



Standardization of Siddha Single drug formulation *Kottai karanthai Chooranam*.

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ABSTRACT

Traditional herbal medicines are getting significant attention in global health debates. In Siddha system of medicine using herbal related medicine predominantly. Standardization of all herbal drugs is needed to maintain the herbal quality. *Kottai Karanthai Chooranam* is a single herbal formulation which is selected by the author for standardisation. Aim and Objective of this study was to assess the quality of the drug *Kottai Karanthai Chooranam* by conducting physicochemical analysis, preliminary phytochemical analysis and other analytical techniques. The physicochemical analysis of the drug *Kottai Karanthai Chooranam* showed $4.033 \pm 2.801\%$ of loss on drying at 105°C , $2.867 \pm 0.4509\%$ of total Ash content, 0.3933 ± 0.2501 of Acid Insoluble Ash, extract values of Alcohol & water is $20.33 \pm 4.869\%$ & $9.33 \pm 0.1153\%$. The drug was free of microbial contamination, heavy metals and pesticide were below deductible limits. The results obtained indicate that the drug is standard quality and can be used as reference standard in laying pharmacopoeia standard.

Keywords:

Siddha medicine, herbal drug, *Kottai Karanthai Chooranam*, Standardization.

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INTRODUCTION

Traditional herbal medicines are naturally occurring, plant derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. Traditional herbal medicines are getting significant attention in global health debates [1]. In India, having a rich heritage of traditional medicine constituting its different components like Siddha, Ayurveda, Unani and Homeopathy [2]. Siddha is the indigenous system of Indian Medicine practiced in South India especially in Tamil Nadu [3]. In general, among the Indian systems of medicine (ISM), Siddha uses herbs predominantly. Great emphasis is given to herbs by our ancient Siddhars. In Bogar Nigandu, about 4,444 diseases are described and various herbs are indicated for these diseases [4].

Herbal formulation of drug administers in the form of liquid, powder, pill or paste. According to the WHO, 60 % of the world's population relies on herbal medicine and about 80% of the population in developing countries depends almost totally on it for their primary health care needs. Phytochemicals and also their chemical analogs have provided abundant clinically useful drugs in the treatment of chronic and acute diseases. And still research is continued to search for newer therapeutic agents from medicinal plants [5]. This increase of demand resulted in the irrational collection of immature plants even though properly identified resulting in loss of quality. Along with adulteration of plants with substandard substances further deteriorated the quality resulting in substandard medicinal preparations [6]. So there is a need for standardization of all herbal drugs to maintain the herbal quality. Therefore it is highly desirable that these drugs should be characterized with modern instruments, based on which the specifications of such drugs can be well standardized on the scientific basis [7].

The term *Chooranam* (Powdered herbal preparation for internal use) is applied to the powder prepared by a single or a combination of two or more herbal ingredients [8,9]. The selected drug *Kottai Karanthai Chooranam* is a single herbal formulation though simple and cost effective, has diverse medicinal properties and used in the of *Karappan* (Eczema), *Sori* (Pruritus), *Sirangu* (Scabies), *Thozh pinigal* (Skin disease), *Maripatta earu Kazhiyum* (Constipation), *Vali* (Arthritis), *Veri* (Psychiatric disorder), *Sinaippu* (Erythema) [10].

Systematic protocols for standardization of *Kottai Karanthai Chooranam* is not available, hence it was decided to evaluate the qualitative and quantitative analysis for *Kottai Karanthai Chooranam* scientifically to prevent its adulteration.

For the standardization of this drug Organoleptic properties, Phytochemical screening, Physicochemical parameters, HPTLC analysis, heavy metal analysis, Pesticide residue, Aflatoxin, specific pathogen, sterility test were carried out to evaluate its quality.

MATERIALS AND METHODS

Plant materials

The sample of drug namely *Kottai Karanthai Samoolam* (Whole plant of *Sphaeranthus indicus*) was collected from the area of Annavasal, Pudukkottai Dt, Tamil nadu. Herbal was authenticated by Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai-47.

