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Chemical Standards and HPTLC Finger Print Profiles of a Siddha Polyherbal Formulation - *Kadukkai Legiyam*

Research Article

Sujith Thatipelli[1], Achintya Kumar Mandal[2], Shakila Ramachandran[3*]

1.Research Assistant (Chemistry), 2. Assistant Research Officer (Chemistry),
3. Research Officer (Chemistry), Department of Chemistry,
Siddha Central Research Institute (Central Council for Research in Siddha, Ministry of AYUSH, Government of India), Anna Hospital Campus, Arumbakkam, Chennai, Tamil Nadu, India.

Abstract

To study physico-chemical, phytochemical and high performance thin layer chromatography of a Siddha drug "*Kadukkai Legiyam*" (KL). The prepared *Kadukkai Legiyam* (KL) was prepared as per the standard operating procedures mentioned in literature. Then the drug was subjected to physicochemical parameters, phytochemical screening, thin layer chromatographic photo documentation (TLC), high performance thin layer chromatographic (HPTLC) finger print profile of hexane, chloroform, ethanol and hydro alcohol (1:1) extracts. Different extracts of the drug showed distinct TLC and HPTLC finger print patterns which will be unique to this drug. This study giving information about physiochemical and phytochemical analysis and HPTLC fingerprint profile of different extracts, the integration spectrum which will useful in standardizing the raw drugs and future comparison studies.

Key Words: *Siddha medicine, Kadukkai legiyam, Terminalia chebula,* TLC, HPTLC.

Introduction

Siddha system of medicine is traditional and ancient medicinal system in India. There is growing importance in traditional health systems in providing healthcare for a wider population across the globe, especially in the developing countries. WHO currently encourages, recommends and promotes traditional as well as natural remedies in all over the world and national healthcare programs, as they are easily available at low cost, no side effects and traditionally acceptable. Traditional healing system around the world that utilizes herbal remedies is an important resource for the discovery of modern drugs. Siddha system has flourished well in South India. The traditional Indian Siddha medicine system supports the importance of medicinal plants to treat diseases.

Kadukkai Legiyam is a polyherbal formulation mentioned in Siddha system of medicine. It is prescribed for treating many illnesses, like anuria, constipation, veneral heat, burning sensation in palm, painful diseases, heat in the anal region, generalized oedema and tuberculosis. Five to ten grams twice daily is the dosage (1).

The purpose of this research is to give information about standardization of *Kadukkai Legiyam* through physicochemical, phytochemical analysis and high performance thin layer chromatograph (HPTLC). Physico chemical study such as loss on drying, ash values, extractive values, pH, phytochemical analysis and HPTLC finger print parameters had been developed for *Kadukkai Legiyam* in different extracts. It will be useful in standardizing the raw drugs as well as the herbal medicines.

Kadukkai mathirai, another traditional Siddha Medicine containing *Terminalia chebula* as major ingredient, is used for the treatment of liver diseases, prokinetic and iron deficiency anemia (2). It has been reported to exhibit hepato protective activity (3). Kadukkai is considered as safe as no adverse effect on biochemical and hematological parameters and histopathology of kidney, liver, and spleen was observed even after administering these drugs for a long period (4) and also exhibit hypo lipidemic activity also (5).

Terminalia chebula is the major ingredient of *Kadukkai Legiyam*. Constituents of *T. chebula* are gallic acid, chebupentol, terchebin, ellagitannin, terchebulin, arjunolic acid, arjungenin, terminoic acid ferulic acid, vanilic acid, *p*-coumaric acid, caffeic acid, fatty acids and tannin (6). It is used for the treatment of various diseases like diabetes, asthma, diarrhea, mouth ulcers, skin allergies, acidity, fever, constipation, to prevent hair loss, dandruff and used for weight loss. The plant possesses multiple pharmacological and medicinal activities, such as antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antimutagenic, antiproliferative, cardioprotective, antiarthritic, anticaries, gastrointestinal motility, wound healing activity (7), antioxidant, radioprotector (8), anti-arthritic (9) and disease modifying activity (10).

*** Corresponding Author:**

Shakila Ramachandran

Research Officer (Chemistry) Department of Chemistry, Siddha Central Research Institute (Central Council for Research in Siddha, Ministry of AYUSH, Government of India), Anna Hospital Campus, Arumbakkam, Chennai-600106, Tamil Nadu, India. India.
Email Id: rshakila@gov.in

Materials and Methods

Plant collection

All the ingredients were purchased from local herbal suppliers and authenticated by the Research Officer (Pharmacognosy) of this Institute.

