



Physicochemical And Phytochemical Evaluation Of The Drug Kamalai Kiyazham – A Siddha Polyherbal Formulation

M. Archana¹, M. D. Saravanadevi²,

¹PG Scholar, Postgraduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-106, Tamilnadu, India.

²Professor, Head of the Department, Postgraduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-106, Tamilnadu, India.

Abstract

Siddha system of medicine is the unique system of medicine which contains various medicines both Externally and Internally for treatment and management. Most of the traditional systems of medicine are effective but there is a lack of Standardization. So there is a need to develop standardization technique. Any drugs should be standardized before clinical use. The aim of this study to estimate quality of Kamalai Kiyazham (KK) by conducting physicochemical analysis and phytochemical analysis. physicochemical properties such as loss on drying, total ash value, acid insoluble ash value, water soluble ash value and extractive values were carried out. The phytochemical properties such as alkaloids, flavonoids, tannins, triterpenes, steroids, Saponins and glycosides were also carried out. The results obtained after physicochemical analysis of test drug showed 4.66 plus/minus 0.60% of Loss on drying at 105 dec C , 3.36 plus/minus 0.40 % of Total Ash, 0.058 plus/minus 0.011 % of Acid insoluble ash etc. The pH value of the drug 6.8 which indicates it is acidic. The results obtained after preliminary phytochemical analysis confirmed the presence of alkaloids, flavonoids, glycosides ,steroids, triterpenoids, phenols, tannins, saponins and sugar.. The present study provides the details physicochemical and phytochemical properties of kamalai kiyazham which are useful in laying down standardization and pharmacopeia parameters. This study was done based on Pharmacopoeial laboratory for Indian Medicine guidelines.

Key words: *Siddha* medicine, kamalai kiyazham, Jaundice, Physicochemical analysis, phytochemical analysis.

Introduction

The *siddha* system of medicine is considered as one of the Traditional systems in India having closely embedded with south Indian culture. *Siddhars* were the masters in natural chemistry and formulated medicinal preparations utilizing various natural resources such as plants, animals and minerals. In *siddha* medicine there are 32 types of internal medicine and 32 types of external medicine. Kudineer (decoction) is one among the 32 types of internal medicine⁽¹⁾. Kudineer is known with other names like kiyazham, kasayam, marunuthneer , guvatham and unneer⁽²⁾. The life span of Kiyazham is 3hours, hence it will be effective at freshly prepared state⁽³⁾. Decoctions are easily diffused and entered into blood circulation rather than any other medications. In order to prepare decoctions without difficulty in sourcing raw material premixed coarse powder of the kudineer formulations are available as kudineer chooranam⁽¹⁾. Kamalai kiyazham is a poly-herbal formulation mentioned in the classical Siddha literature for the medication of kamalai (jaundice)⁽⁴⁾. Kamalai also known as icterus, is a yellowish pigmentation of the skin and sclera due to high bilirubin levels. The most common symptoms of jaundice are itchiness, pale faces and dark urine⁽⁵⁾. The mortality rate for Liver disease shows 2 million deaths per annum and it accounts for 4% of all deaths (across worldwide 1 out of every 25 deaths); approximately two-third of all liver-related deaths occur in

men⁽⁶⁾. As per World Health Organization (WHO), more than 80% of global population uses plants or their products as the primary source of medicinal agents⁽⁷⁾.

Kudineer chooranam is referred to the coarse powder prepared by a single or a combination of two or more medicinal plant ingredients. It is simple and affordable. Kiyazham is the most efficient dosage form of medication in the Siddha system of medicine. Decoction are made by pouring water to dry herbal /plant parts or fresh ones and then dehydrated so that the water content is greatly reduced to 1/16th or 1/8th or 1/4th or 1/2th of its initial volume⁽⁸⁾. Thus, the present study deals with standardization of Siddha herbal formulation, Kamalai Kiyazham a Siddha drug mentioned in the text anubava vaithiya deva ragasiyam which is used to treat pandurogam (anaemia) and kamalai (jaundice) and its related symptoms⁽⁴⁾. Till now there is no clear documentation available on standardization and phytochemical investigation aspect of this formulation. This is proved through the systematic standardization of the test drug by physicochemical and phytochemical evaluation according to PLIM guidelines.

2 Materials and methods

Selection of the trial drug

For this present study, the Poly-herbal formulation "Kamalai Kiyazham" a compound drug preparation for Kamalai (jaundice) has been chosen from classical Siddha literature "Anubava Vaithiya Deva Ragasiyam." Publisher: J.Seetharam prasath, Publication: B.Rathna nayakkar &sons. Thirumagal achagam. Page no: 382⁽⁴⁾

kamalai kiyazham consists of the following ingredients.

