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PHYTOCHEMICAL STANDARDISATION OF KAICHUKKATTAI CHURANAM – A SIDDHA COMPOUND FORMULATION

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ABSTRACT

One of the major bottlenecks in the wider acceptance of herbal drugs from developing countries is the inadequacy or lack of standardization which embodies the total information and the controls that are necessary to guarantee consistency of composition of the product ensuring their quality. Working with standardized material will be helpful for scientists and doctors for obtaining genuine drug and authentically prepared compound formulations and thus standardisation research studies occupies an important place in both clinical and drug research programmes. The aim of the present study is to establish a standard for an important Siddha formulation, Kaichukkattai churanam using physico-chemical and phytochemical parameters and to detect Catechin as marker compound. The study also aims to evaluate the shelf-life period of the formulation. The physico-chemical characteristics of the drug like loss on drying at 105˚C, ash value, acid insoluble ash, water soluble ash, solubility in water & alcohol and pH of water extract were determined. The fluorescent studies and preliminary phyochemical analysis were carried out. HPTLC photodocumentations, 3D densitometric chromatograms and fingerprinting profiles were documented. Catechin was isolated as marker compound from the formulation and HPTLC profile of the churanam with marker compound was documented. Shelf-life period of the churanam was determined as per ICH guidelines. The parameters obtained during the analysis of the formulation serves the purpose of reasonable and dependable standards for this formulation. Catechin was identified as marker compound and shelf-life period was found to be 1.62 years. The observations laid down a platform for the standardization and will help us to determine the genuineness of Kaichukkattai churanam. Standardization ensures the quality of medicines and gives authenticity to the medicines prepared by the manufacturers, prescribing physicians and consumers and the results obtained can be used to check and ensure the quality of the medicine.

KEYWORDS: Kaichukkattai churanam, Standardisation, Pharmacopoeial, HPTLC studies, Marker compound, Shelf-life studies.

INTRODUCTION

Indigenous systems of medicine, which are believed to be one of the most ancient, are well organized traditional health-care for rural as well as urban populations in India. Plant materials are used throughout the world as home remedies and raw materials for the pharmaceutical industry. But there is no uniformity seen in the aspects of quality, efficacy and drug safety in the manufacturing practices. Moreover physical, chemical and biological variations affect the drug efficacy. It is therefore essential to ensure the quality of medicinal plant materials and formulations. So standardisation research studies occupies an important place in both clinical and drug research programmes since it provides approach data for obtaining genuine drug and authentically prepared compound formulations.

Pharmacopoeial standards are important and are mandatory for the implementation of the drug testing

provisions under the Drugs and Cosmetic Act, 1940 and Rules there under. These standards are also essential to check samples of drugs available in the market for their safety and efficacy.

The World Health Organization (WHO), in a number of resolutions, emphasized the need to ensure quality control of herbal drugs by applying suitable standards including modern techniques and has published "Quality Control Methods for Medicinal Plant Materials"[1]. Indian System of Medicine deals with both preventive and curative aspects of life in a most comprehensive way and presents a close similarity to the WHO's concept of health propounded in the modern era. The rich biodiversity of India present a unique repertoire. Considering biodiversity as unlimited, the native population plunders it, particularly in the case of medicinal plants where there is a spurt in demand. The large numbers of industries continue their indiscriminate use of precious medicinal plants and this exhausts the plant stock. Export of raw drugs is also going up every year. The scarcity of the medicinal plants leads to the problem of adulteration or substitution of drugs. Genuineness or authenticity of any herbal drug needs to be standardized using approved parameters.

In this paper, an attempt has been made to standardize an important Siddha formulation Kaichukkattai churanam following WHO and FDA guidelines. Kaichukkattai churanam is an important widely used Siddha drug particularly for disorders of digestive system. Churanam is a fine powder of one or more drugs. Kaichukkattai churanam is taken up with the purpose of laying down the pharmacopoeial standards for quality control. In the present study, different parameters like Physico-chemical parameters, High Performance Thin Layer Chromatogram (HPTLC), Identification of marker compound and the shelf-life period of the formulation were followed for the purpose of standardisation.

MATERIALS AND METHODS

1. Reagents and Chemicals

All the reagents, chemicals and solvents used were of GPR grade.

