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Physiochemical and phytochemical analysis of *Karanthai legium* - Siddha herbomineral formulation

Research Article

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Abstract

Siddha system of medicine is one of the holistic systems. The consumption of traditional medicine is also in upward trend. To manufacture any herbal drug the quality and safety of the drug is to be estimated. *Karanthai legium* (KL) is one of the herbomineral formulation indicated for *kiranthi (Skin disease), karappan (Eczema), soolai (Pain), yoni putru (Cervical cancer), kunmam (Ulcer).* This study aims to develop standardization of *Karanthai legium* as per PLIM (Pharmacopeia Laboratory of Indian Medicine) guidelines. The test drug KL focused on Physico-chemical analysis, Phytochemical Analysis, Oraganoleptic character, Instrumental analysis such as heavy metal analysis, HPTLC Analysis, Aflatoxin, Pesticide residue, Specific pathogen and Microbial contamination. Physiochemical analysis report showed loss of drying KL is 14.96%, total ash is 0.92%, acid insoluble ash is 0.17%, water soluble ash is 0.50%,pH of KL is 5.45.The drug KL had HPTLC fingerprint analysis revealed the presence of nine phytocomponents at 254nm, eight phytocomponents at 366nm and six phytocomponents at 520nm.The heavy metal analysis showed no trace of heavy metal residue in KL. This study revealed the data regarding the physicochemical characterisation of *Karanthai legium*, which will be helpful in standardising the drugs and for further comparison studies.

Keywords: *Spaeranthus amaranthoicdes*, *Karanthai legium*, Siddha medicine, TLC, Physico-chemical analysis.

Introduction

Siddha system is one the traditional medicine has flourished well in South India. The traditional system supports the importance of medicinal plants to treat diseases (1).It based on herbal, mineral and metal preparation of drugs. Among them, herbal are in demand and many adulteration in raw drugs. One of the major issues that Siddha practitioners encounter is the lack of formal quality management guidelines for herbal medicines and their formulations (2). Hence, standardization of Siddha drug is needed for commercialization of Siddha formulations (3). This ensures the formulation's safety, quality and purity and it's used to explain all the steps required during the manufacturing process and quality control that results in a reproducible quality (4).

Siddha medicine is classified into internal (32 types) and external (32 types) medicine (5). Among them, *Legium* is one of the internal medicine, a semisolid preparation obtained by merely mixing up a powdered medicine or drug with honey, ghee or jiggery(6). *Karanthai legium*(KL) is one of the herbomineral drug mentioned in bogar aruliya vithiya saram 700 indicates for *kiranthi (Skin disease), karappan (Eczema), soolai (Pain), yoni putru (Cervical cancer), kunmam (Ulcer).The* plant *Spaeranthus amaranthoicdes* has major part in preparation having presence of steroids, alkaloids, sugars, phenolics, flavonoids, saponins, tannins.It is also one of the rejunivating drug, which prevent from disease and promote longevity of life.

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 commercialisation 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.

The aim of the study to analyse *Karanthai legium* by PLIM guidelines through physicochemical, phytochemical analysis and high performance thin layer chromatograph (HPTLC).This study will make a fingerprint for the *Karanthai legium* for further research in future.

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Materials and Methods

Procurement and Authentication of raw drugs

The raw drugs were purchased from local raw drug seller, Chennai and authenticated by botanist (NISMB5352022), National Institute of Siddha, Chennai.

Table: 1 Ingredients and their quantities of *Karanthai legium* (KL)