Preparation of drug

The *Kottai Karanthai Samoolam* were completely dried, then powdered separately and sieved by white cloth which is mentioned as *Vasthirakayam* in classical Siddha text. The sieved ingredient powders were mixed thoroughly to get the *Chooranam* and stored in a clean and airtight glass container.

Place of work

The Organoleptic properties, Phytochemical screening, Physicochemical parameters, HPTLC, Pesticide residue, Aflatoxin, specific pathogen, sterility test analysis of kottai Karanthai chooranam was carried as per standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99. The analysis of heavy metals and trace elements were estimated by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai.

PHARMACOGNOSTIC STUDIES

Organoleptic Character Analysis

Colour, odour, taste and nature of the drug were noted.

Physicochemical evaluation

All the Physicochemical parameters were carried out as per the methods mentioned in standard books [11,12].

Phytochemical analysis

All the Phytochemical analysis were carried out as per the methods mentioned in standard books [13].

Heavy metal analysis

Heavy metals like lead, cadmium, mercury and arsenic and nutritional elements like ferrous, copper, zinc, potassium and calcium were studied [14].

Thin Layer Chromatography and High-Performance Thin Layer Chromatography

Thin Layer Chromatography 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 pencils. Micro pipette was used to spot the sample for TLC applied sample volume 10-microliter 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 were observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm^[15].

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 a high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. 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 Phyto therapeutics. To detect the presence of various Phyto constituents in formulation *Kottai Karanthai Chooranam* showed the presence of Phenol, Tannin and Saponin.

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 fingerprint was developed for the detection of phytoconstituents present in each sample^[16].

Pesticide Residue

All the pesticide residue were carried out as per the methods mentioned in standard procedure^[17,18] ..

Aflatoxin

To detect the presence of B1, B2, and G1, G2 in *Kottai Karanthai Chooranam* were carried out as per the methods mentioned in standard procedure^[19] .

Sterility test

To determine the sterility of the *Kottai Karanthai Chooranam* were carried out as per the methods mentioned in standard procedure^[18] .

Specific pathogen

To determine the specific pathogen of *KKC* were carried out as per the standard procedure^[18] .

RESULTS AND DISCUSSION

Organoleptic characters

Organoleptic characters on *Kottai Karanthai Chooranam* revealed that the drug appears solid state, greenish brown in colour, nature of the powder is moderately fine and strong characteristic odour. The observed result was tabulated in Table no :1 & Figure no:1.

Physicochemical evaluation

The loss on drying test is used to determine the amount of volatile matter (i.e water drying off from the drug). Moisture is one of the major factors responsible for deterioration of the drugs^[20]. The percentage of loss on drying of *Kottai Karanthai Chooranam* was 4.033 ± 2.801 % (Normal range 1 - 20 %). Low moisture content is always desirable for higher stability of drugs. As per the results the loss on drying of *Kottai Karanthai Chooranam* is low, so the stability of the *Kottai Karanthai Chooranam* is higher.

The Ash limit Tests are designed to measure the amount of the residual. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the drug^[20]. Acid insoluble ash use to detect the contamination from sand or soil^[20]. The total Ash content and Acid Insoluble Ash values of *Kottai Karanthai Chooranam* were 2.867 ± 0.4509 % (Normal range: 1-25%) and 0.3933 ± 0.2501 (Normal range: 0.1 – 10%). The total ash value of *Kottai Karanthai Chooranam* is low, it implies that the inorganic constituents are low and indicates the purity of the drug.

Extractive value determined the amount of active constituents in a given amount of medicinal plant material when extracted with solvent. In that particular solvent depends upon the nature of the drug and solvent used. The determination of water soluble and alcohol soluble extractives is used as a means of evaluating crude drugs^[20]. The extract value of Alcohol in *Kottai Karanthai Chooranam* is 20.33 ± 4.869 % and water is 9.33 ± 0.1153 %.

The observed result was tabulated in Table no :2.

Phytochemical analysis

To detect the presence of various Phyto constituents in formulation *Kottai Karanthai Chooranam* showed the presence of Phenol, Tannin and Saponin. Presence of the above components in phytochemical analysis increases the therapeutic value of *Kottai Karanthai Chooranam*. The observed result was tabulated in Table no :3 & Figure no: 2.3.4.5.