2. Preparation of Kaichukkattai churanam

The churanam was prepared by the Department of Pharmacy, Siddha Central Research Institute, Chennai by the method described in Siddha Formulary of India Part II. The ingredients of the formulation are Kaichukkattai (*Acaia catechu* Willd., stem extract – 3 parts), Ilavangapattai (*Cinnamomum zeylanicum* Blume, Stem bark - 3 parts), Kirampu (*Syzygium aromaticum* (Linn.) Merr. & L. M. Perry, Flower bud - 2 parts) and Catikkai (*Myristica fragrans* Houtt., kernel – 1 part). The ingredients are properly cleaned, dried, finely powdered and sieved separately. They are accurately weighed and then all mixed together. The powder is fine to the extent of at least 80 µm mesh. The finer powder has better therapeutic value. The churanam prepared is stored in the air tight containers. Kaichukkattai churanam and its ingredients are given in Fig. 1.

Fig. 1: Kaichukkattai churanam and its ingredients.

3. Organoleptic evaluation
Organoleptic evaluation refers to the assessment of formulation by colour, odour, taste, texture etc. The organoleptic characters of the sample were carried out based on the method described by Siddique et al.[2]

4. Determination of physico-chemical parameters
The physico-chemical analysis such as determination of loss on drying at 105°C, total ash content, acid insoluble ash, extractable matter in water and alcohol and pH of water extract were carried out by standard methods.[1,3]

5. Preliminary phytochemical analysis
For preliminary phytochemical studies, 5g of drug was successively extracted using Soxhlet apparatus with petroleum ether, chloroform, ethanol and water. The extracts were concentrated by distilling off the solvents under reduced pressure. The presences of different phytoconstituents were determined by standard procedure.[4,5] The qualitative chemical tests were carried out for identification of the nature of different phytoconstituents present in the formulation.

6. Fluorescence analysis
One mg of the churanam was treated with petroleum ether, benzene, acetone, ethyl acetate, ethyl alcohol, methyl alcohol and distilled water and then fluorescence characters were detected in visible, short UV and long UV light.[6]

7. Development of High Performance Thin Layer Chromatographic (HPTLC) profile
Preparation of the extract
The chloroform extract of the churanam was used for HPTLC studies. 1 gm of the churanam was extracted in 10 ml chloroform. This solution was used for HPTLC.

HPTLC Instrument
The extract was applied as bands on the plate with Camag microlitre syringe attached with Automatic TLC Sampler 4 (ATS4). The TLC plate used was aluminium sheet precoated with silica gel 60 F_{254}. The Camag twin trough chamber was used for developing the plate. Camag visualiser was used for photodocumentation. Camag TLC scanner installed with WINCATS software was used for fingerprint development in the UV and visible region after derivatisation with vanillin-sulphuric acid.

Procedure
10µL and 15 µL each of the extract was applied on the TLC plate as 10 mm bands in two tracks of 10 mm distance. The plate was developed in the solvent system, Toluene; Ethyl acetate (5:1). The developed plate was air dried and visualized under UV 254 and 366 nm. The TLC chromatograms were documented. Then the plate was scanned under UV 254 and 366 nm. The fingerprints were recorded. The plate was then derivatised using vanillin- sulphuric acid. The chromatograms under white light and fingerprint profile at 575 nm were recorded.

8. Isolation of Catechin from Kaichukkattai churanam
10 gm of the churanam was taken in a 500 ml beaker containing 100 ml distilled water. It was boiled with constant stirring for complete dissolution and filtered through a filter paper. Then it was evaporated to 50 ml and allowed to stand for 24 hours. The obtained precipitate was filtered using a filter paper. The aqueous filtrate was discarded. The residue was dissolved in ethanol and filtered. The ethanolic extract was evaporated to dryness and the residue was dissolved into hot water (500 ml). It was allowed to stand for 24 hours. The precipitate was filtered and dried in air. The process of re-crystallization from water was repeated thrice.

9. Confirmation of the isolated compound as Catechin
The identity of the compound isolated as Catechin was established by using melting point, specific colour reactions in ethanolic solution, Colour reactions of the compound on paper, elemental analysis, determining UV absorption maxima in methanol and IR spectral properties.

Determination of melting point
The melting points were determined in open capillaries and are uncorrected.