Ingredients in *Kadukkai Legiyam*

Kadukkai Legiyam contains 20 different ingredients as listed in the Table 1.

Preparation of *Kadukkai Legiyam*

Powdered the ingredients from Sl.no.1 to 17 separately and taken the drug as per the quantity mentioned in the table 1. Added ghee in to sugar and heated. To this, the powdered ingredients were added and stirred continuously till obtaining the consistency of legiyam. Removed from heat and allowed to cool. Added with honey and mixed well for homogeneity.

Table 1: Ingredients of *Kadukkai Legiyam*

S. No.	Tamil Name	Botanical Name	Part Used	Quantity (in g)
1	Kadukkai	*Terminalia chebula* Retz.	Fruit rind	350
2	Civathai	*Operculina turpethum* (L.) Silva Manso	Root	70
3	Karisalai	*Eclipta prostrata* L.	Whole plant	70
4	Vallarai	*Centella asiatica* (L.) Urban	Whole plant	70
5	Puliyarai	*Oxalis corniculata* L.	Leaf	70
6	Nila avarai	*Cassia angustifolia* Vahl.	Leaf	70
7	Katukurohini	*Picrorrhiza kurroa* Royle ex Benth	Root & Rhizome	9
8	Kirampu	*Syzygium aromaticum* (L.) Merr. & Perry	Flower bud	9
9	Thippilli	*Piper longum* L.	Fruit	9
10	Cirunakappu	*Cinnamomum wightii* Meissn.	Flower bud	9
11	Valuluvai	*Celatrus paniculatus* Willd.	Seed	9
12	Karkatasringi	*Pistacia integerrima* Stewart ex Brandis	Gall	9
13	Karunjeeragam	*Nigella sativa* L.	Seed	9
14	Milagu	*Piper nigrum* L.	Fruit	9
15	Thandrikkai	*Terminalia bellirica* L.	Fruit rind	9
16	Kotamalli	*Coriandrum sativum* L.	Fruit	9
17	Thalisapattiri	*Taxus baccata* auct. non L.	Leaf	9
18	Sugar	*Saccharum officinarum*	-	1550
19	Ney (Ghee)	*Bos indicus*	-	800
20	Ten (Honey)	*Apies mellifera*	-	400

Physicochemical parameters

All the physiochemical parameters of KL were carried out as per standard methods (11).

Phytochemical Screening

All the Qualitative Phytochemical tests of KL were done by using the standard methods (12).

Chemicals, solvents and materials

Analytical grade solvents *n*-hexane, chloroform, toluene, ethyl acetate, ethanol and formic acid were purchased from Merck. For visualizing purpose vanillin (1 g) dissolved in sulphuric acid (5 ml) in ethanol (95 ml) (VSA) was used.

Preparation of extracts for TLC/HPTLC

The drug (2 g) was packed in a thimble made up of Whatman filter paper and kept in a Soxhlet extractor. Extracted successively with *n*-hexane, chloroform, ethyl acetate and ethanol (each 50 ml). After extraction, filtered the extracts and concentrated in vacuo using rotary evaporator (Buchi Laboratory Technique Limited, Switzerland) at 40°C and finally made up to 10 ml in standard flasks. In the case of hydro alcohol extract 5 g of sample were soaked for 24 h in 50% of alcohol (50 ml ethanol 50 ml water). At the end of extraction, each extract was passed through Whatman filter paper no. 1. The yellowish green filtrates obtained were concentrated in vacuo using rotary evaporator (Buchi Laboratory Technique Limited, Switzerland) at 40°C. The final products were sticky dark-brown substances, which were redissolved in corresponding solvents and stored in sample vials and used for HPTLC fingerprint profile.

Mobile Phases

The mobile phases for *n*-hexane extract, *hexane : ethyl acetate* (10:1.6 *v/v)*; for chloroform, *toluene* : *ethyl acetate : formic acid* (9:1:0.5, *v/v/v*); for ethanol, *toluene : ethyl acetate : formic acid* (6:2:0.6, *v/v/v*); for hydro alcohol extract, *toluene* : *ethyl acetate* : *methanol* (3:4:3, *v/v/v*).