Table No:1 Ingredients and Botanical name of Kamalai kiyazham

	Ingredients	Botanical Name	Quantity
1	Kadukkai thol	<i>Terminalia chebula</i>	35 g
2	Nellikai thol	<i>Phyllanthus emblica</i>	35 g
3	Thandrikkai thol	<i>Terminalia bellerica</i>	35 g
4	Vembin pattai	<i>Azadiracta indica</i>	35 g
5	Nilavembu	<i>Andrographis paniculata</i>	35 g
6	Aadadhoda	<i>Justicia adatoda</i>	35g
7	Seenthilkodi	<i>Tinospora cordifolia</i>	35 g

Collection of the drug material

The raw drugs kadukkai (*Terminalia chebula*), nellikkai(*Phyllanthus emblica*), thandrikkai (*Terminalia bellerica*) were bought from authenticated country store in Chennai. Nilavembu(*Andrographis paniculata*), Aadathoda(*Justicia adatoda*) was collected from tirunelveli district, Tamilnadu. Seenthil(*Tinospora cordifolia*),vembin pattai(*Azadiracta indica*) was collected from Tenkasi district of Tamil Nadu.

Identification and authentication of the drugs

All drugs were recognized and authenticated by Botany and Gunapadam (pharmacologist) experts, Government Siddha Medical College, Arumbakkam, Chennai. The identified product samples were maintained in the PG Gunapadam laboratory for future references.

Purification of the drug

All the crude drugs specified here have been purified as per Siddha literature. All impurities such as sand and dust have been removed.

Preparation of the kamalai kiyazham⁽⁴⁾:

All the dried drugs will be taken in an equal quantity.

Then the drugs will be crushed into a coarse powder

Finally the prepared medicines are kept in an air tight package and labelled as KK .30g of prepared chooranam will be added with 240ml of water and heated at low flame till the water condensed to 30ml.

Fig:1



Fig :2



Dosage: 30 ml

Adjuvant: Honey

Indications: Anemia, jaundice

Organoleptic characters

State, nature, smell, touch, flow property, appearance of the drug was noted.

These following studies were done at Noble Research Solutions, Perambur at Chennai.

2.1. Physicochemical Evaluation ⁽⁹⁻¹⁰⁾

Loss on drying

The test drug was accurately weighed 2gm of the KK preparation was placed in a tarred in evaporation vessel. The sample was dried at 105°C for 5 hours and then weighed.

Determination of Total Ash

The test drug KK was accurately weighed 2g in silica cup and heated in an oven at 400 °C until it turns white which shows absence of carbon. The percentage of total ash was calculated based on the weight of the air-dried drug.

Determination of Acid-insoluble ash

The ash obtained by the total ash test was boiled with 25 ml of dilute hydrochloric acid for 6minuts. The insoluble material was then collected in a crucible and washed with hot water and ignited to a constant mass. The percentage of acid insoluble ash was calculated from the weight of air-dried ash.

Determination of Alcohol Soluble Extract

The test sample KK was soaked in 100 ml of alcohol in a closed bottle for 24 hours, shaken frequently for during six hours and left to stand for 18 hours. Filter rapidly, taking care to avoid loss of solvent, evaporate 25 ml of the filtrate to dryness in a tar-based flat-bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extract of the air-dried drug.

Determination of Water Soluble Extract

The test sample KK was soaked in 100 ml of chloroform water in a closed bottle for 24 hours, shaking frequently for six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking care to avoid loss of solvent, evaporate 25 ml of the filtrate to dryness in a tar-based shallow dish and dry at 105°C, to constant weight and weight. Calculate the percentage of water-soluble extract of the air-dried drug.

pH determination

About 5g of the test sample KK was dissolved in 25 ml of distilled water and filtered. The resulting solution was allowed to stand for 30minutes and then the pH was assessed.

Particle Size Determination by Microscopic Method Methodology

Particle size determination was done by optical microscopic method. In which the sample was dissolved in the sterile distilled water (dilution approximately 1/100). The diluted sample was mounted on a slide and fixed in the appropriate position. A light microscopic image was taken with a scale micrometer to obtain the average particle size. At least 30 observations were made to determine the average particle size of the sample.