Colour reactions in ethanolic solution
Colour reactions were carried out by adding the reagents to an ethanolic solution of the compound to confirm the class of compound.[7]

Colour reactions of the compound on paper
The paper chromatography of the compound in six different solvent systems -BAW, PhOH, t-BAW (t-butanol : aceticacid: water, 3:1:1), 15% HOAc, 50% HOAc and water- was carried out using Whatmann No.1 sheets. The spot of the compound on a paper chromatogram was observed in visible and UV light by fuming or spraying with suitable reagents.[7]

UV spectral analysis
UV absorption was recorded in spectroscopic grade methanol in a Varian, CARY 100 BIO UV-Vis Spectrophotometer.

IR spectral analysis
The IR spectrum was recorded in KBr.

10. Identification of Catechin as Marker compound
An HPTLC method for identifying the isolated compound Catechin as marker compound was established in the alcohol extract of Kaichukkattai churanam. The mobile phase used was Toluene: Ethyl acetate (1:4) and the volume applied for Churanam was 18 µl and Catechin15 µl. The developing reagent used was vanillin-sulphuric acid. The experiment using HPTLC instrument was conducted as discussed earlier.

11. Stability study

Stability study of the churanam was conducted to determine the shelf- life period.[8] The samples were analysed by determining the Physico-chemical parameters in the 0^{th}, 1^{st}, 3^{rd} and 6^{th} months. Five containers of the churanam of 50 g each were packed and stored well. Samples were withdrawn at the intervals of 0, 1, 3, and 6 months. Basic analytical parameters including loss on drying at 110°C, ash values, pH value, water soluble extractives and alcohol soluble extractives were evaluated at regular intervals.

Based on the values obtained at different stages; intercept, slope, expected time (in months) for 10% of degradation were calculated for individual parameters of the formulation. As India falls in Zone III; the mean obtained of these months was multiplied with 3.3 to extrapolate shelf-life period.

RESULTS

The organoleptic characters were found to be brown fine powder with pungent taste and odour.

The Physico-chemical parameters obtained for Kaichukkattai churanam are given in Table 1.

Table 1: Physico-chemical parameters of Kaichukkattai churanam.

Sl. No.	Parameter	Result
1.	Loss on Drying at 105°C %	8.65
2.	Total Ash Content %	5.09
3.	Acid Insoluble Ash %	0.86
4.	Water Soluble Extractive %	29.09
5.	Alcohol Soluble Extractive %	30.36
6.	pH of water extract	7.95

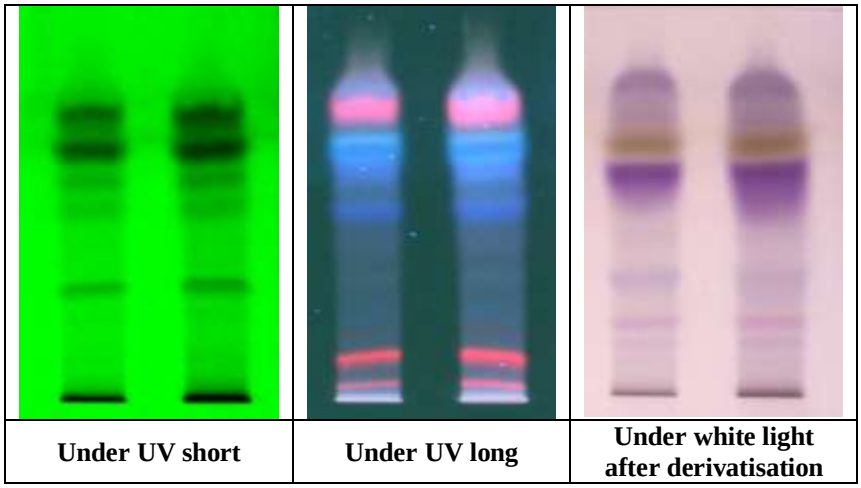
The preliminary phytochemical analysis of Kaichukkattai churanam showed the presence of Alkaloids, Anthracenes, Cardiac glycosides, Flavonoids, Glycosides, Lignins, Lipids, Phenols, Proteins, Quinones, Starch, Steroids, Sugars, Tannins and Terpenoids.

Fluorescence behaviour of different extracts of Kaichukkattai churanam in visible, UV short and UV long lights are given in Table 2.