The presence of phenolic compounds in the *Kottai Karanthai Chooranam* indicates that these medicine might be anti- microbial agents and antiseptic or bactericidal properties. Phenols have been responsible for having the ability to block specific enzymes that cause inflammation and immune enhancer and hormone modulators.

Tannins antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. This finding is supported in treating skin disease and also prevents the formation of infection.

Heavy metal analysis

Heavy metal analysis through ICP-OES results was tabulated in Table no: 4. The Heavy metals like lead, mercury, nickel, copper, arsenic and cadmium were found below detection level. Hence it may be safe for human consumption. It also shows the presence of physiologically important minerals like Calcium, Copper, Iron, Potassium, Manganese, Sodium, Phosphorus and Zinc which are within ppm limit.

High Performance Thin Layer Chromatography Analysis

The HPTLC analysis Which is used for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances^[23].

The HPTLC analysis of the sample showed that presence of seven prominent peaks corresponds to presence of seven versatile Phyto components present in which peak 4 occupies the major percentage of area of 28.83 which denotes the abundant existence of such compound. The observed result was tabulated in Table no: 5 & Figure no:(6,7).

Pesticide Residue

Presence of pesticide causes serious side effects in human beings. Organo chlorine cause abnormalities of liver function, skin (chloracne), and the nervous system and produce cancer^[24]. Organophosphorus acetylcholinesterase inhibition can cause acute parasympathetic system dysfunction, muscle weakness, seizures, coma, and respiratory failure^[25]. Pyrethroids produce tremors of the whole body, aggressive behaviour, hypersensitivity and ataxia, choreoathetosis-salivation syndrome (CS) and motor dysfunction, male fertility dysfunction and cardiac toxicity^[26].

Pesticide analysis of *Kottai Karanthai chooranam* showed there was no presence of pesticides residues such as Organo chlorine, Organophosphorus and Pyrethroids in *Kottai karanthai Chooranam*. It indicated the quality of the ingredients of *Kottai Karanthai Chooranam*. The observed result was tabulated in Table no :6.

Aflatoxin

Aflatoxin is a toxin from *Aspergillus flavus* and *Aspergillus parasiticus* having the chemical formula $C_{12}H_{12}O_6$ and may cause hepatic carcinoma in human beings^[20]. Aflatoxin analysis of *Kottai Karanthai chooranam* reveals free from Aflatoxin B1, B2 and Aflatoxin G1, G2. It indicated the quality of the ingredients of *Kottai Karanthai Chooranam*. The result was tabulated in Table no :7.

Table 1: Organoleptic Character of Kottai Karanthai Chooranam (KKC)

State	Solid
Nature	Moderately fine
Odor	Strong characteristic
Touch	Dry
Flow Property	Non- Free flowing
Appearance	Greenish brown

Figure 1: Organoleptic character



Table 2: Physico-chemical analysis

S.NO	PARAMETERS	PERCENTAGE
1.	Loss on Drying at 105 °C (%)	4.033 ± 2.801
2.	Total Ash (%)	2.867 ± 0.4509
3.	Acid insoluble Ash (%)	0.3933 ± 0.2501
4.	Water soluble Extractive (%)	9.33 ± 0.1153
5.	Alcohol Soluble Extractive (%)	20.33 ± 4.869
6.	Particle Size	Completely passes through sieve size of 1 mm (90 % passes through 400 micro meter sieve and 10% passes through 1mm sieve)

Table 3: Phytochemical Investigation

S.NO	TEST	OBSERVATION
1.	Alkaloids	-
2.	Flavonoids	-
3.	Glycosides	-
4.	Steroids	-
5.	Triterpenoids	-
6.	Coumarin	-
7.	Phenol	+
8.	Tannin	+
9.	Protein	-
10.	Saponins	+
11.	Sugar	-
12.	Anthocyanin	-
13.	Betacyanin	-

