

Biochemical, Physicochemical, Preliminary Phytochemical Evaluation of *Kadukkai Poo Chooram*

Rishipriya. C. R¹, A. Manoharan², P. Sathish Kumar³

¹PG Scholar, Department of Pothu Maruthuvam, Government Siddha Medical College and Hospital,
Palayamkottai, Tirunelveli - 627002, Tamilnadu, India

²Professor and HOD, Department of Pothu Maruthuvam, Government Siddha Medical College and Hospital,
Palayamkottai, Tirunelveli – 627002, Tamilnadu, India

³Lecturer, Department of Pothu Maruthuvam Government Siddha Medicine College and Hospital,
Palayamkottai, Tirunelveli -627002, Tamilnadu, India

Corresponding Author: Rishipriya. C. R

DOI: <https://doi.org/10.52403/ijrr.202308115>

ABSTRACT

Background: *Kadukkai Poo Chooram* is a poly herbal formulation in Siddha system of medicine mainly indicated for the management of bleeding hemorrhoids. According to Siddha system, medicine is a substance that helps to alleviate or eradicate the disease, gives strength to the body and normalizes the functions of the body. In Siddha system mainly the herbal formulations gained worldwide attention due to its long-term benefits in terms of overall wellness with lesser side effects. But there is striving for global acceptance due to lack of scientific validation and documentation. To overcome these limitations and ensure the quality, safety and therapeutic efficacy modern methods can be incorporated.

Aim and Objective: The aim of this study is to evaluate the biochemical, phytochemical and physicochemical properties of *kadukkai poo choornam*.

Methodology: The extraction of the drug was done by soxhelt method and phytochemical screening was done for the presence of alkaloids, glycosides, phenols, terpenoids carbohydrates, quinones, steroids, Saponins and proteins. Biochemical analysis was carried out to detect the presence of sulphate, chloride, carbonate, starch, ferrous iron, tannic acid, phosphate, zinc, reducing sugar and albumin. The Physicochemical analysis also carried out using standard procedures.

Results: The phytochemical screening revealed the presence of alkaloids, glycosides, phenols, terpenoids, carbohydrates, tannins and quinones. The developed qualitative densitometric HPTLC fingerprint can be used as an identifying biological marker for the drug extracts. In biochemical analysis the presence of Sulphate, chloride, reducing sugar, tannins and unsaturated compound. Physicochemical analysis revealed the values of total ash, acid insoluble ash, water soluble ash, sulphated ash, pH, volatile oil, foaming index and swelling index.

Conclusion: This study is an attempt to validate the therapeutic efficacy of *kadukkai poo choornam* for the management of hemorrhoids. And the above study can be used in further clinical studies and drug standardization.

Keywords: *Kadukkai poo choornam*, *biochemical analysis*, *physicochemical*, *phytochemical screening*, *Polyherbal formulation*, *hemorrhoids*

INTRODUCTION

The siddha system of medicine is a traditional medical system that uses a holistic approach to provide preventive, promotive, curative, rejuvenating and rehabilitative health care. This ancient system of medicine consists of many herbal, herbomineral and metallic preparations.

Herbal preparations gained worldwide attention due to its long-term benefits in terms of overall wellness with lesser side effects. The medicinal plants used in different diseases and ailments are the richest bio reservoirs of various phytochemicals. The medicinal properties of the plants are determined by the phytochemicals. Nature is a unique source of structures of high phytochemical diversity representing phenolics (45%), terpenoids and steroids (27%) and alkaloids (18%) as major group of phytochemicals. Although, these components seem to be non-essential to the plant producing them, they play a vital role in survival by mediation of ecological interactions with competitors, protect them from diseases, pollution, stress, UV rays and also contribute for colour, aroma and flavour with respect to the plant. The metabolites produced by the plants to protect themselves from biotic and abiotic stresses that turned into medicines that people can use to treat various diseases. Kadukkai poo choornam is a Polyherbal Siddha medicine preparation mainly indicated for the treatment of bleeding hemorrhoids. The ingredients of

the drug include leaf gall of Kadukkai (*Terminalia chebula*), kirambu (*Syzygium aromaticum*), lavanga pattai (*Cinnamomum verum*). Identification of phytoconstituents in the plant material helps to predict the potential pharmacological activity of that plant. This study mainly deals with collection, extraction and qualitative phytochemical, biochemical and physicochemical analysis of Kadukkai Poo Chooranam, a poly herbal siddha preparations.