Table 2: Fluorescence behaviour of different extracts of Kaichukkattai churanam.

Sl. No.	Extractives	Source of light		
		Visible light	Short UV	Long UV
1	Petroleum ether	Brown	Greenish brown	Greenish brown
2	Benzene	Colourless	Light yellow	Colourless
3	Acetone	Colourless	Light yellow	Colourless
4	Ethyl acetate	Light blue	Light yellow	Light blue
5	Ethyl alcohol	Light Brown	Bluish brown	Brown
6	Methyl alcohol	Brown	Yellowish brown	Bluish brown
7	Distilled water	Light Brown	Light brown	Brown

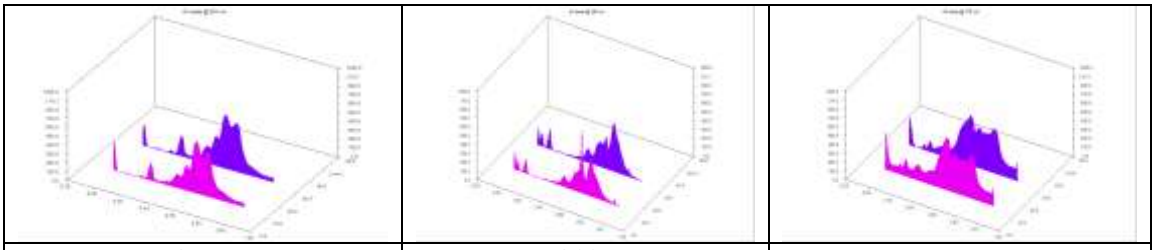
The HPTLC study of the chloroform extract of the plant material was carried out. The mobile phase used was Toluene: Ethyl acetate (5:1) and the volume applied was 10 µl in Track 1 and 15 µl in Track 2. The plates were viewed under UV short, UV long and developed in vanillin-sulphuric acid reagent. HPTLC profile of Kaichukkattai churanam is given in Figure 2.



Under UV short	Under UV long	Under white light after derivatisation

Fig. 2: HPTLC photodocumentation profiles of the chloroform extract of Kaichukkattai churanam.

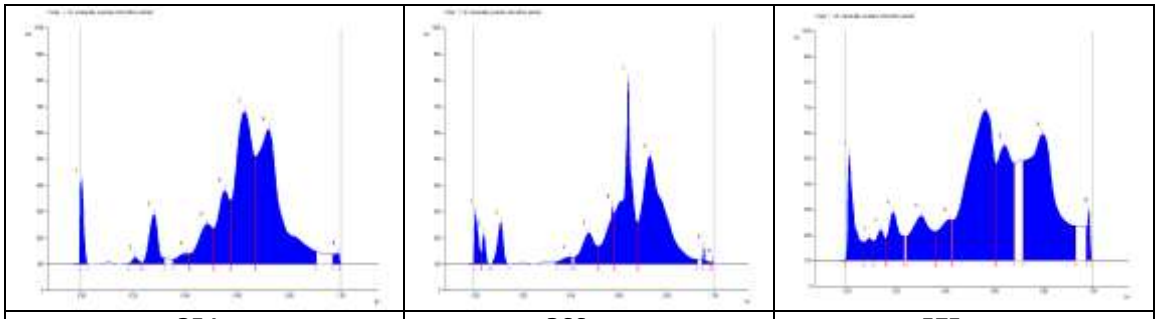
The 3D densitometric chromatogram of 10 µl and 15 µl of chloroform extract of Kaichukkattai churanam is given in Fig.3.



at 254 nm	at 366 nm	at 575 nm

Fig. 3: 3D densitometric chromatogram of 10 µl and 15 ml of chloroform extract of Kaichukkattai churanam.

The HPTLC fingerprinting profiles of 10 µl of chloroform extract of Kaichukkattai churanam is given in Fig.4 and R_f values of major compounds are given in Table 3.



at 254 nm	366 nm	575 nm

Fig. 4: HPTLC fingerprint profiles of 10 ml chloroform extract of Kaichukkattai churanam.

Table 3: HPTLC data of 10 µl chloroform extract of Kaichukkattai churanam showing R_f values and peak areas of major compounds.