Instrument

For HPTLC, aluminium plate precoated with Silica gel 60F_{254} (Merck) of 0.2 mm thickness was used. Automatic sampler ATS4 for extract application on TLC plate, twin trough chamber (10 × 10 cm) for plate development, visualizer for photo documentation under UV-visible conditions, Scanner 4 with winCATS software for finger prints, TLC plate heater for derivatization (all from CAMAG, Switzerland) were used.

Procedure for TLC/HPTLC

All the extracts (5, 10 & 15 µl) were applied separately in 4 different TLC plates of size 6 cm x10 cm as 8 mm bands at a height of 10 mm from bottom, 15 mm in X-axis. The TLC plates were developed in the respective mobile phases finalized. The developed plates were air dried, viewed under UV 254 nm and 366 nm and the images were documented followed by scanning under λ254/366 wavelengths using deuterium lamp in absorption and Hg lamp in fluorescence mode. Then the plates were dipped in a dip tank containing VSA reagent and heated at 105°C till the appearance of colored spots. Immediately the derivatized TLC plates were photo documented and scanned at a wavelength of 520 nm using W lamp in absorption mode.

Results and Discussions

Physico-chemical Studies

All the physicochemical results of KL were carried out in duplicate and the mean values are presented in the Table 2. Since KL is a legiyam, the loss on drying was estimated as 14.29% which denotes the high moisture including volatile substances. The total ash was determined as 1.68 % which shows that 98.32 % of the drug is organic in nature and only 1.68 % is inorganic in nature and in which 1.11 % are water soluble in nature. More than half of the drug (55.99 %) is soluble in water and about one third of the drug (35 %) is soluble in alcohol. The drug is acidic in nature (pH = 4.39) and the reducing sugar is 7.68 % and total sugar 16.93 %. Pharmacopoeial standards are an essential requirement for any herbal drugs (13).

Table 2: Physico chemical values of KL

Sl.No	Parameters	Mean values
1	Loss on drying at 105° C (%, w/w)	14.29
2	Total Ash (%, w/w)	1.68
3	Water soluble ash (%, w/w)	1.11
4	Acid insoluble ash (%, w/w)	0.08
5	Water soluble extractive (%, w/w)	38.18
6	Alcohol soluble extractive (%, w/w)	26.25
7	pH (10% solution)	4.39
8	Reducing sugar (%, w/w)	7.68
9	Total sugar (%, w/w)	16.93

Phytochemical Screening

The results of qualitative phytochemical test of KL are presented in the Table 3. The phytochemical tests revealed that the drug is rich in all categories of phytochemicals because of which the drug may have wide variety of therapeutic uses.

Table 3: Phytochemicals of KL

S.No	Phytochemical	Inference
1	Phenol	+
2	Tannin	+
3	Flavonoids	+
4	Triterpenoids	+
5	Proteins	-
6	Glycosides	+
7	Reducing sugar	-
8	Anthraquinones	+
9	Quinones	+
10	Alkaloids	+
11	Saponins	+
12	Cardiac glycoside	+
13	Coumarin	+
14	Acids	+

(Note: + indicates present and – indicates absent)

Chromatographic studies

The TLC of *n*-hexane extract of KL (Fig. 1) showed four spots at R_f 0.05, 0.09, 0.20, 0.28, (all green) under UV 254 nm; twelve spots at R_f 0.05 (sky blue), 0.06 (light pink), 0.08 (sky blue), 0.13 (Brown), 0.16 (Pink), 0.23, 0.27 (all Red), 0.30 (Blue), 0.40 (Green), 0.67, 0.74, 0.82(all Blue), under UV 366 nm; six spots at R_f 0.22 (Ash), 0.27 (Black), 0.30 (Light Yellow), 0.39 (Ash), 0.86 (Black) & 0.91 (Light Yellow) under white light (after dipping in VSR).TLC profile of successive chloroform extract of KL (Fig. 1) showed four spots at R_f 0.10, 0.13, 0.21, 0.26 (all green) under UV 254 nm; twelve spots at R_f 0.07 , 0.11 (all Sky Blue), 0.13 (Light Green), 0.15 (Blue), 0.18 (Pink), 0.36, 0.40, 0.44, 0.48 (all Sky Blue), 0.55 (Ash), 0.61 (Blue),0.74 (Blue) under UV λ 366 nm; six spots at R_f 0.22, 0.27, 0.30, 0.39, 0.86, 0.91(all are of ash color), under white light (after dipping in VSR).