Figure 2: Qualitative Phytochemical Investigation

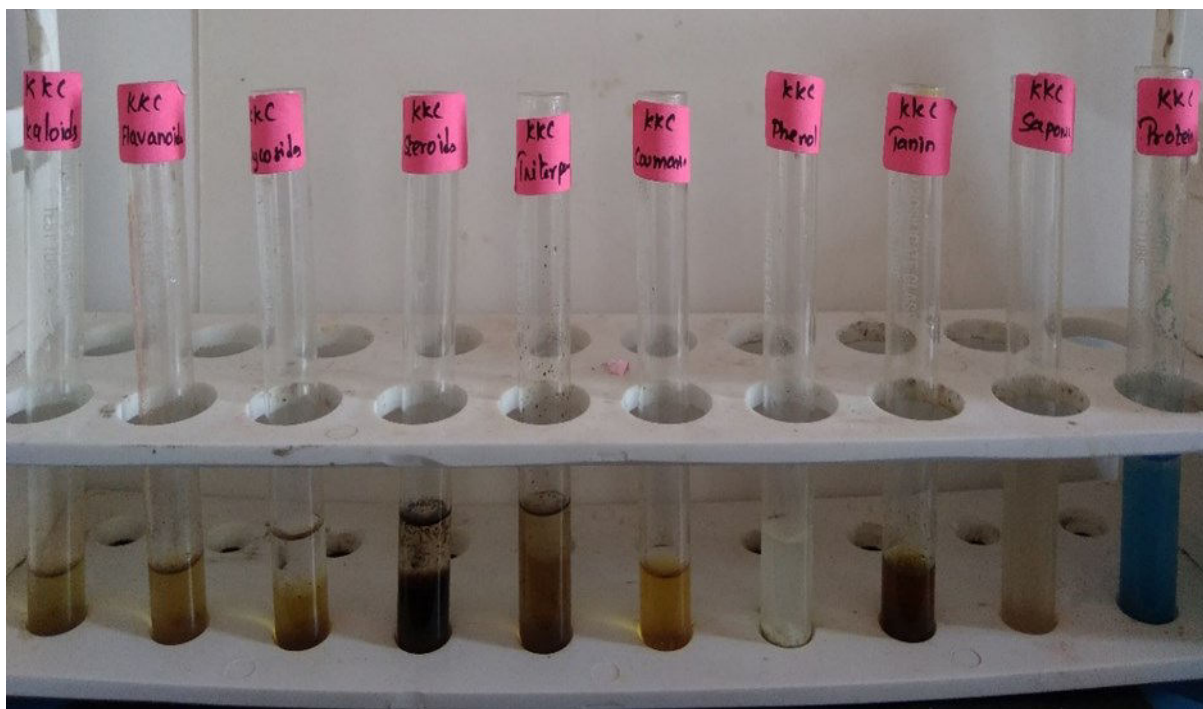


Figure 3 Test for Coumarin, Phenol, Tannins, Saponin, Proteins

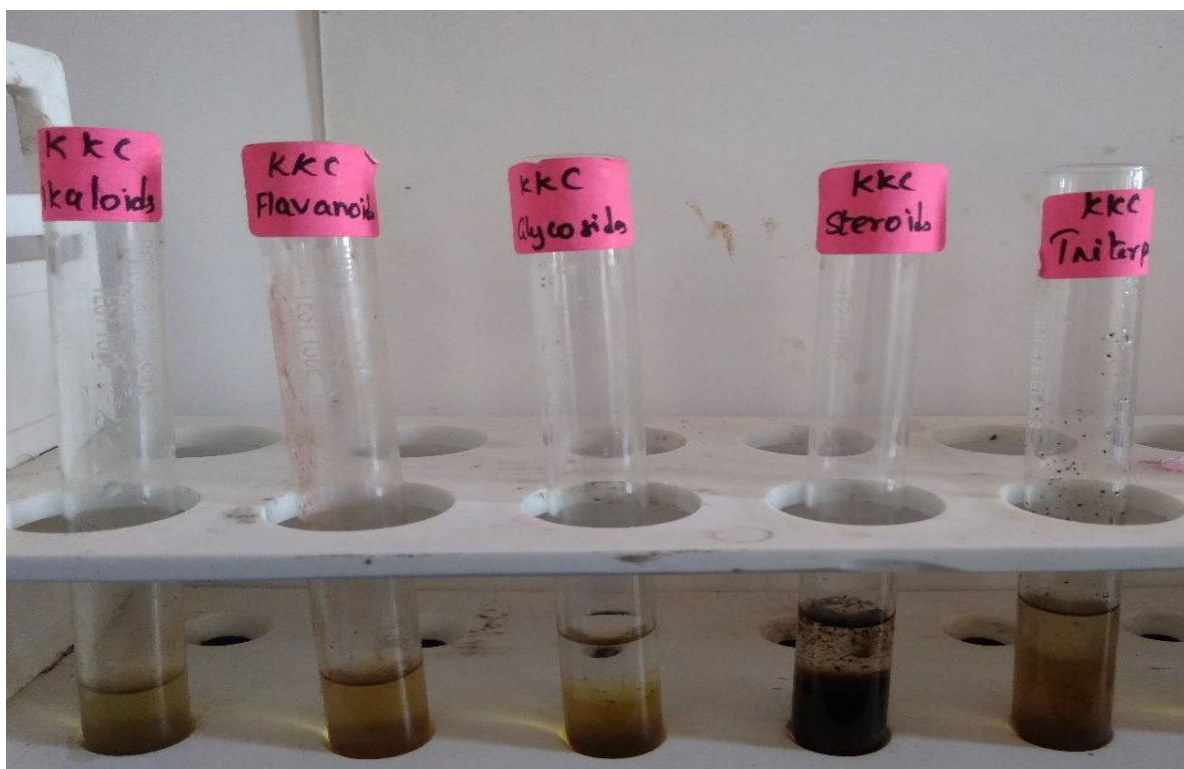


Figure 4: Test for Carbohydrates & Anthocyanins.