S.	Plant	Botanical	Famil	Part	Quantity
1	*Chukku*	*Zingiber*	Zingib	Rhizo	*¼ Balam*
2	*Milagu*	*Piper nigrum L.*	Pipera	Fruit	*¼ Balam*
3	*Thipilli*	*Piper longum*	Pipera	Fruit	*¼ Balam*
4	*Kadukkai*	*Terminalia*	Combr	Fruit	*¼ Balam*
5	*Thandrik*	*Terminalia*	Combr	Fruit	*¼ Balam*
6	*Vasambu*	*Acorus calamus*	Aracea	Root	*¼ Balam*
7	*Indhuppu*	*Rock salt*	-	Salt	*¼ Balam*
8	*Valuluvai*	*Celastrus*	Celastr	Seed	*¼ Balam*
9	*Kostam*	*Saussurea*	Astera	Root	*¼ Balam*
10	*Karunjee*	*Nigella sativa*	Ranun	Seed	*¼ Balam*
11	*Omam*	*Trachyspermum*	Apiace	Seed	*¼ Balam*
12	*Arasu*	*Ficus religiosa*	Morac	Seed	*¼ Balam*
13	*Elam*	*Elettaria*	Zingib	Seed	*¼ Balam*
14	*Manjal*	*Curcuma longa*	Zingib	Rhizo	*¼ Balam*
15	*Karboga*	*Psoralea*	Fabace	Seed	*¼ Balam*
16	*Seviyam*	*Piper nigrum L.*	Pipera	Root	*¼ Balam*
17	*Vaividan*	*Embelia ribes*	Myrsin	Seed	*¼ Balam*
18	*Anai*	*Scindapsus*	Aracea	Root	*¼ Balam*
19	*Jathikai*	*Myristica*	Myrist	Fruit	*¼ Balam*
20	*Shenbag*	*Michelia*	Magno	Flower	*¼ Balam*
21	*Thalisam*	*Abies*	Pinace	Leaf	*¼ Balam*
22	*Athimath*	*Glycyrrhiza*	Fabace	Root	*¼ Balam*
23	*Jathipath*	*Myristica*	Myrist	Root	*¼ Balam*
24	*Karantha*	*Spaeranthus*	Astera	Root	200grams
25	*Lavagam*	*Syzygium*	Myrtac	Flower	*¼ Balam*
26	Ghee				50 ml
27	Honey				50 ml

Procedure

Except the root of *karanthai*, all of the afore mentioned raw drugs (Table 1) (each*¼ Balam)* were refined, ground, and converted into a fine powder. *Karanthai* root was washed, dried, and ground into a powder. The same amounts of powdered *karanthai* root to the net weight of the aforementioned raw drugs is taken and mixed well. This powder was added slowly and thoroughly combined with melted ghee. Finally, stirred with honey (7).

Standardisation according to PLIM Guideline

As per AYUSH - PLIM guideline (8), the trial drug *Karanthai legium (KL)* was tested its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug.

Organoleptic Characters

The organoleptic characters of the sample drug were evaluated.1gm of the KL was taken and the state, appearance, nature, odour, and other morphological characters were viewed by the naked eye under natural light and results were noted. (Table 2)

Physicochemical Analysis

Physicochemical studies like total ash, water-soluble ash, acid soluble ash, acid-insoluble ash, water, and alcohol soluble extract, loss on drying at 105^{o}C were done at Siddha Central Research Institute, Anna Government Hospital Campus, Arumbakkam, Chennai. Results were noted in (Table 3).

Preliminary Phytochemical Screening of *Karanthai Legium*

The preliminary phytochemical screening test was carried out for each extract (aqueous and ethanol extract) of KL as per the standard procedure mentioned here under. Phytochemical studies like the detection of alkaloids, carbohydrates, saponins, phenols, and tannins, Detection of Flavonoids, diterpenes, Quinones, Gum, and Mucilage were done at The Tamilnadu DR. M.G.R.Medical University, Chennai. The Preliminary phytochemical studies of aqueous & ethanol extract of *KARANTHAI LEGIUM* were done using standard procedures. The present study reveals that the bioactive compounds were present in all the extracts of *KL*. The results were presented in tables (Table 4)

Instrumental Analysis of *Karanthai Legium*

The test drug *KARANTHAI LEGIUM* (KL) was analysed to generate the fingerprint using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) in SIDDHA CENTRAL RESEARCH INSTITUTE, Anna Govt. Hospital Campus, Arumbakkam, Chennai.

Thin-layer Chromatography Methodology (TLC)

Applied 10 µl chloroform extract of drug KL on TLC plate using Camag's ATS4 applicator and

developed by the mobile phase, Hexane: Ethyl acetate (7.5:2.5, v/v) up to 9 cm distance.

Sample Preparation for TLC

Sample (1 g) was dissolved in 10 ml of chloroform and then sonicated for 15 minutes and filtered. This solution was used for TLC.