TLC photo documentation of the successive ethanol extract of KL (Fig. 1) showed four spots at R_f 0.14, 0.40, 0.54 (all green) under UV λ 254 nm; 10 spots at R_f 0.08 (Blue), 0.18 (Sky Blue), 0.27 (Sky blue), 0.32 (Blue), 0.38 (Sky Blue), 0.50 (Light Green), 0.59, 0.71 (all Blue), 0.80 (Sky Blue), & 0.92 (Blue) were seen under UV λ 366 nm; three spots with R_f 0.13 (Light Brown), 0.60 (Ash), & 0.69 (Ash) were found under white light (after dipping in VSR). TLC profile of the hydro alcohol extract of KL (Fig. 1) shows six spots at R_f 0.04, 0.13, 0.21, 0.36, 0.57, 0.69 (all green) were found under UV λ 254 nm; four spots at R_f 0.04 (Sky Blue), 0.14 (Blue), 0.36 (blue) & 0.40 (Sky Blue) were seen under UV λ 366 nm; and five spots with R_f 0.07 (Black), 0.15 (Black), 0.25,0.36 (all Ash) &0.43 (Light Pink) were found under white light (after dipping in VSR).

HPTLC profile of hexane extract (Fig. 2) under UV 254 nm, major peaks (4, 12 and 13) appeared at R_f 0.19 (area 38.40%), 0.86 (16.49%) and 0.89 (14.25%); under 366 nm, major peaks (5, 7, 9 and 10) at R_f 0.26 (area 12.54%), 0.39 (12.72%), 0.67 (17.37%) and 0.71 (11.96%); HPTLC profile of chloroform extract (Fig. 3) under UV 254 nm, major peaks (6, 7 and 14) appeared at R_f 0.20 (area 39.60%), 0.26 (18.25%) and 0.95 (13.76%); under 366 nm, major peaks (4, 5 and 6) at R_f 0.37 (area 53.54%), 0.40 (18.44%) and 0.45 (16.86%); HPTLC profile of ethanol extract (Fig. 4) under UV 254 nm, major peaks (3, 7 and 8) appeared at R_f 0.15 (area 21.48%), 0.42 (24.49%) and 0.56 (33.48%); under 366 nm, major peaks (2, 4 and 13) at R_f 0.21 (area 10.90%), 0.28 (20.52%) and 0.82 (14.24%); HPTLC profile of hydro alcohol extract (Fig. 5) under UV 254 nm, major peaks (2, 3, 5 and 8) appeared at R_f 0.13 (area 13.87%), 0.22 (18.11%), 0.36 (11.55%) and 0.69 (20.31%) under 366 nm, major peaks (2, 3, 4 and 5) at R_f 0.33 (area 12.27%), 0.38 (18.08%), 0.41 (28.10%) and 0.93 (34.37%); after derivatization with VSR, at 520 nm, showed major peaks (2, 3, 5 and 6) at R_f 0.12 (29.43%), 0.17 (25.24%), 0.40 (10.38%) and 0.43 (15.99%).