MATERIALS AND METHODS

Collection and Authentication

Required raw drugs are collected from in and around Tirunelveli. It was identified and authenticated by the Medicinal botanists at GSMC, Palayamkottai. The ingredients of the trial drug were purified according to the proper procedures that are mentioned in Siddha classical literature. The purified ingredients are powdered separately and mixed together. And the drug was labelled as Kadukkai Poo Chooranam.

Ingredients of Kadukkai Poo Chooranam

SL No	Botanical name	Family	Common name	Parts used
1	<i>Terminalia chebula</i>	Combretaceae	Kadukkai poo	Leaf gall
2.	<i>Syzygium aromaticum</i>	Myrtaceae	Kirambu	Flower bud
3.	<i>Cinnamomum verum</i>	Lauraceae	Lavanga pattai	Stem bark

Physicochemical Analysis methods:

The physicochemical analysis is mainly used for detecting adulteration or improper handling of drugs. Physicochemical analysis such as determination of loss on drying, total ash value, water soluble ash, acid insoluble ash, sulphated ash, pH value, volatile oil, alcohol soluble extractives, water soluble extractives were carried out by standard procedures.

Biochemical Analysis methods:

5 grams of drug was weighed accurately and placed in a 250 ml clean beaker, 50ml of distilled water added to it and dissolved well. Then it is boiled for 10 minutes, cooled and filtered to a 100ml volumetric

flask and then it is made into 100 ml with distilled water. This solution was used for the analysis.

Phytochemical Analysis methods:

The authenticated plants are purified, coarsely powdered, extracted with ethanol solvent at room temperature. The alcohol extract was chemically tested for phytochemical constituents using standard procedures recommended by Harbone.

High Performance Thin Layer Chromatography (HPTLC):

Developing solvent system

A number of solvent systems were tried and a system which gave the maximum

resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the solvent system.

Sample application

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F254 pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Visualizer and

the images were captured under UV light at 254 nm and 366 nm.

Densitometry

The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The Rf values and finger print data were recorded with win CATS software associated with the scanner.

Post chromatographic derivatization

The plate was derivatized using vanillin-sulphuric acid reagent, heated at 1050 C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the Rf values and finger print data were documented.

RESULTS AND DISCUSSION

Table:1 Physicochemical Analysis

SI NO	Parameters	Result
1	LOD at 105 C	7.5%
2	Total Ash	5.08%
3	Acid insoluble ash	0.05%
4	Water soluble ash	1.6%
5	Sulphated Ash	8.84%
6	PH of Aqueous extract	5.68
7	Volatile oil	2.5%
8	Water soluble extractives	25.48%
9	Swelling index	4
10	Alcohol soluble extractives	29.16%
11	Foaming index	333.33

pH:

The pH obtained by the study of KPC is 5.68(weak acidic). The permeability coefficient and distribution coefficient are more in weakly acidic drugs than weakly basic drugs. Acidic drugs are get better absorbed in stomach.

Total Ash:

The total Ash value represents the organic and carbon matter present in the drug is converted to Ash at temperature 450°C or above. The total ash value of KPC is 5.08%.

Acid insoluble ash:

The acid insoluble ash value of KPC (0.05%) shows very small amount of inorganic component is insoluble in acid. It indicates that adulteration of raw ingredients by substances such as silica and rice husk is very less.

Water soluble ash:

Decreased Water-soluble Ash is a part of the total ash content, which is soluble in water. It is a good indicator of either previous extraction of water-soluble salts in the drug or incorrect preparation. The water-soluble ash value of KPC is 1.6%

Water soluble extract value:

Water soluble extract value plays an important role in evaluation of crude drugs. The value of KPC is 25.48%.