Scanning

After development, the plate was photo documented using Camag's TLC Visualiser under UV 254 nm and UV 366 nm and then scanned using Camag's Scanner 4 at (D2 lamp/Absorption mode, Hg lamp/Fluorescent mode) fingerprint profiles of the extract were documented. Then the plate was dipped in 5% vanillin-sulphuric acid reagent followed by heating at 105^{0}C till the development of coloured spots. The plate was then photo-documented in white light and scanned at 520 nm for fingerprint profile. (Table 5)

High Performance Thin Layer Chromatography (HPTLC)

Sample preparation

The sample KL was prepared in polar solvent sonicated and refluxed the solution was filtered with Whatman 41 paper and re-filtered with a syringe filter (0.45 µ). Filtered solutions were applied to HPTLC 60 silica gel glass-backed layers (Merck).

Scanning

For the fingerprinting, a Camag TLC scanner 3 linked to Win CATS software was set at 350 nm, after multi-wavelength scanning between 250 and 400 nm in the absorption mode had first been tried. A chromatographic finger print was developed for the detection of phytoconstituents present in each sample and the irrespective Rf values were tabulated.

Test for Heavy Metals

To determine the heavy metals such as mercury, arsenic, lead, and cadmium concentrations in the test drug was performed by Atomic Absorption Spectroscopy [AAS] Model AA 240 series. KL sample was digested with 1 mol/L HCl for determination of arsenic and mercury. Results were tabulated in (Table 9)

Microbial Contamination

Test for Specific Pathogen

Test sample KL was directly inoculated into the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by the pour plate method. The plates were incubated at 37oC for 24 - 72h for observation. The presence of specific pathogens is identified by their characteristic colour concerning the pattern of colony formation in each differential media. Results were tabulated in (Table 10)

Pesticide Residue Analysis

Extraction Test sample *Karanthai legium* was extracted with acetone and followed by homogenisation for a brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample *Karanthai legium (KL)* was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent had almost completely evaporated. The balance residue will be dissolved using toluene and filtered through a membrane filter. Results were noted and tabulated in (Table 11)

Aflatoxin

Aflatoxins-producing fungi can contaminate crops in the field, at harvest, and during storage. Exposure to aflatoxins causes an increased risk of liver cancer. The procedure for the detection of Aflatoxin was recommended by WHO (2007).

Procedure

Standard aflatoxin was administered in volumes of 2.5 L, 5 L, 7.5 L, and 10 L to the surface of a pre-coated TLC plate. Similar to this, the test sample (KL) was placed, allowed the spots to dry, and then the chromatogram was developed in an unsaturated chamber with a solvent system made up of a chloroform, acetone, and isopropyl alcohol (85: 10: 5) mixture until the solvent front had moved not less than 15 cm from the origin. Mark the solvent off, then take the plate out of the developing chamber and let it air dry. Examine the plate under 365nm UV light to find the spots on it. Results were tabulated in (Table 12).

Results

Organoleptic character of *Karanthai legium* were evaluated and showed in table 2. It was done to assess the quality of the poly herbal sample.

Table 2: Organoleptic characters of *Karanthai legium* (KL)

S.NO	PARAMETER	RESULT
1	State	Semisolid
1	Nature	Smooth Surface
2	Odor	Aromatic
3	Touch / Consistency	Soft
4	Flow Property	Non- free flowing
5	Appearance	Intense Greenish

The physicochemical analysis of *karanthai legium* was done and noted in table 3.

Table 3: Physicochemical parameters of *Karanthai legium*

S.NO	NAME OF THE TEST	Value (%)
1	Loss of Drying (% w/w)	14.96
2	Total ash (% w/w)	0.92
3	Water soluble ash(% w/w)	0.50
4	Acid insoluble ash (% w/w)	0.17
5	Water soluble extractives (% w/w)	33.32
6	Alcohol soluble extractives (% w/w)	42.02
7	Hexane extractive value (% w/w)	43.61
8	pH(10% solution)	5.45
9	Rancidity	Nil

Phyto-chemical Screening

The Preliminary phytochemical studies of Aqueous and Ethanol extract of *Karanthai Legium* (KL) were done using standard procedures. The results were presented in tables 4. The present study reveals that the bioactive compounds were present in all the extracts of KL.