Solubility test

A pinch of sample was taken in a dry test tube and to it 2ml of the solvent was added and shaken well for about a minute and the results were observed. The experiment was conducted with solvents such as Chloroform, ethanol, water, ethyl acetate, hexane, dimethyl sulphide (DMSO) and the results were observed individually.

2.2 Phytochemical analysis ⁽¹¹⁻¹²⁾

Alkaloid test:

Mayer's Test

To 5ml test sample KK, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Coumarin test:

To 5ml test sample KK, 1 ml of 10% sodium hydroxide was added. The presence of coumarins was showed by the formation of yellow colour.

Saponin test:

To 5ml test sample KK, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation shows the presence of saponins.

Tannin test:

To 5ml test sample KK, Ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

Glycoside test:

Borntreger's Test

Test drug KK was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydro- lysate was subjected to the following tests. To 2 ml of filtered hydro-lysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour shows presence of glycosides.

Flavonoid test:

To 5ml test sample KK about 5 ml of dilute ammonia solution was added followed by addition of few drops of conc. Sulphuric acid. Appearance of yellow colour shows the presence of Flavonoids.

Phenol test:

Lead Acetate test

5ml test sample for KK: 3ml of 10% lead acetate solution was added. A large white precipitate indicated the presence of phenolic compounds.

Steroid test:

2ml of chloroform and a few drops of concentrated sulfuric acid were added to 5 ml of test sample KK. The top layer of the test tube turned red and the sulfuric acid layer was yellow and fluorescent green. This indicated the presence of steroids.

Triterpenoid test:**Liebermann- Burchard test**

5ml of test sample KK was mixed with chloroform solution and a few drops acetic anhydride were added and then mixed well. 1 ml of concentrated sulphuric acid was added from the sides of the test tube, the appearance of a red ring indicates the presence of triterpenoids.

Test for Cyanine's

To 5ml test sample KK, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour shows the presence of anthocyanin.

Carbohydrate test: Benedict's test

0.5 ml Benedict's reagent was added to 5 ml of test sample KK. The mixture is heated in a boiling water bath for 2 minutes. A typical coloured precipitate indicates the presence of sugar.

Protein test: Biuret Test

1ml of 1% copper sulphate and then 5% sodium hydroxide were added to 3 ml of KK extracts; the formation of purple –violet colour indicates the presence of proteins.

3. Results**Organoleptic characters**

The drug KK was coarsely powdered and the results were mentioned in Table 2.

Table No 2: Organoleptic characters

	Specification		
1	State	Solid	Liquid
2	Nature	Coarse Fibrous	Non Viscous
3	Odour	Characteristic	Aromatic
4	Touch	Hard Texture	Non greasy
5	Flow property	Non free flowing	Free flowing
6	Appearance	Brownish	Dark Brownish

Physicochemical parameters

The results for physicochemical analysis were tabulated in Table 3:

Table no 3: Results of physicochemical evaluation of KK

s.no	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	4.66 ± 0.60
2.	Total Ash (%)	3.36 ± 0.40
3.	Acid insoluble Ash (%)	0.058 ± 0.011
4.	Water soluble Extractive (%)	28.2 ± 0.519
5.	Alcohol Soluble Extractive (%)	6.6 ± 0.655
6.	pH	6.8

Solubility profile :

The drug KK for solubility profile was given in table: 4

Table no 4: Solubility Profile

S.No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

Qualitative phytochemical evaluation of KK

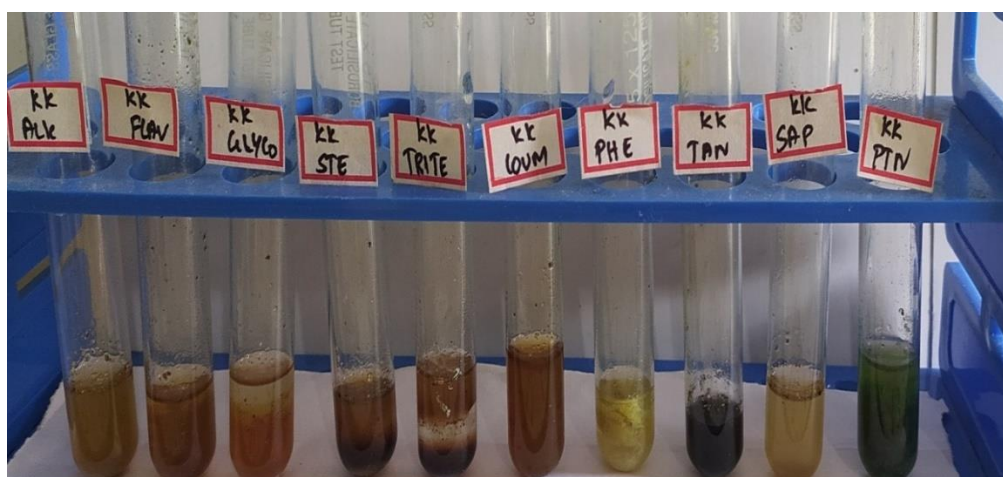
The results for the analysis of phytochemical present in the given sample were mentioned and the outcome was tabulated in table: 5