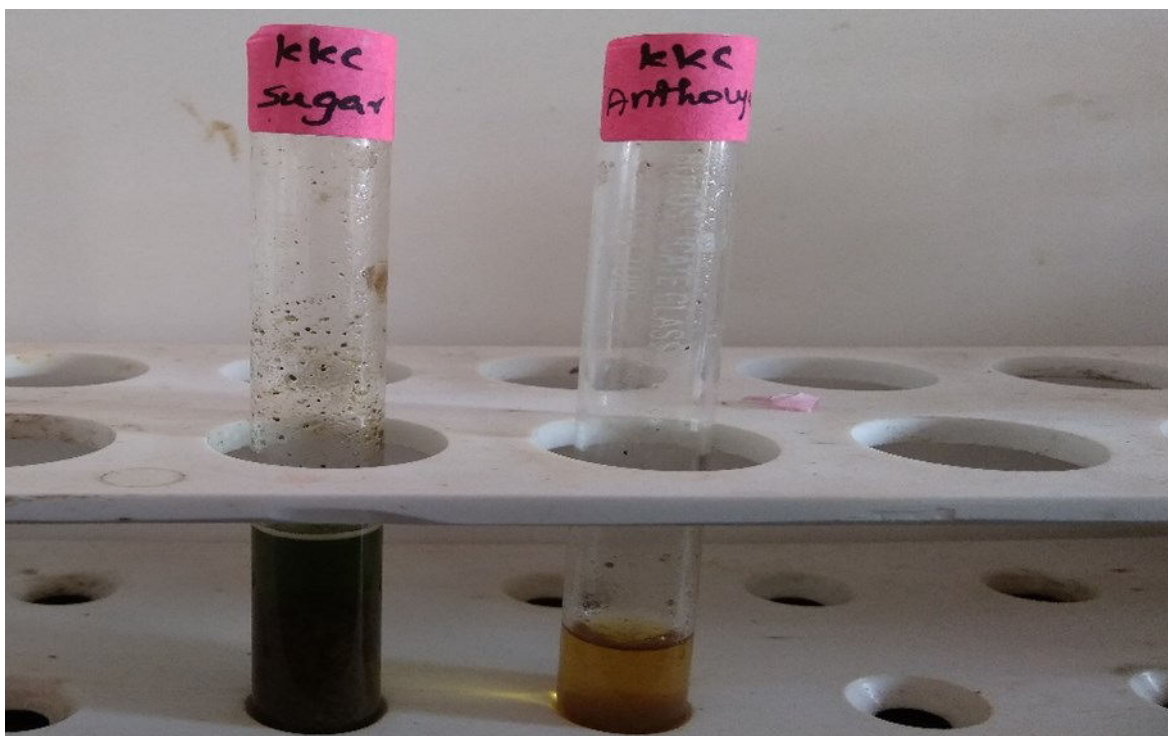


Figure 5: Test for Coumarin, Phenol, Tannins, Saponin and Protein

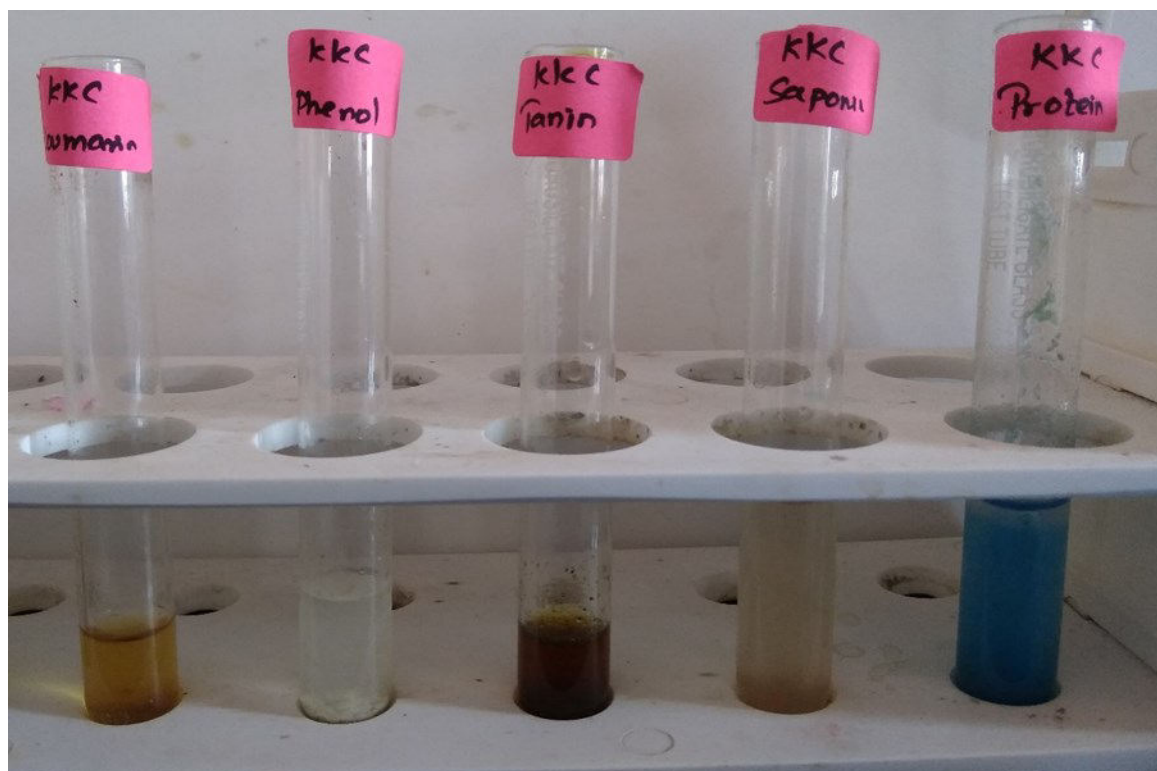


Table 4: Inductively Coupled Plasma Optical Emission Spectrometry Analysis of Kottai Karanthai Chooranam (KKC)

S.NO	ELEMENT NAME	STANDARD VALUE	OBTAINED VALUE
1.	As	188.979	BDL
2.	Ca	315.807	24.150mg/L
3.	Cd	228.802	BDL
4.	Cu	327.393	BDL
5.	Fe	238.204	2.340mg/L
6.	Hg	253.652	BDL
7.	K	766.491	120.821mg/L
8.	Mg	285.213	01.020mg/L
9.	Na	589.592	13.110mg/L
10.	Ni	231.604	BDL
11.	Pb	220.353	BDL
12.	P	213.617	58.541 mg/L
13.	Zn	213.856	01.587 mg/L