Sulphated ash

Sulphated ash value test uses a procedure to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulphuric acid. The limit of sulphated ash content is 11% ,thus the sulphated ash value of the formulated drug is below the limit and considered to be safe.

Alcohol soluble extract value:

Alcohol soluble extract value signifies the presence of amount of fats, lipids some steroids in drug. The alcohol soluble extract value of KPC (29.16%) is more than the water-soluble extract, thus alcohol is the better solvent of extraction for the formulation than water.

Swelling index:

Swelling index is the volume in milliliters taken up by the swelling of 1 gram of herbal material under specified conditions. Swelling improves the absorption and it also determine the purity of drug material. Swelling index of KPC is 4.0

Volatile oil:

Volatile oils are odorous volatile principles of plant sources, evaporate when exposed to air at ordinary temperature. These represent the essence of active constituents of plants. The volatile oil content in KPC is 2.5%.

Foaming index:

The foaming index is determined by measuring the height of the foam produced by the equivalent of 1 g of herbal drug or herbal drug preparation under the stated test conditions. The foaming index of KPC is 333.3.

Table:2 Biochemical Analysis

PARAMETERS	OBSERVATION
Calcium	Absent
Sulphate	Present
Chloride	Present
Carbonate	Absent
Starch	Absent
Ferric iron	Absent
Ferrous iron	Absent
Phosphate	Absent
Albumin	Absent
Tannic acid	Present
Unsaturated compounds	Present
Reducing sugar	Present
Amino acid	Absent
Zinc	Absent

Sulphates

Sulphates are salts of sulphuric acid. They dissolved in a bowel prep solution work as a laxative by drawing water into the bowel, causing it to empty out. Aluminium potassium sulphate is an inorganic sulphate salt, when it is combined with tannic acid and injected into hemorrhoids, blood flow to the hemorrhoid is interrupted, and a quick hemostatic effect and shrinkage of hemorrhoid develops. With time persistent fibrosis develops due to sterile inflammation; then adhesion and fixation of the mucosa and the submucosal layer to the muscular layer is promoted. Finally, bleeding and prolapse of the hemorrhoid disappear.

Chloride

Chloride is the predominant anion in intracellular fluid and one of the most important extracellular anions. It contributes to many body functions including the maintenance of osmotic pressure and acid-base balance, muscular and nervous activity and movement of solutes between fluid compartments. It plays a role in the digestion of food by supporting the production and release of hydrochloric acid in stomach.

Unsaturated Compounds

Unsaturated compounds are chemical compounds that contains carbon – carbon double bond or triple bond. The high reactivity of unsaturated compounds is manifest by their easy addition of certain simple molecules such as halogens, has long

been one of the salient characteristics of organic of organic chemistry. Unsaturated compounds are more reactive than saturated compounds.

Reducing Sugar

A sugar that serves as a reducing agent due to its free aldehyde or ketone functional groups in its molecular structure. Sugar is an energy source and a necessary structural

component of living cells. Our body require healthy sugar levels to function properly.

Tannins

Tannins are water soluble polyphenols that are present in many plants. Tannins have been reported to exert physiological effects such as to accelerate blood clotting, reduce blood pressure, decreases the serum lipid levels and modulate immune response.

Table:3 Phytochemical Analysis

PHYTOCHEMICALS	TEST	RESULTS
Saponin	Froth Test	Absent
Tannin	Lead acetate test	Present
Phenol	Alcohol ferric chloride test	Present
Terpenoids	Neller's Test	Present
Alkaloids	Mayer's Test	Present
Flavonoids	Shinado's Test	Present
Steroids	Libermann Burchard Test	Absent
Glycosides	Modified Borntrager 's Test	Present
Carbohydrate	Molisch's Test	Present
Quinone	Sodium hydroxide Test	Present
Proteins	Xanthoproteic test	Absent

Phenol

Polyphenols are a group of secondary metabolites involved in the hydrogen peroxide scavenging in plant cells. These compounds are known for their notable potential activity against various human viruses and also have immunomodulatory and anti-inflammatory activities.

