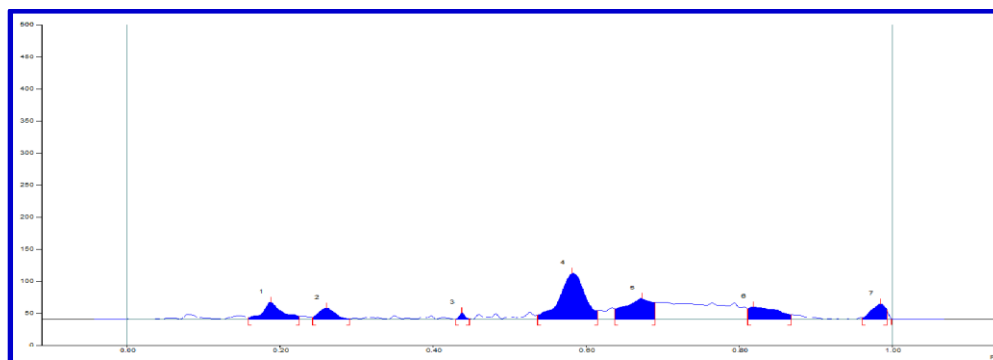


Figure 7: HPTLC finger print of alcohol extract at 254 nm (Absorbance mode)

Table 5: R_f values of alcohol extract at 254 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.16 R_f	3.3 AU	0.19 R_f	26.9 AU	13.15 %	0.23 R_f	5.4 AU	580.8 AU	11.73 %
2	0.24 R_f	4.0 AU	0.26 R_f	18.0 AU	8.81 %	0.29 R_f	0.9 AU	325.8 AU	6.58 %
3	0.43 R_f	0.1 AU	0.44 R_f	10.7 AU	5.22 %	0.45 R_f	1.0 AU	62.4 AU	1.26 %
4	0.54 R_f	7.4 AU	0.58 R_f	72.2 AU	35.23 %	0.62 R_f	14.0 AU	1982.2 AU	40.02 %
5	0.64 R_f	17.3 AU	0.67 R_f	32.9 AU	16.08 %	0.69 R_f	26.3 AU	988.9 AU	19.96 %
6	0.81 R_f	17.4 AU	0.82 R_f	19.9 AU	9.73 %	0.87 R_f	7.9 AU	643.1 AU	12.98 %
7	0.96 R_f	3.2 AU	0.99 R_f	24.1 AU	11.77 %	0.99 R_f	14.8 AU	370.2 AU	7.47 %

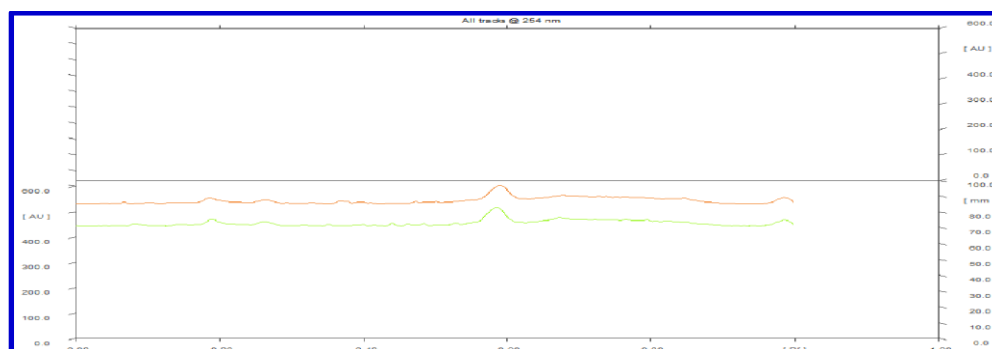


Figure 8: Densitometric chromatogram of alcohol extract at 254 nm (Absorbance mode)

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HPTLC finger printing analysis of Alcohol extract at 254 nm (Absorbance mode), the sample reveals the presence of 7 prominent peaks corresponds to presence of 7 versatile phytocomponents present with in it. R_f value of the peaks

ranges from 0.16 to 0.96. Further the peak 4 and 6 occupies the major percentage of area of 40.02% and 12.98% which denotes the abundant existence of such compound.