Table 5: High Performance Thin Layer Chromatography (HPTLC) Analysis Of Kottai karanthai Chooranam

PEAK	Start	Start	Max	Max	Max	End Rf	End	Area	Area
1.	-0.06	57.7	-0.05	83.1	10.69	-0.04	44.8	784.4	2.78
2.	-0.04	47.3	-0.02	80.9	10.40	-0.00	68.6	1539.6	5.46
3.	-0.00	69.0	0.01	83.0	10.68	0.08	2.1	2484.9	8.81
4.	0.09	3.0	0.18	178.4	22.94	0.25	1.2	8134.3	28.83
5.	0.33	15.0	0.41	47.0	6.04	0.50	10.9	3265.6	11.58
6.	0.59	2.9	0.66	214.9	27.63	0.71	49.5	6912.6	24.50
7.	0.82	58.7	0.89	90.5	11.63	0.94	1.8	5090.3	18.04

Figure 6: High Performance Thin Layer Chromatography (HPTLC) Analysis Of Kottaikaranthai Chooranam

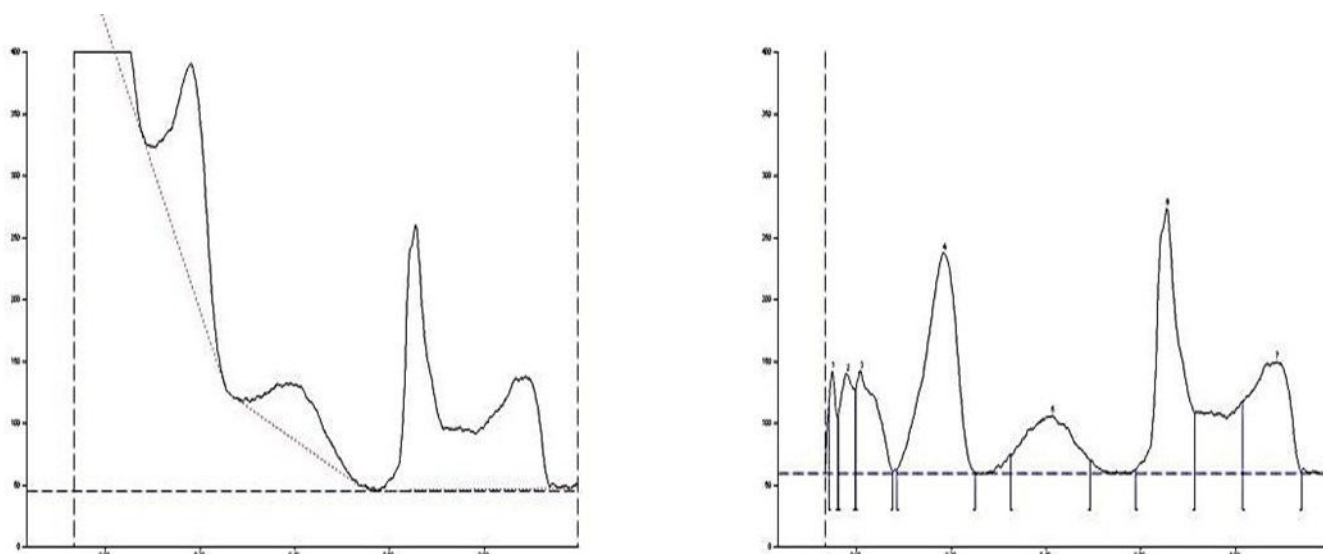


Figure 7: TLC Visualization of KKC - TLC plate visualization at 366 nm



Table 6: Aflatoxin Analysis of Kottai Karanthai Chooranam

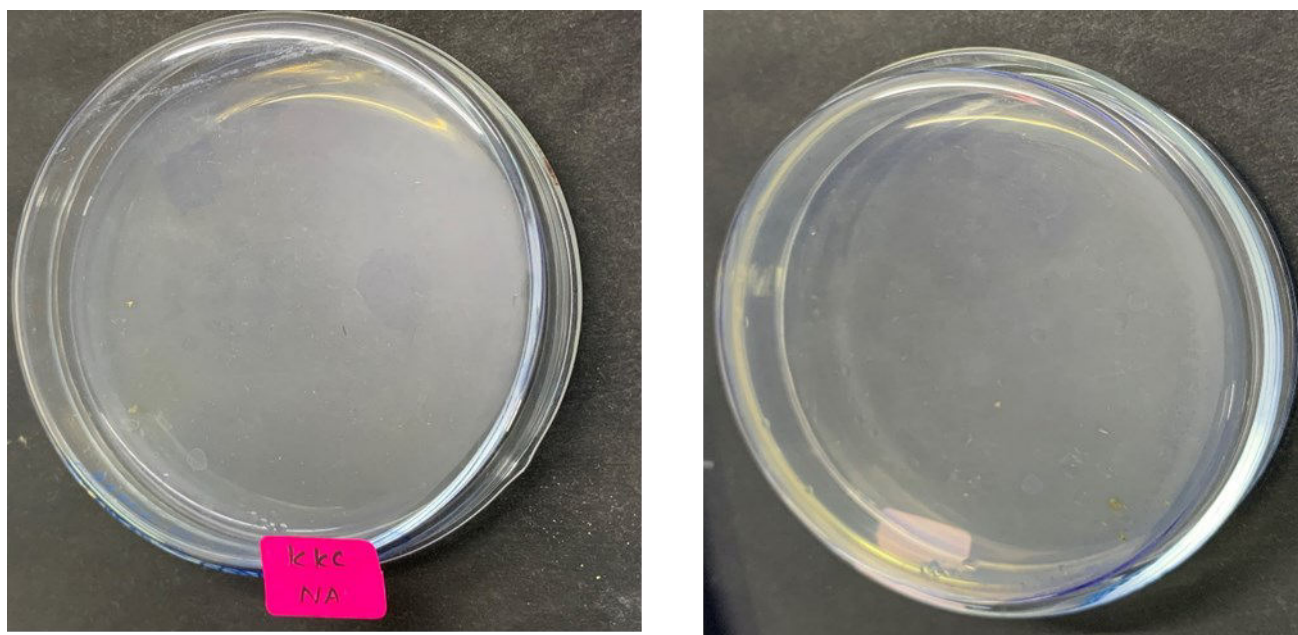
	Kottai Karanthai Chooranam	AYUSH Specification Limit
B1	Not Detected – Absent	0.5 ppm
B2	Not Detected – Absent	0.1 ppm
G1	Not Detected – Absent	0.5 ppm
G2	Not Detected – Absent	0.1 ppm

Table 7: Pesticide Residue

PESTICIDE RESIDUE	SAMPLE KKC	AYUSH LIMIT (mg/kg)
I.Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg
Beta BHC	BQL	0.1mg
Gamma BHC	BQL	0.1mg
Delta BHC	BQL	0.1mg
DDT	BQL	1mg/
Endosulphan	BQL	3mg/ kg
II.Organophosphorus Pesticides		
Malathion	BQL	1mg/
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/
III. Organo carbamates		
Carbofuran	BQL	0.1mg
III.Pyrethroid		
Cypermethrin	BQL	1mg/ kg

Table 8: Sterility Test

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10 ³ CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10 ³ CFU/g	

Figure 8: Sterility test for Pour Plate Method**Table 9: Test for Specific Pathogen**

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH Specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

CONCLUSION

For the quality assured herbal product, the standardization is required. Standardization of *Kottai Karanthai Chooranam* (single herbal formulation) has been carried out according to WHO guidelines and standard procedures. In standardization, the above-mentioned parameters i.e. authenticity, physical parameter, phytochemical analysis, aflatoxin, pesticide residue, sterility test, specific pathogen test and heavy metal analysis gives the quality for *Kottai Karanthai Chooranam*. HPTLC tool is mostly used for identification of the compound of *Kottai Karanthai Chooranam*. These parameter results are sufficient to authenticate and standardize *Kottai Karanthai Chooranam*. The result obtained from *Kottai Karanthai Chooranam* could be utilized as reference for developing standard formulation of great efficacy.

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