Terpenoids

Terpenoids are the largest and most diverse group of naturally occurring compounds. It has been found to be useful in the prevention and therapy of several diseases, including cancer, and also have antimicrobial, antifungal, antiparasitic, antiviral, antiallergic, antihyperglycemic, anti-inflammatory and immunomodulatory properties.

Alkaloids

Alkaloids are secondary metabolites which carry one or more nitrogen atoms. Most of them are derived from amino acid precursors. Alkaloids have diverse physiological effects such as antibacterial, anti-inflammatory, analgesic, anti tumour activities.

Flavonoids

Flavonoids strengthen the veins, which may help reduce the risk of hemorrhoids. Micronized Purified Flavonoid Fraction (MPFF) has a variety of significant anti-inflammatory, anti-oxidant and venoprotective action. It reduces venous inflammation by inhibiting leukocyte rolling, adhesion and migration and inhibit the synthesis of inflammatory mediators. It also improves venous tone and lymphatic drainage by modulating noradrenergic signalling and reducing norepinephrine metabolism.

Glycosides

Glycosides are organic molecules which can be isolated from plant or animal sources, which on enzymatic hydrolysis gives one or more sugar moieties. Glycosides isolated from different medicinal plants showed in vivo analgesic effects. The intra peritoneal administration of this glycosides showed statistically significant analgesic activity in carrageenin induced rat paw edema model. They are traditionally used in modern medicine because of their cardiotoxic, purgative, analgesic, anti-arrhythmic and demulcent action.

Carbohydrates

Human body breaks down carbohydrates to glucose. Glucose or blood sugar is the main source of energy for our body cells, tissues and organs, Carbohydrates are ubiquitous and perform a wide array of biological roles. Carbohydrates based or modified therapeutics are used extensively in cardiovascular and hematological treatments ranging from inflammatory diseases and anti-thrombotic treatments to wound healing.

Quinones

Quinones are small secondary metabolites synthesized by a broad range of organisms. Perception of these aromatic molecules in plants involves membrane bound LRR-RLKs (Leucine -rich repeat receptor like kinases) to induce downstream cellular responses in plants such as calcium fluxes, specific gene expression and MAPK (Mitogen-activated protein kinases) activation. The anthranoid compounds, which can be chemically described as dihydroxy-anthraquinones, dianthrone and anthrone, possess a laxative effect. Because of their chemical structure, they are carried unabsorbed to the large bowel, where metabolism to active aglycones takes place.

HPTLC RESULTS

HPTLC Study of Kadukkai Poo choornam revealed the presence of different phytochemical constituents and different components by the detailed fingerprinting. And the results were interpreted based on area coverage of peak and number of peaks and Rf value of peaks.

HPTLC analysis was carried out by the solvent mixture of Toluene: Ethyl acetate

(5:2, v/v). Analysis of samples after derivatization using vanillin sulphuric acid viewed under uv short, uv long and white light at different wavelengths (254 nm, 366nm,575 nm) are presented in fig 1. Rf values obtained from HPTLC fingerprint scanned at wavelengths 254 nm,366nm and 575nm is given in fig 2, fig 3 and fig 4 respectively.

HPTLC fingerprinting analysis of alcoholic extract of KPC at 254 nm, the sample explore the presence of 8 prominent peaks corresponds to the presence of 8 versatile phytochemicals present within it. Rf value of the peak's ranges from 0.01Rf – 0.64Rf. Then, the peak 8 occupies the major percentage of area of 71.88%.

HPTLC fingerprinting analysis of alcoholic extract at 366 nm, the sample reveals the presence of 7 prominent peaks corresponds to presence of 7 versatile phytochemicals present within it. Rf value of the peak ranges from 0.01Rf – 0.80Rf. Further the peak 5 occupies the major percentage of area of 45.59% and 33.56%.