Tab no 5: Phytochemical analysis of KK

S.NO	TEST	OBSERVATION
1.	ALKALOIDS	+
2.	FLAVANOIDS	+
3.	GLYCOSIDES	+
4.	STEROIDS	+
5.	TRITERPENOIDS	+
6.	PHENOL	+
7.	TANIN	+
8.	SAPONIN	+
9.	SUGAR	+
10.	BETACYANIN	+

(+) -> Indicates Positive and (-) -> Indicates Negative

Figure 3:Qualitative phytochemical investigation



4. Discussion:

Standardization of the drugs is more essential to drive the efficacy, potency of the drug. The standardization of KK was achieved through various procedures like analysis the organoleptic characters, physicochemical characters and phytochemical analysis. The drug KK was coarsely powdered (Fig:1) with hard texture and brownish colour. Fresh preparation (Fig:2) of its extract shows non greasy, dark brown with aromatic odour. Oral bio-availability depends on several factors including aqueous solubility, drug permeability etc. The drug KK soluble in specific solvent like ethanol, water and dimethyl sulfoxide (DMSO). Thereby it proves its efficiency of solubility increasing in bio-availability in the stomach indirectly. The results derived from the physicochemical evaluation divulge that loss on dry in value of KK was 4.66 % which indicates low moisture content could increase the stability and shelf life of the drug which is suitable for medicine preparation. The Total Ash value of KK was 3.36 % which indicates the purity of the drug. Acid insoluble ash value of KK was 0.058 % which ensures the trial is not contaminated with siliceous material like sand, dust. The water soluble extractive value of KK was 28.2 % which represents easy facilitation of diffusion and osmosis mechanism. The alcohol soluble extractive value of KK was 6.6 % which indicates that the drug has good quality, purity and no adulteration. The pH value of KK was 6.8% which indicates that the drug is acidic nature. In oral administration the acidic nature of the drug enhances rapid absorption in the stomach.

The result of the qualitative phytochemical analysis indicates that the formulation KK reveals the presence of alkaloids, flavonoids, glycosides steroids, triterpenoids, phenols, tannins, saponins, sugar and betacyanin. Alkaloids are used as the anti-hepatocarcinogenic effects, through various mechanisms including inhibition of proliferation, metastasis and angiogenesis, changing cell morphology, promoting apoptosis and autophagy, triggering cell cycle arrest, regulating various cancer-related genes as well as pathways and so on⁽¹³⁾. Flavonoids is used as against non-alcoholic fatty liver disease (NAFLD) and its related disorders have been observed in both animal and human studies. Flavonoids prevent hepatosteatosis by increasing fatty acid oxidation in the liver⁽¹⁴⁾. Glycosides, alkaloids and terpenoids are increasingly important for their medicinal value in liver fibrosis therapy⁽¹⁵⁾. Steroids improve survival in fulminant autoimmune hepatitis, drug-induced, or indeterminate ALF, and whether this benefit varies according to the severity of illness⁽¹⁶⁾. Triterpenoids are studied for their antioxidant and anti-inflammatory and regulates apoptosis that attributes its therapeutic effects in numerous diseases. It is used for hyperglycaemia, liver fibrosis, wound healing, cerebral ischemia, dementia, metabolic syndrome and obesity⁽¹⁷⁾. Saponin used in the management of liver disorders⁽¹⁸⁾. The sugars are the carbohydrate which gives instant energy to the body is present in the test drug KK. Numerous plants have been reported to possess hepatoprotective activity due to the presence of phytochemicals like alkaloids, flavonoids, glycosides, tannins, betacyanin and saponins, etc. However the presence of these phytoconstituents hence proved that the trial drug will be effective in treating various disorders.

5. Conclusion

Standardization of Kamalai Kiyazham was done as per PLIM guidelines and standardized procedure. The obtained results of standardization of Siddha herbal formulation KK by different parameters such as organoleptic characters, physicochemical parameters, and phytochemical analysis will be useful as tool for authentication and analysis their safety and quality of herbal drug. These standardization parameters could be considered as a reference standard of this drug for quality control assessment in future.