Sl.No.	254 nm		366 nm		575 nm	
	R_f value	Area (%)	R_f value	Area (%)	R_f value	Area (%)
1.	0.00	3.60	0.00	3.63	0.01	5.30
2.	0.21	0.52	0.04	1.88	0.09	1.38
3.	0.28	5.36	0.11	4.43	0.14	2.14
4.	0.41	1.47	0.39	1.28	0.19	4.36
5.	0.49	7.22	0.48	7.75	0.30	7.14
6.	0.55	10.64	0.58	8.44	0.42	4.14
7.	0.63	31.98	0.64	28.61	0.56	31.20
8.	0.72	38.42	0.73	43.18	0.64	13.44
9.	0.99	0.79	0.96	0.72	0.80	29.71
10.			0.99	0.10	0.99	1.21

Catechin was isolated from Kaichukkattai churanam by the methods given in Materials and methods. The melting point of isolated Catechin was 175-6^{0}C. The identity was confirmed by direct comparison (m.m.p) with authentic sample. Mixed melting point with an authentic sample did not show lowering.

Colour reactions of the compound in ethanolic solution is given in Table 4, Colour reactions of the compound on paper in Table 5, the results of elemental analysis in Table 6 and IR spectrum in Figure 5.

Table 4: Colour reactions of the compound in ethanolic solution.

Aq. NaOH	Con. H_2SO_4	Mg-HCl (Shinoda test)	Na/Hg and HCl
Yellow changing to red and brown	Red	None	None

Table 5: Colour reactions of the compound on paper.

Reagent Light	None visible	None UV	NH_3 visible	NH_3 UV	$AlCl_3$ visible	$AlCl_3$ UV	Na_2CO_3 visible	$NaHBO_4$ visible	$ArSO_3H$ visible
Compound	Colour less	Colour less	Colour less	fluorescent pale blue black	Colour less	Colour less	Colour less	Colour less	brown

Table 6: Elemental Analysis (CHNS Analysis) of the Compound.

Sl. No.	Element	Percentage (%)
1.	Carbon (C)	54.32
2.	Hydrogen (H)	4.33
3.	Oxygen (O)	41.35
4.	Nitrogen (N)	Not Detected

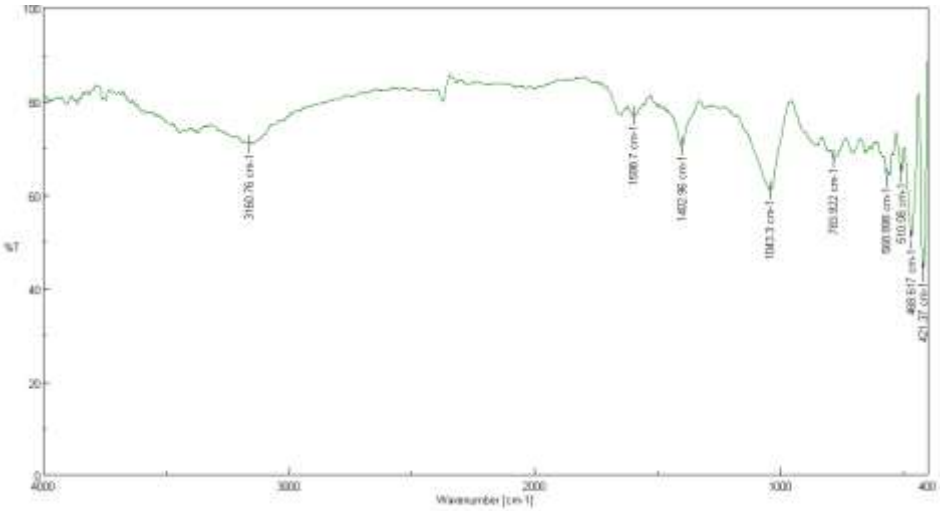


Fig. 5: IR spectrum of Catechin.

The IR spectral result is as follows:
IR (ν_{max}, KBr): 3160, 1598, 1402, 1043, 783 cm^{-1} (Figure 6).
The spectra and other results confirm that the compound isolated is Catechin and the chemical structure of Catechin is given in Figure 7.