HPTLC fingerprinting analysis of alcoholic extract at 575 nm, the sample reveals the presence of 9 prominent peaks corresponds to the presence of 9 prominent peaks corresponds to presence of 9 versatile phytochemicals present within it. Rf value of the peak ranges from 0.00Rf – 0.62 Rf. Further the peak 9 occupies the major percentage of area of 58.87%.

The approach of fingerprint analysis through HPTLC has become the effective technique for quality control of herbal drugs. It serves as a device of identification, authentication and quality control of herbal drugs.

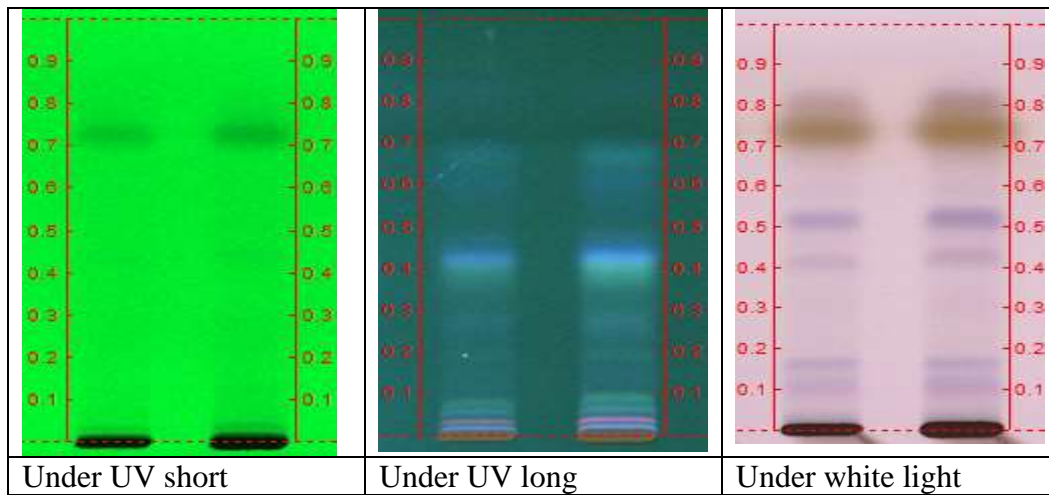


Fig 1: HPTLC profile of alcohol extract of Kadukkai Poo Chooranam viewed in UV short; UV long; White light after derivatisation using vanillin-sulphuric acid; Solvent system –Toluene:Ethyl acetate –(5:2); volume applied; Track 1-5 ul; Track 2-10 ul