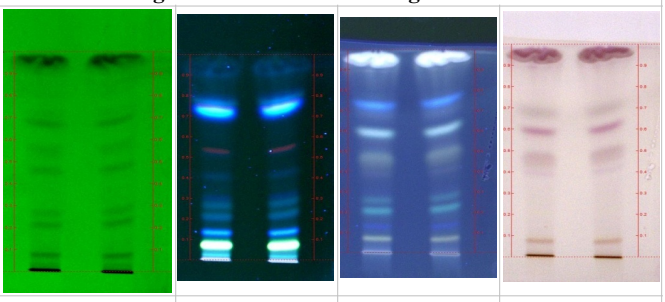
Table No 4: Phytochemical Analysis of *Karanthai legium* (Aqueous and Ethanol extract)

S.No.	Phyto-chemicals	Test Name	H_2O Extract	Ethanol Extract
1	Alkaloids	Dragendorff's Test Wagner Test	+ ve + ve	+ ve + ve
2	Carbo-hydrates	Molisch's Test Benedict Test	+ve +ve	+ ve + ve
3	Phenols	Ferric Chloride Test	+ve	+ ve
4	Tannins	Gelatin Test	+ve	-ve
5	Flavonoids	Alkaline Reagent Test Lead acetate	+ve +ve	+ ve + ve
6	Diterpenes	Copper Acetate Test	+ve	+ ve
7	Quinones	Test for Quinones	-ve	-ve
8	Gum & Mucilage	Test for Gum & Mucilage	-ve	-ve
9	Saponin	Foam Test	-ve	-ve

Instrumental Analysis

TLC and HPTLC fingerprinting can be employed for the purpose of creating quality standards for polyherbal compositions (9,10). For sample KL fingerprint was mentioned below.

Figure no.1: TLC Chromogram of KL



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

Rf and color of spots

Table 5: TLC Rf and colour of spots

λ=254 nm		λ=366 nm		λ=366 nm (Derivatized)		λ=520 nm (Derivatized)	
Rf	Colour	Rf	Colour	Rf	Colour	Rf	Colour
0.07	Green	0.01	Cyan blue	0.06	Yellow	0.07	Brown
0.22	Green	0.07	Fluorescent green	0.13	Blue	0.22	L.yellow
0.27	Green	0.13	Fluorescent Blue	0.22	Green	0.41	Pink
0.46	Green	0.22	Blue	0.28	Green	0.48	Violet
0.57	Green	0.27	Blue	0.41	L. violet	0.60	Violet
0.68	Green	0.31	Blue	0.48	Ash colour	0.69	Grey
0.73	L. green	0.42	Blue	0.60	White		
		0.53	Blue	0.69	Grey		
		0.55	Pink	0.75	Blue		
		0.73	Fluorescent blue				

HPTLC Finger Printing Analysis of KL

Fig no 7: 3D Chromatogram of 254 nm

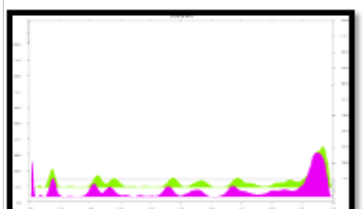


Fig no 8: HPTLC @ 254 nm

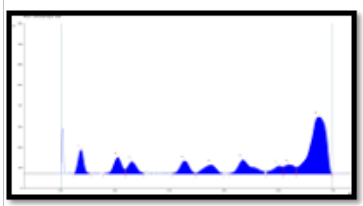


Table 6: Peak Table @254 nm

Track 1, ID: karantai legium, chcl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.4 AU	0.08 Rf	117.3 AU	14.89 %	0.11 Rf	0.2 AU	2125.2 AU	7.23 %
2	0.16 Rf	0.1 AU	0.21 Rf	80.6 AU	10.23 %	0.24 Rf	22.5 AU	2072.8 AU	7.05 %
3	0.24 Rf	22.7 AU	0.26 Rf	58.4 AU	7.42 %	0.31 Rf	6.2 AU	1690.8 AU	5.75 %
4	0.41 Rf	2.5 AU	0.46 Rf	62.7 AU	7.96 %	0.50 Rf	4.1 AU	1859.8 AU	6.33 %
5	0.50 Rf	4.6 AU	0.56 Rf	42.9 AU	5.44 %	0.60 Rf	0.0 AU	1760.3 AU	5.99 %
6	0.62 Rf	1.1 AU	0.67 Rf	68.8 AU	8.48 %	0.75 Rf	10.8 AU	3016.7 AU	10.27 %
7	0.76 Rf	11.3 AU	0.80 Rf	37.5 AU	4.76 %	0.82 Rf	34.6 AU	1210.8 AU	4.15 %
8	0.82 Rf	34.9 AU	0.84 Rf	44.5 AU	5.65 %	0.87 Rf	33.6 AU	1394.7 AU	4.75 %
9	0.87 Rf	33.7 AU	0.95 Rf	277.1 AU	35.18 %	1.00 Rf	1.3 AU	14244.0 AU	48.48 %