The UV spectral result is as follows:
UV (λ_{max}, nm) in methanol - 220 nm and 277 nm

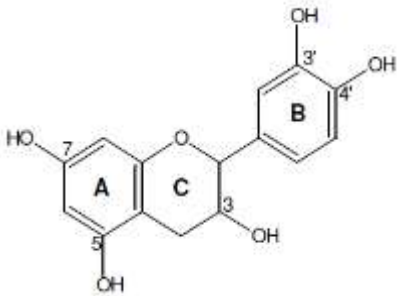


Fig. 6: Chemical structure of Catechin.

Catechin (Fig. 6) was isolated and characterised from the aqueous plant extract as discussed earlier. An HPTLC study of the alcohol extract of the isolated Catechin was carried out along with the alcoholic churanam extract on silica gel G 60 F_{254} precoated aluminium sheet using Toluene: Ethyl acetate (1:4) as the mobile phase.18 µl of churanam extract was spotted on Track 1 and 15 µl Catechin dissolved in alcohol was spotted on Track 2. HPTLC photodocumentation is given in Fig.7 and the R_f values and colour observed are given in Table 7.Catechin was detected as a brown spot with R_f value 0.29 (Table 7) when the chromatogram was observed under white light at 575 nm after derivatisation using vanillin-sulphuric acid.

Fig. 7: HPTLC photodocumentation profies of the alcohol extract of Kaichukkattai churanam and Catechin under white light at 575 nm after derivatising using vanillin- sulphuric acid.

Table 7: R_f values at 575 nm and colour observed for Kaichukkattai churanam and Catechin.

Sl. No.	Kaichukkattai churanam			Catechin	
	R_f value at 575 nm	Colour observed	Area %	R_f value at 575 nm	Colour observed
1.	0.16	Light brown	1.05	--	--
2.	0.29	Brown	14.03	0.29	Brown
3.	0.42	Light brown	1.88	--	--
4.	0.49	Light purple	1.62	--	--
5.	0.79	Purple	25.66	--	--
6.	0.95	Light purple	4.38	--	--

The data for calculating the real time shelf-life studies are given in Table 8.

Table 8: Shelf-life studies of Kaichukkattai churanam.

Sl. No.	Test	Result			
		0^{th} month	After 1 month	After 3 months	After 6 months
1	Loss on drying at 105˚C (%)	8.65	8.78	9.00	11.24
2	Total ash (%)	5.09	4.90	4.85	4.57
3	Acid insoluble ash (%)	0.86	0.85	0.80	0.68
4	Water soluble extractives (%)	29.09	29.00	28.25	27.80
5	Alcohol soluble extractives (%)	30.36	29.00	28.51	27.58
6	pH of water extract	7.95	7.50	6.92	6.51

The real time shelf-life study was conducted considering of 10 % degradation rate in physico-chemical parameters and found that the stability period for the churanam was 1.62 years. No considerable change was observed in organoleptic characters even after 6 months.

DISCUSSION

The organoleptic and physico-chemical parameters help to a great extent for the purpose of standardization. Deterioration time of the formulation depends on the amount of water present in it. If the water content is high, the drug can be easily deteriorated due to contamination by fungal colonies.[9] The loss on drying determined at 105°C was found to be 8.65 % (Table 1).This shows that the raw materials used for the preparation of formulation was dried properly. Ash values are a significant pharmacognostic tool which aids to decide quality and purity of crude drugs. The ash content is the residue remaining after incineration, which represents the inorganic constituents present in the drug. The total ash includes both physiological ash which is derived from the plant tissue itself and non-physiological ash, which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash measures the amount of silica present as sand and silicaceous earth. The total ash value (Table 1) obtained shows the amount of inorganic contents in the drug and lesser acid-insoluble ash indicates the purity of the drug. The Extractive values such as water soluble extractive and alcohol soluble extractive indicate the amount of chemical constituents present and are useful for the determination of exhausted drugs and is an important tool to check quality of the drug (Table 1). The pH of water extract shows the acidity or alkalinity of the drug. The pH value obtained is 7.95, which shows that the water extract is slightly alkaline.

Preliminary phytochemical analysis reveals that the drug contains so many phytochemicals and the medicinal effect of the drug may be due to the synergistic effect of these chemical constituents. Fluorescence analysis of the drug treated with different solvents helps to detect various chromophores present in the test drugs. Fluorescence behaviour of the drug in different solvents were observed under day light, short UV (254 nm) and long UV (366 nm). The extracts showed different fluorescent properties (Table 2). Colour variation was observed can be used as a standard parameter for quality control of the drug.