Acknowledgement:

Authors would like to express our special thanks to The Tamilnadu Dr. M.G.R. Medical University, Chennai and all the faculties of Department of Gunapadam as well as the Principal of Government Siddha Medical College, Chennai, Tamilnadu who gave us the great opportunity to carry out this project on the Standardization of Kamalai Kiyazham. And we also express our special thanks to Nobel research solution, Chennai.

Source of Funding: None

Ethical Approval: Approved

References

1. Thiyagarajan R, Gunapadam thathuvaguppu, Dept. of Indian medicine and homeopathy, 9th edition 2016 .pg:58
2. Devaasirvatham samuvel ,marunthusei eyalum kalaium, indian medicine –homeopathy, 2016.
- 3.Thirunarayanan T, Introduction to Siddha Medicine, Centre for Traditional Medicine &Research, 2016, page,no:122.
- 4.seetharam prasath J,anubava vaithiya deva ragasiyam ,thirumagal achagam,1991.pg no:382.
5. Abbas MW, Shamshad T, Ashraf MA, Javaid R. Jaundice: a basic review. Int J Res Med Sci. 2016 Ma3. Aswini P, Manimekalai J, Devi S, Velpandian V. Standardization and Physico-chemical analysis of classical poly-herbal Siddha formulation-“maaradaippuku chooranam”. International Journal of Pharmaceutics and Drug Analysis. 2022 Jul 7:18-26. y;4(5):1313-9.
6. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. Journal of Hepatology. 2023 Mar 27.
- 7.Manimekalai J. *Preclinical Evaluation of Styptic, Hematinic and Anti-Oxidant activity of Siddha Herbal Formulation “Raja Elathi Chooranam” in Animal Models* (Doctoral dissertation, Government Siddha Medical College, Chennai).
8. Mattummal R, Gopi DK, Parameswaran SR, Narayana SK. Bioactive molecules in siddha polyherbal nilavembu kudineer alleviating symptoms of dengue/chikugunya. Traditional Medicine Research. 2018;3(5):215.
- 9.India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
- 10.Protocol for testing of Ayurvedic siddha and unani medicines[Internet]. Ghaziabad: Department of AYUSH, Pharmacopoeial Laboratory for Indian medicines; 2008. P.49-50. Available from: https://www.researchgate.net/publication/24944109.Protocol_for_Testing_of_Ayurvedic_Siddha_and_Unani_medicines
11. N.Srikanth et al, chemical characterization of an Ayurvedic herbo-mineral preparationMahalakshmi vilasRas, Jour of Ayur and Int Med, vol-10, Issue- 4, oct-dec 2019,pg262- 268
- 12.Brain KR, Turner TD, The practical Evaluation of phyto-pharmaceuticals.Bristol: Wright scientechnica; 1975:36-45
13. Liu C, Yang S, Wang K, Bao X, Liu Y, Zhou S, Liu H, Qiu Y, Wang T, YuH. Alkaloids from traditional Chinese medicine against hepatocellular carcinoma. Biomedicine & Pharmacotherapy. 2019 Dec 1;120:109543.
14. Akhlaghi M. Non-alcoholic fatty liver disease: beneficial effects of flavonoids. Phytotherapy Research. 2016 Oct;30(10):1559-71.
15. Ma X, Jiang Y, Wen J, Zhao Y, Zeng J, Guo Y. A comprehensive review of natural products to fight liver fibrosis: alkaloids, terpenoids, glycosides, coumarins and other compounds. European Journal of Pharmacology. 2020 Dec 5;888:173578.
16. Karkhanis J, Verna EC, Chang MS, Stravitz RT, Schilsky M, Lee WM, Brown Jr RS, Acute Liver Failure Study Group. Steroid use in acute liver failure. Hepatology. 2014 Feb;59(2):612-21.
17. Nagoor Meeran MF, Goyal SN, Suchal K, Sharma C, Patil CR, Ojha SK. Pharmacological properties, molecular mechanisms, and pharmaceutical development of asiatic acid: a pentacyclic triterpenoid of therapeutic promise. Frontiers in pharmacology. 2018 Sep 4;9:892.
18. Chaudhary J, Jain A, Dahiya R. Saponins in Management of Hepatic Disorders: A Review. The Natural Products Journal. 2022 Jun 1;12(3):2-12.