Alcohol extract at 366 nm

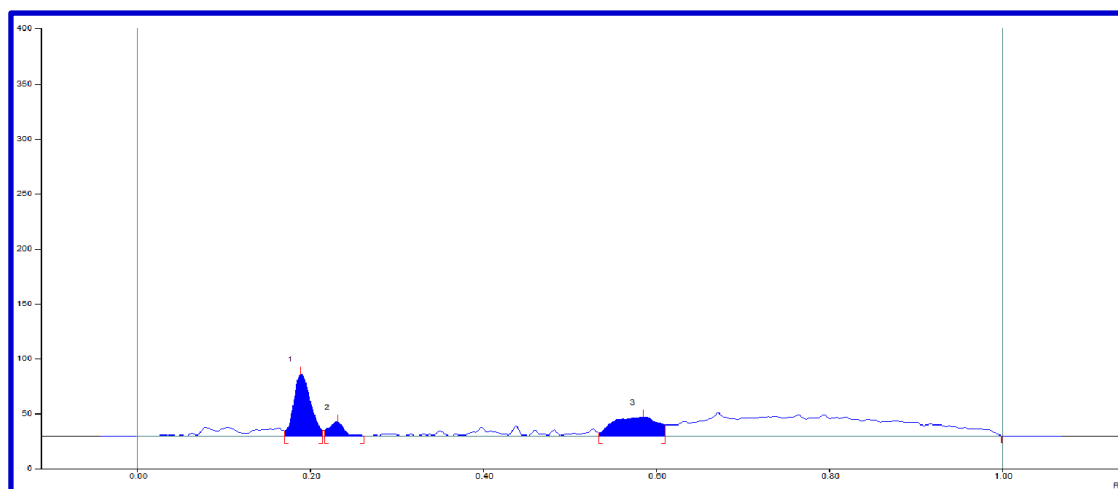
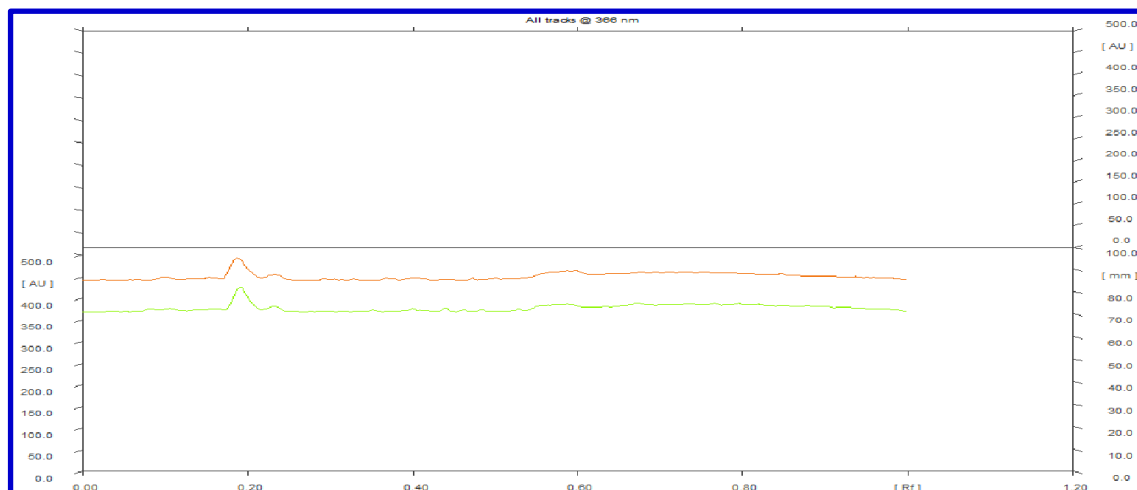


Figure 9: HPTLC finger print of alcohol extract at 366 nm (Absorbance mode)

Table 6: R_f values of alcohol extract at 366 nm (Absorbance mode)