Figure 1. TLC photo documentation of different extracts of KL

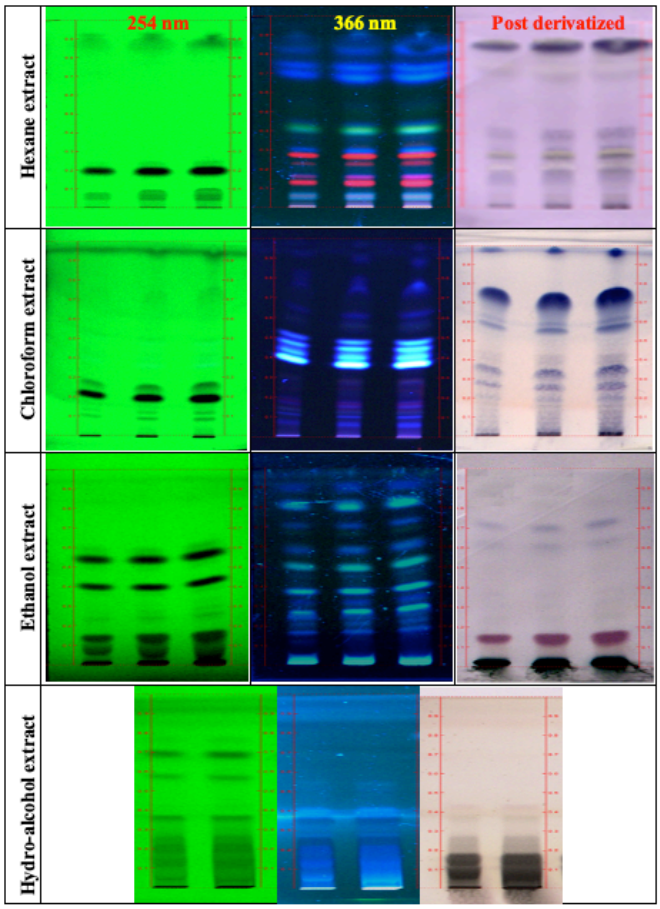


Figure 2. HPTLC finger print profile of hexane extract of KL

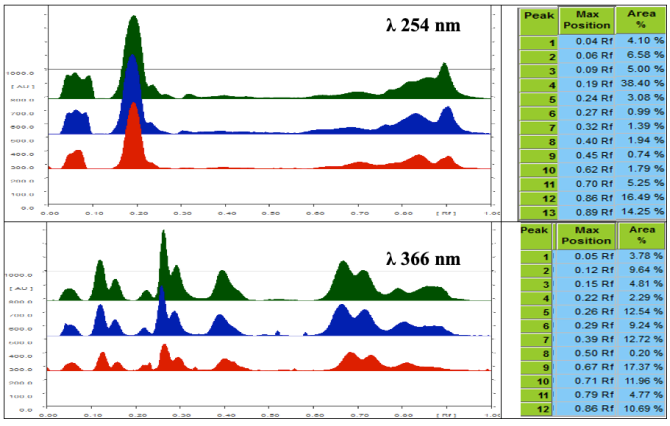
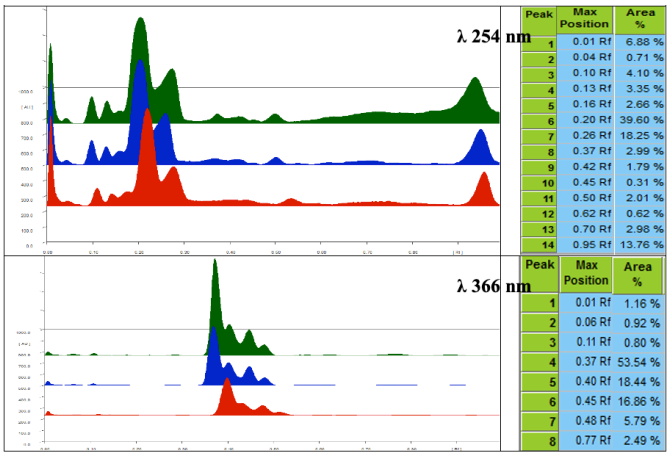


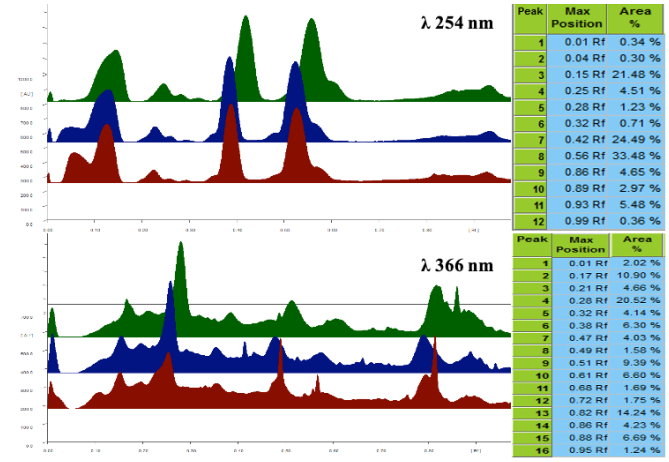
Figure 3. HPTLC finger print profile of chloroform extract of KL



Peak	Max Position	Area %
1	0.01 Rf	6.88 %
2	0.04 Rf	0.71 %
3	0.10 Rf	4.10 %
4	0.13 Rf	3.35 %
5	0.16 Rf	2.66 %
6	0.20 Rf	39.60 %
7	0.26 Rf	18.25 %
8	0.37 Rf	2.99 %
9	0.42 Rf	1.79 %
10	0.45 Rf	0.31 %
11	0.50 Rf	2.01 %
12	0.62 Rf	0.62 %
13	0.70 Rf	2.98 %
14	0.95 Rf	13.76 %