HPTLC is a valuable quality assessment tool for the chemical evaluation of herbal products. HPTLC patterns show separation of compounds present in the chloroform extract of the formulation. HPTLC fingerprint enables a particular drug to be identified and this method may be applied to identify the drug. The results of the HPTLC studies of the chloroform extract of the drug (Figure 2, 3 and 4) serve an important parameter for quality assessment of the drug and the HPTLC finger printing obtained serves as a chemical standard to check the quality of the formulation. HPTLC fingerprint of Kaichukkattai churanam at 254 nm revealed 9 spots, at 366 nm 10 spots and after derivatisation using vanillin-sulphuric acid reagent at 575 nm 10 spots (Table 3). Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters. HPTLC is one of the sophisticated instrumental techniques for qualitative and quantitative analysis of the herbs and herbal drugs. Hence this method serves the purpose of standardisation to a great extent.

Catechin was isolated from Kaichukkattai churanam by the methods given in Materials and methods. Colour reactions in ethanolic solution (Table 2) and on paper (Table 3) showed that the compound is Catechin. Elemental analysis (Table 4) showed that the molecular formula is $C_{21}H_{20}O_{12}$ and molecular mass is calculated as 464. Many functional groups can be identified by their characteristic vibration frequencies and hence the IR spectrum is the simplest and often a diagnostic method of assigning a compound to its class. IR spectroscopy is used as a 'fingerprinting device' for comparing a compound with its authentic sample and hence has much importance in the complete identification of the compound. The UV (λ_{max}, nm) in methanol showed maxima at 220 nm and 277 nm confirms that the compound is Catechin.

Catechin, the marker compound isolated from the formulation, is a medium polar compound and hence was not moved in less polar solvent system such as Toluene; Ethyl acetate (5:1). Hence other solvent systems were tried and Toluene: Ethyl acetate (1: 4) was found to be suitable. But in this solvent system majority of spots observed in the fingerprints are missing. Hence the HPTLC results obtained in both the solvent systems together may be utilised for quality assessment of the churanam.

Marker compound means chemical constituents within a medicinal plant that can be used to verify its potency or identity. Sometimes, the marker compounds may be described as active ingredients or chemicals that confirm the correct botanical identity of the raw material. A chromatographic fingerprint of a herbal medicine is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and/or chemical characteristics. By using chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of drug. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic component of the herbal drug.

The spot corresponding to Catechin was found in Kaichukkattai churanam also at R_f value = 0.29 and the peak area was found to be 14.3 % suggesting that Catechin is one of the major components present in the churanam. These results show that Catechin may be used as a marker compound for Kaichukkattai churanam.

To determine the shelf-life period, the physico–chemical parameters were determined in the 0^{th}, 1^{st}, 3^{rd} and 6^{th} months (Table 8). Stability study is aimed at assuring that the product remains within specifications established to ensure its identity, strength, quality and purity. It can be interpreted as length of time under specific conditions and storage that a product will remain within the pre-defined limits for all its important characteristics. The main purpose of conducting stability testing of pharmaceutical products is to ensure the efficacy and quality of active compounds in product and to establish the shelf-life or expiration period as well as to support the label claim. The stability data on any dosage form includes selected parameters that together form the stability profile. This stability profile is the basis for assigning the storage conditions and shelf-life to pharmaceutical products. The design of the stability study for the finished product should be based on the knowledge of the behavior and properties of the drug substance and the dosage form[10,11,12]. As per ICH guideline, countries comes under climatic zones I and II having climatic condition 21°C/ 45% RH and 25°C/60% RH respectively. Countries comes under climatic zones III and IV having climatic condition 30°C/35% RH and 30°C/70% RH. India comes under climatic zone III & IV[8]. The real time shelf-life study for the churanam was found to be 1.62 years for in our country which comes under climatic zone III & IV. No considerable change was observed in organoleptic characters and microbial load even after 6 months.

CONCLUSION

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is an important parameter of herbal drug standardization for the proper identification of medicinal plants. The present HPTLC fingerprinting profiles along with Physico-chemical parameters, catechin as marker compound and the shelf-life period of the formulation may be utilised as diagnostic tools to identify and to determine the quality and purity of the formulation in future studies.

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