254nm

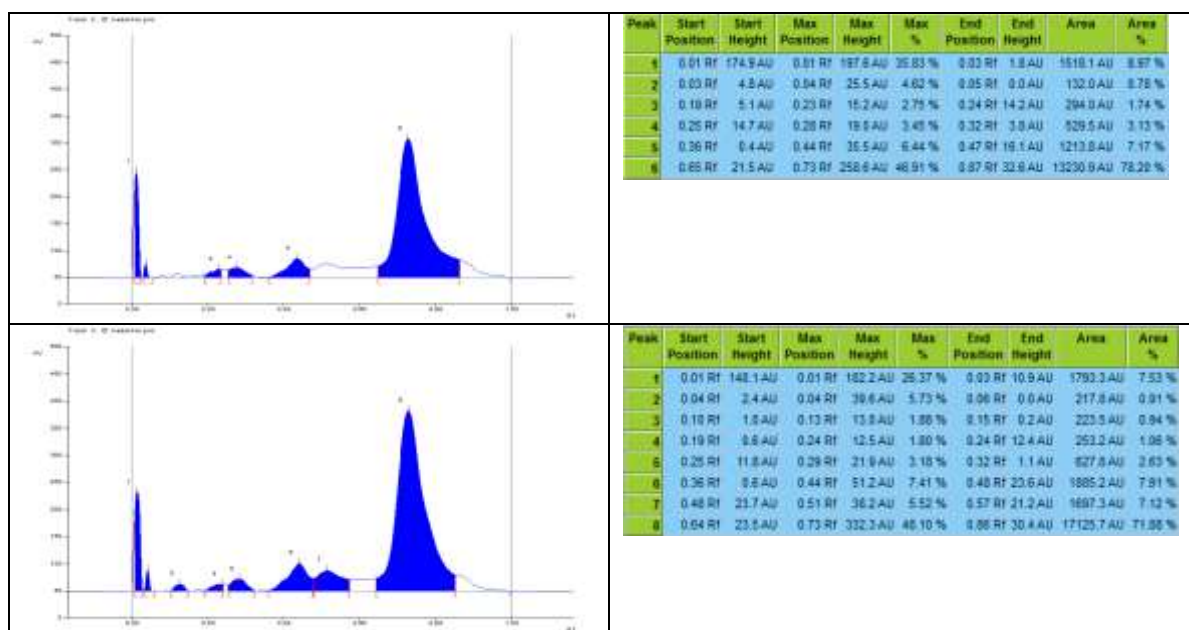
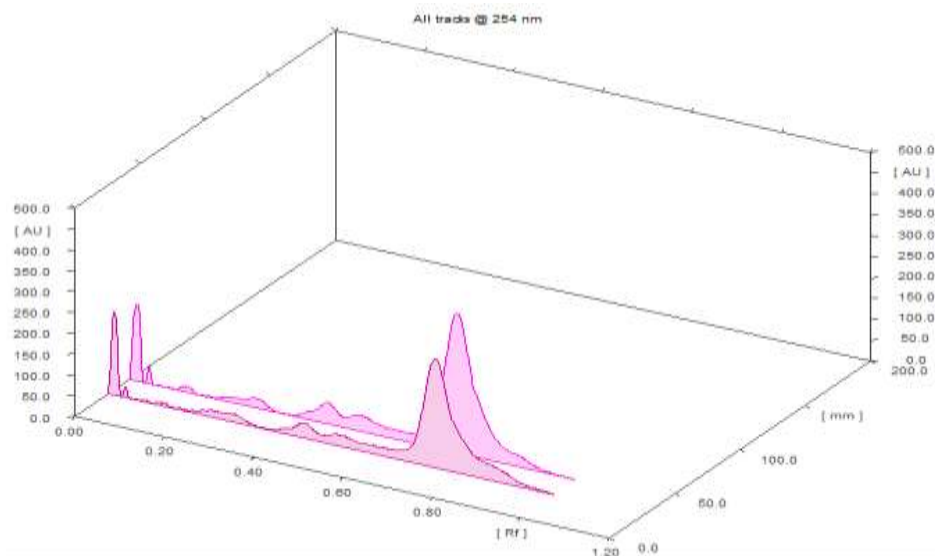


Fig 2: HPTLC fingerprint profile of 5ul and 10ul of alcohol extract of Kadukkai Poo Chooranam of 254 nm after derivatisation