Peak	Max Position	Area %
1	0.01 Rf	1.16 %
2	0.06 Rf	0.92 %
3	0.11 Rf	0.80 %
4	0.37 Rf	53.54 %
5	0.40 Rf	18.44 %
6	0.45 Rf	16.86 %
7	0.48 Rf	5.79 %
8	0.77 Rf	2.49 %

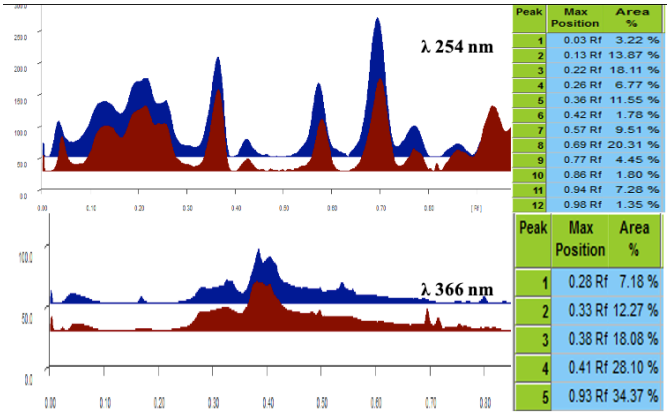
Figure 4. HPTLC finger print profile of ethanol extract of KL



Peak	Max Position	Area %
1	0.01 Rf	0.34 %
2	0.04 Rf	0.30 %
3	0.15 Rf	21.48 %
4	0.25 Rf	4.51 %
5	0.28 Rf	1.23 %
6	0.32 Rf	0.71 %
7	0.42 Rf	24.49 %
8	0.56 Rf	33.48 %
9	0.86 Rf	4.65 %
10	0.89 Rf	2.97 %
11	0.93 Rf	5.48 %
12	0.99 Rf	0.36 %

Peak	Max Position	Area %
1	0.01 Rf	2.02 %
2	0.17 Rf	10.90 %
3	0.21 Rf	4.66 %
4	0.28 Rf	20.52 %
5	0.32 Rf	4.14 %
6	0.38 Rf	6.30 %
7	0.47 Rf	4.03 %
8	0.49 Rf	1.58 %
9	0.51 Rf	9.39 %
10	0.61 Rf	6.60 %
11	0.68 Rf	1.69 %
12	0.72 Rf	1.75 %
13	0.82 Rf	14.24 %
14	0.86 Rf	4.23 %
15	0.88 Rf	6.69 %
16	0.95 Rf	1.24 %

Figure 5. HPTLC finger print profile of hydro-alcohol extract of KL



Peak	Max Position	Area %
1	0.03 Rf	3.22 %
2	0.13 Rf	13.87 %
3	0.22 Rf	18.11 %
4	0.26 Rf	6.77 %
5	0.36 Rf	11.55 %
6	0.42 Rf	1.78 %
7	0.57 Rf	9.51 %
8	0.69 Rf	20.31 %
9	0.77 Rf	4.45 %
10	0.86 Rf	1.80 %
11	0.94 Rf	7.28 %
12	0.98 Rf	1.35 %

Peak	Max Position	Area %
1	0.28 Rf	7.18 %
2	0.33 Rf	12.27 %
3	0.38 Rf	18.08 %
4	0.41 Rf	28.10 %
5	0.93 Rf	34.37 %

The approach of fingerprint analysis through HPTLC has become the effective technique for quality control of herbal drugs due to its simplicity, flexibility and reliability. It serves as a device for identification, authentication and quality control of herbal drugs. The development of chromatographic finger prints acts as a significant role in the quality control of complex herbal medicines (14,15). Ellagic and gallic acid in *Triphala churnam*, in which *T. chebula* is one of the ingredients, were estimated by HPTLC (16).

Conclusion

Kadukkai Legiyam is a combination of 20 herbs. This study gives information about physicochemical, phytochemical analysis and HPTLC fingerprint profile of different extracts which will be useful in quality assessment of the drug and batch comparison studies.

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Conflict of Interest

The authors declare no conflict of interest.

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