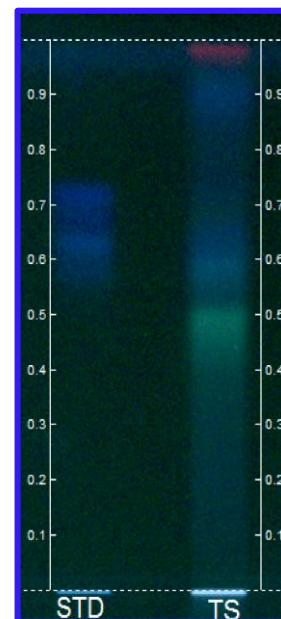
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.17 Rf	4.5 AU	0.19 Rf	56.4 AU	65.26 %	0.22 Rf	5.1 AU	902.5 AU	49.67 %
2	0.22 Rf	5.3 AU	0.23 Rf	12.7 AU	14.66 %	0.26 Rf	0.0 AU	181.2 AU	9.97 %
3	0.53 Rf	2.4 AU	0.59 Rf	17.4 AU	20.08 %	0.61 Rf	9.9 AU	733.3 AU	40.36 %

**Figure 10:** Densitometric chromatogram of alcohol extract at 366 nm (Absorbance mode)**Report**

HPTLC finger printing analysis of Alcohol extract at 366 nm (Absorbance mode), the sample reveals the presence of 3 prominent peaks corresponds to presence of 3 versatile phytochemicals present with in it. R_f value of the peaks ranges from 0.17 to 0.53. Further the peak 1 occupies the major percentage of area of 49.67% which denotes the abundant existence of such compound.

Aflatoxin Detection of Koraikkizhangu Kudineer:

TLC plate was developed using Chloroform: Acetone: Water (14: 2: 0.2) as mobile phase. After development allow the plate to dry in air, record the finger print and densitometric chromatogram of the test sample and standard sample scanned at 366 nm.



Solvent: Chloroform: Acetone: Water (14:2:0.2)

Track 1: Std (10 μ l); **Track 2:** Test sample (Koraikkizhangu Kudineer) (15 μ l)

Figure 11: Aflatoxin Detection

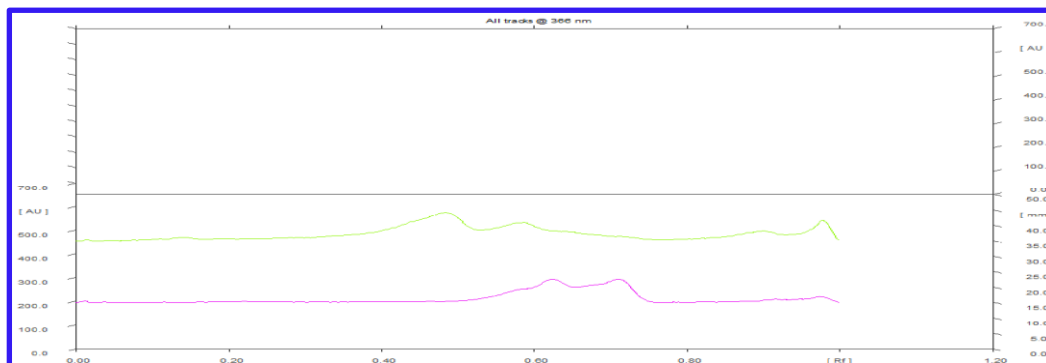


Figure 12: Densitometric chromatogram of Test sample (Koraikkizhangu Kudineer) and Standard (Aflatoxins) at 366 nm