Fig no 9: 3D Chromatogram of 366nm

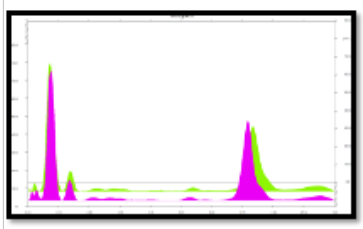


Fig no: 10 HPTLC @366 nm

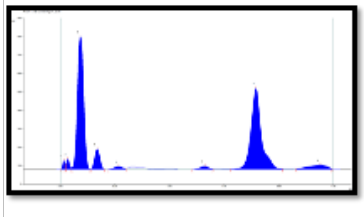


Table 7: Peak Table @ 366 nm

Track 1, ID: karantai legium, chcl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.6 AU	0.01 Rf	48.1 AU	3.36 %	0.02 Rf	28.0 AU	352.2 AU	1.02 %
2	0.02 Rf	28.8 AU	0.03 Rf	56.4 AU	3.94 %	0.04 Rf	8.3 AU	585.7 AU	1.69 %
3	0.04 Rf	8.5 AU	0.07 Rf	721.4 AU	50.36 %	0.11 Rf	0.5 AU	14767.3 AU	42.67 %
4	0.11 Rf	0.4 AU	0.14 Rf	110.4 AU	7.71 %	0.16 Rf	0.3 AU	1905.9 AU	5.51 %
5	0.17 Rf	0.0 AU	0.21 Rf	15.5 AU	1.08 %	0.24 Rf	5.5 AU	423.6 AU	1.22 %
6	0.48 Rf	0.2 AU	0.53 Rf	17.1 AU	1.19 %	0.56 Rf	1.9 AU	455.7 AU	1.32 %
7	0.63 Rf	0.8 AU	0.72 Rf	439.4 AU	30.68 %	0.82 Rf	2.5 AU	14674.6 AU	42.41 %
8	0.87 Rf	3.5 AU	0.95 Rf	24.0 AU	1.67 %	1.00 Rf	2.5 AU	1439.7 AU	4.16 %

Fig no 7: 3D Chromatogram of 254 nm

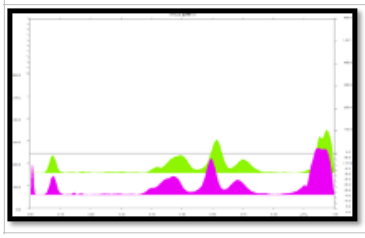


Fig no 8: HPTLC @ 254 nm

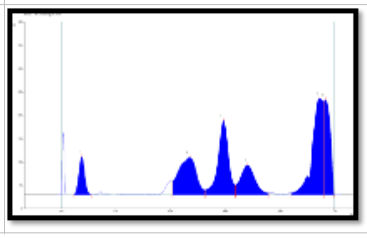


Table 6: Peak Table @254 nm

Track 1, ID: karanthai legium, chc3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.4 AU	0.08 Rf	81.9 AU	10.30 %	0.11 Rf	0.1 AU	1551.7 AU	5.98 %
2	0.41 Rf	30.8 AU	0.47 Rf	80.4 AU	10.11 %	0.53 Rf	10.5 AU	4590.7 AU	17.69 %
3	0.53 Rf	10.6 AU	0.60 Rf	159.4 AU	20.03 %	0.64 Rf	21.5 AU	5444.6 AU	20.97 %
4	0.64 Rf	21.5 AU	0.68 Rf	63.8 AU	8.01 %	0.76 Rf	4.0 AU	2994.5 AU	11.54 %
5	0.84 Rf	4.2 AU	0.95 Rf	206.9 AU	26.01 %	0.96 Rf	90.5 AU	7206.5 AU	27.76 %
6	0.96 Rf	200.5 AU	0.97 Rf	203.3 AU	25.55 %	1.00 Rf	10.8 AU	4170.0 AU	16.06 %

Heavy Metal Analysis of KL

The heavy metal analysis showed that there were no traces of heavy metal residues such as lead, arsenic, cadmium, mercury to ensure the safety of the drug KL.