366nm

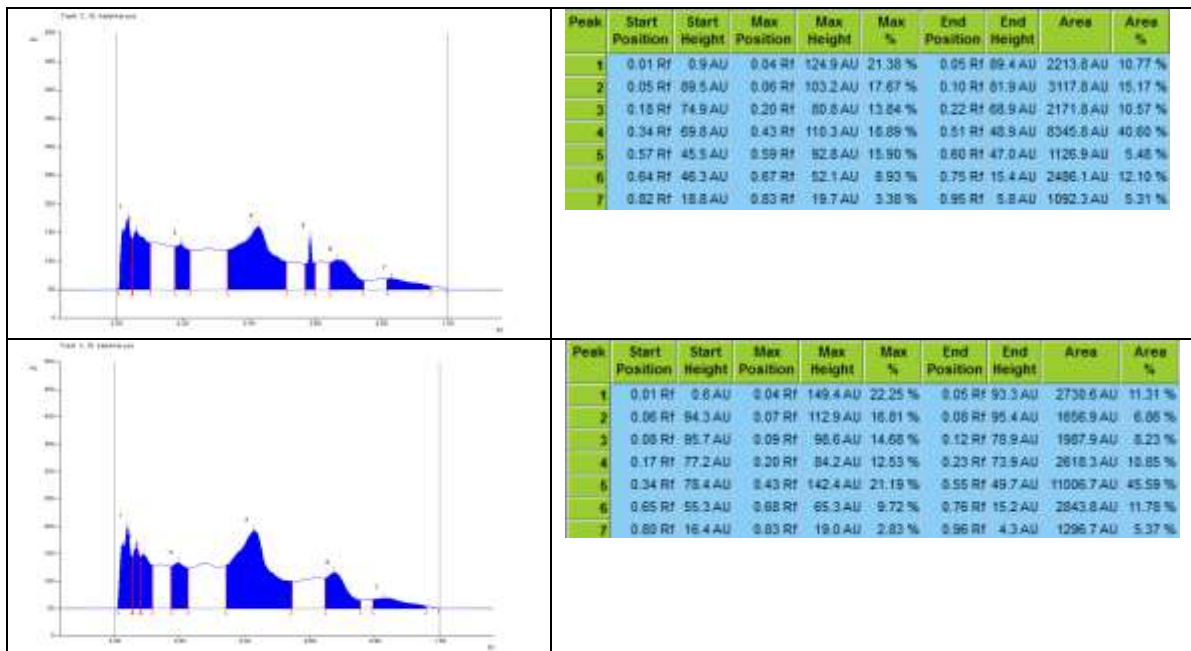
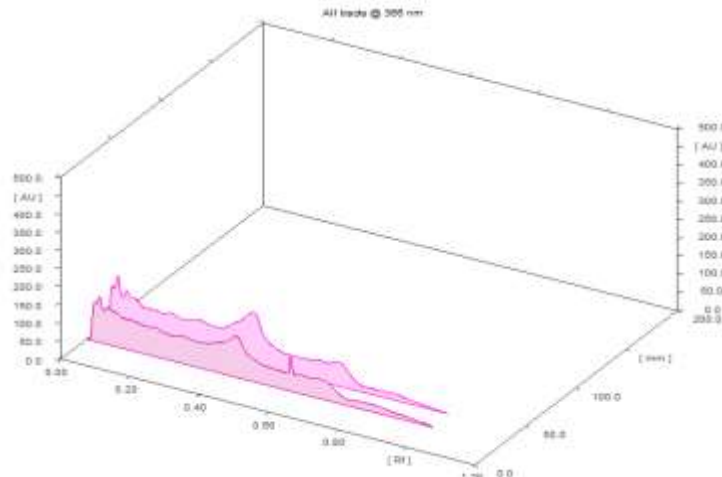
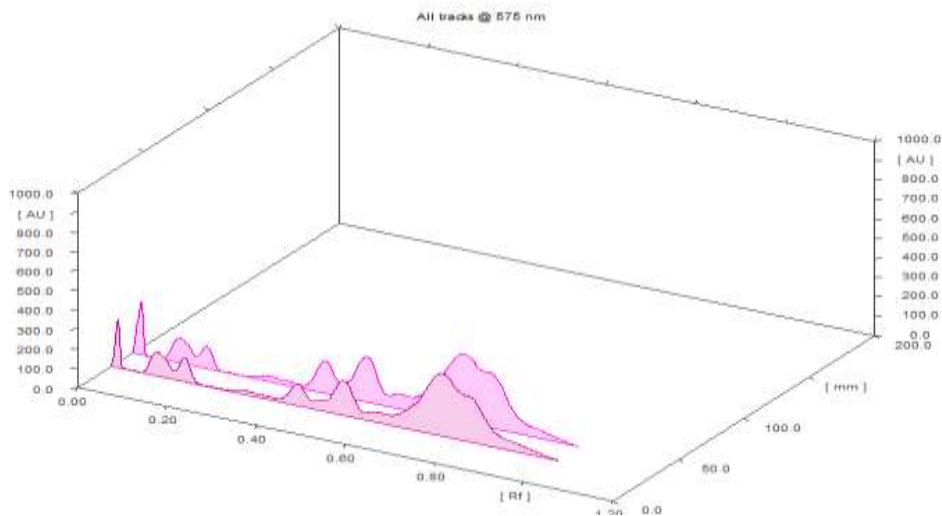