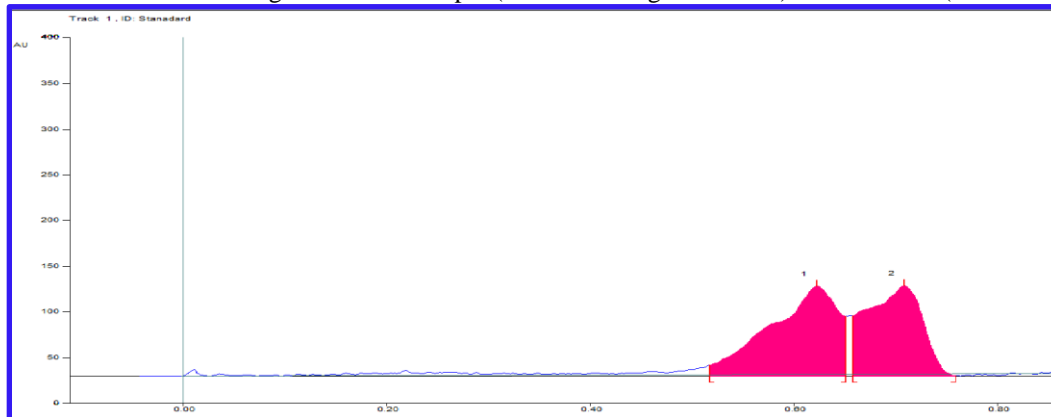


Figure 13: HPTLC finger print of Standard (Aflatoxins) at 366 nm

Table 7: R_f values of Standard (Aflatoxins) at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.36 Rf	25.8 AU	0.48 Rf	120.0 AU	36.83 %	0.53 Rf	44.8 AU	8512.1 AU	49.92 %
2	0.54 Rf	48.5 AU	0.58 Rf	78.0 AU	23.94 %	0.65 Rf	36.9 AU	4471.5 AU	26.22 %

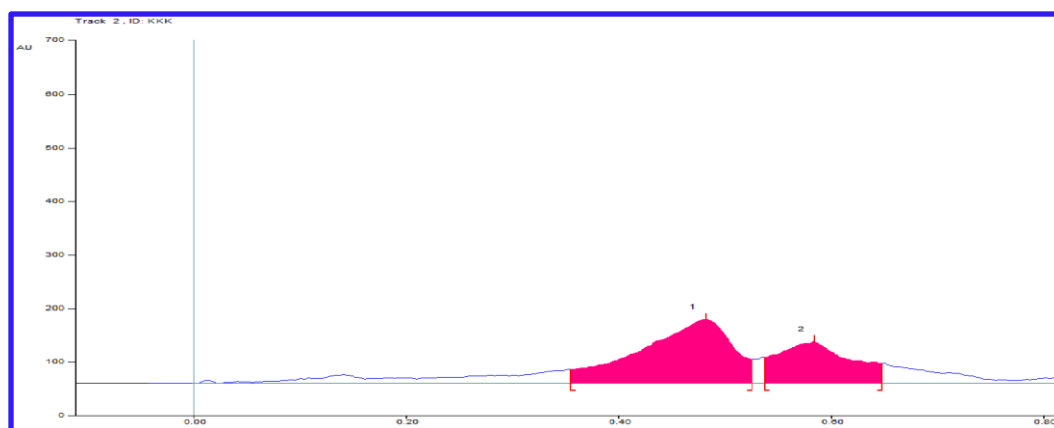


Figure 14: HPTLC finger print of Test sample (Koraikkizhangu Kudineer) at 366 nm

Table 8: R_f values of Test sample (Korai Kizhangu Kudineer) at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.36 Rf	25.8 AU	0.48 Rf	120.0 AU	36.83 %	0.53 Rf	44.8 AU	8512.1 AU	49.92 %
2	0.54 Rf	48.5 AU	0.58 Rf	78.0 AU	23.94 %	0.65 Rf	36.9 AU	4471.5 AU	26.22 %

Report

HPTLC finger printing analysis of Test sample (Koraikkizhangu Kudineer) and Standard (Aflatoxins) at 366 nm, the sample reveals the presence of 2 prominent peaks. R_f value of the peaks ranges from 0.36 to 0.54. Further the peak occupies the major percentage of area of 49.92% and 26.22%.

Interpretation

There is no evidence for the presence of any of the Aflatoxins B1, B2, G1 and G2.

4. Conclusion

The present study on physicochemical parameters, TLC and HPTLC analysis and Aflatoxin detection provides important information which can be used as a fingerprint of herbal Siddha medicine Koraikkizhangu Kudineer.

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