Table 9: Heavy metal analysis of KL

S.No	PARAMETERS	UNITS	RESULTS
1	Lead as Pb	mg/kg	BDL (DL:0.1)
2	Arsenic as As	mg/kg	BDL (DL:0.1)
3	Cadmium as Cd	mg/kg	BDL (DL:0.1)
4	Mercury as Hg	mg/kg	BDL (DL:0.1)

BDL: Below Detectable Level; DL: Detectable level

Microbial Contamination

No growth /colonies were observed in any of the plates inoculates with the *Karanthai legium* (KL). This revealed that the drug KL was free from the viable microorganisms and the absence of total bacterial and fungal count which may indicates that the drug KL has good quality and safer drug.

Table 10: Microbial contamination of KL

S.No	PARAMETERS	UNITS	RESULTS
1	APC – Bacteria	cfu/g	1.25×10^4
2	APC – Fungi	cfu/g	5×10^3

APC: Aerobic Plate Count; cfu: colony forming unit

Pesticide Residue of KL

The drug KL showed that there were no traces of pesticides residues such as Organochlorine, Organo phosphorus, Organocarbamates and pyrethroids in the sample provided for analysis.

Table 11: Pesticide residue of KL

Pesticide residue	Sample KL	AYUSH Limit (mg/kg)
I. Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo-Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL - Below quantification limit

Aflatoxin for KL

The results shows that there were no spots were being identified in the test sample KL loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2,Aflatoxin G1 and Aflatoxin G2.

Table 12: Aflatoxin for *Karanthai Legium*

AFLATOXIN	Sample KL	AYUSH SPECIFICATION LIMIT
B1	Not Detected- Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected- Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected- Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected- Absent	0.1 ppm (0.1mg/kg)

Discussion

Traditional wisdom has claimed that herbal preparations are safe to use, and more and more people throughout the world are consuming them. It is critical to standardise herbal formulations in order to evaluate the drug's effectiveness, safety, and purity(9).The drug *Karanthai* (*Spaeranthus amaranthoicdes*) is one of the *kaya karpam* (rejuvenating herb) for prevention of disease and increase longevity of life The loss on drying of sample KL is 14.96% indicates the stability and shelf life of the drug KL is good. The total ash value of KL is 0.92% shows that the drug KL 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 KL is 0.17%. Hence, it represents the superior quality of the drug KL.

The percentage of soluble matters present in the drug is determined by the values of water soluble ash for KL is 0.50%.Water-soluble extractive value plays an important role in evaluation of crude Drugs and it is 33.32 % in *Karanthai legium*. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value and it is42.02% for *Karanthai legium*. Hexane extractive value plays an important role in evaluation of crude Drugs and it is 43.61 % in *Karanthai legium* .The pH of the drug *Karanthai legium* is 5.45 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 KL. The Phytochemical screening of KL reveals the presence of Alkaloids,Carbohydrates,Phenols, Tannins, Flavonoids, Diterpenes. TLC finger printing analysis of the sample

KL reveals the presence of seven prominent peaks corresponds to presence of three versatile phytocomponents present within it. Rf value of the peaks ranges from 0.07 to 0.73.

The sample KL has HPTC finger printing analysis revealed the presence of nine versatile phytocomponents present at 254nm. Rf value of the peaks ranges from 0.05 to 0.87.At 366nm, presence of eight versatile phytocomponents present with Rf value of the peaks ranges from 0.01 to 0.87. At 520nm, presence of six versatile phytocomponents present with Rf value of the peaks ranges from 0.05 to 0.96.

The heavy metal analysis showed that there were no traces of heavy metal residues such as lead, arsenic, cadmium, mercury to ensure the safety of the drug KL. As a result of these practises, pesticide residues from agricultural fumigations, soil treatments, and pre- or post-harvest periods accumulate on medicinal plants. Absence of total bacterial and fungal count which may indicates that the drug KL has good quality and safer drug. Organo chlorine, Organo phosphorus, and Pyrethroid residues were not detected in the sample KL. From Aflatoxin study, the drug KL is non toxic and there is no contamination and does not act as a carcinogenic property.

Conclusion

This study ensures that *Karanthai legium* (KL) has all properties of a *legium*, specified in the PLIM guidelines. It gives information about physicochemical, phytochemical analysis and HPTLC fingerprint extracts which will be useful in quality assessment of the drug, purity and batch comparison studies. Hence, the Phytochemical and physicochemical analysis of the drug is the first step for further assessing toxicological and validating pharmacological activities.

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Conflict of Interest

The authors have declared no conflict of interest in this study.

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