Fig 3: HPTLC fingerprint profile of 5 ul and 10 ul of alcohol extract of Kadukkai Poo Choornam at 366 nm after derivatisation

575nm



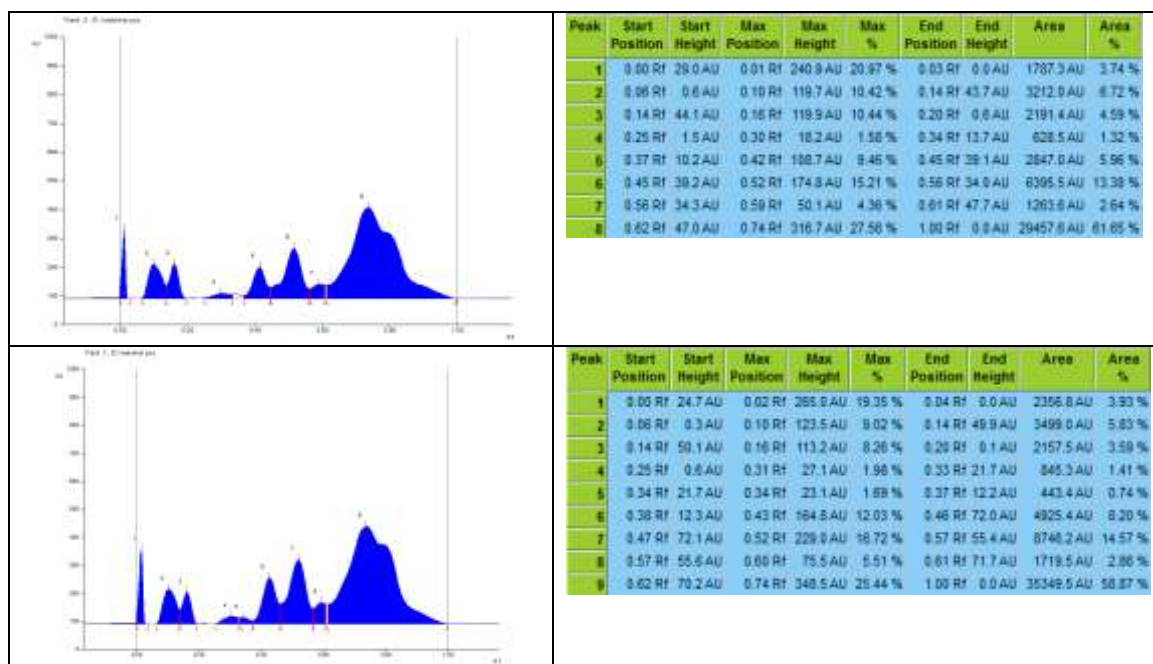


Fig 4: HPTLC fingerprint profile of 5 ul and 10 ul of alcohol extract of Kadukkai Poo Choornam at 575 nm after derivatisation.

CONCLUSION

Kadukkai Poo Choornam is a combination of 3 drugs. This study gives information about physicochemical, biochemical, phytochemical analysis and HPTLC fingerprint profile of the trial drug in different extracts which will be useful in quality assessment of the drug and batch comparison studies. The results also confirms that the presence of therapeutically potent compounds in the study drug.

Declaration by Authors

Acknowledgement: The authors are grateful to The Tamil Nadu Dr MGR Medical University, Chennai, Siddha Regional Research Institute, Trivandrum, The Principal and the faculties of Post Graduate Department of Pothu maruthuvam, Government Siddha Medical College and hospital, Palayamkottai, Tirunelveli.

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Rishipriya. C. R, A. Manoharan, P. Sathish Kumar. Biochemical, physicochemical, preliminary phytochemical evaluation of *Kadukkai Poo Chooranam*. *International Journal of Research and Review*. 2023; 10(8): 903-912.
DOI: <https://doi.org/10.52403/ijrr.202308115>
