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Qualitative and quantitative analysis of siddha herbal Formulation Kabasura kudineer in various concentrations

Research Article

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Abstract

Introduction: Siddha system is one of the ancient systems coming practised in southern part of India, North and Eastern parts of Sri Lanka. The Siddha medicines are categorised as *Aga marundhugal* (Internal Medicine) and *Pura marundhugal* (External Medicine).One among them is *kudineer* (Decoction). *Kabasura kudineer chooranam* (KSK) is a polyherbal formulation and it contains 15 ingredients. It is indicated for *Iyyasuram* and it used for fever with Respiratory disease. Objective and Methods: To analyse the presents of different phytoconstituents, develop HPTLC fingerprint and quantify the amount of phenols and alkaloids in *Kabasura Kudineer* prepared at 1/4 (sample A), 1/8 (Sample B) and 1/16 (Sample C) reduction concentrations. Result: Phytochemical analysis of *Kabasura Kudineer* revealed the presence of alkaloid, phenol, flavonoid and tannin in Sample A, B and C. The R_f value obtained by TLC and HPTLC fingerprint for Sample A are 0.02, 0.04, 0.79, Sample B is 0.04, 0.80 and Sample C is 0.04, 0.43, 0.051 and 0.71. The amount of Alkaloid present in Sample A, B, C 1.61, 1.03, 1.44 and phenol present in Sample A, B, C 17.62, 19.71, 18.60 Conclusion: The Analytical parameters along with TLC photo documentation, HPTLC fingerprinting profile and UV Spectrometer in various concentration of *Kabasura Kudineeer* are different in different reduction concentration ratio. Further specific chemical markers can be quantified using HPTLC and the therapeutic efficacy can be explored in further studies.

Keywords: *Kabasura kudineer,* HPTLC fingerprint, TLC fingerprint, UV Spectrometer, Phytochemical, *Iyyasuram*.

Introduction

In the present scenario, the public are seriously concerned in their health and protection from the diseases. Siddha system is one of the ancient systems coming practised in southern part of India, North and Eastern parts of Sri Lanka. The Siddha medicines are categorised as *Aga marundhugal* (Internal Medicine) and *Pura marundhugal* (External Medicine). The 32 types of *Aga marundhugal* include *Saaru (Juice), Surasam (Juice with mild heating), Karkam (Paste), Ilagam* etc. One among them is *kudineer* (Decoction) (1).

It is defined as a decoction of either dry or wet crude drugs boiled in 16 parts of water which is reduced to either 1/2 or 1/4 or 1/8 or 1/16 or 1/24 parts (2). *Kudineer* is prepared in definite ratio such as 1/2, 1/4, 1/6, 1/8 and so on which are indicated for emesis, gargling, eye application (drops), enema, perspiration respectively (3). It means that each definite ratio has its own significance in its administrative of medications pertaining to its curative property.

Kabasura kudineer chooranam (KSK) is a polyherbal formulation and it contains 15 ingredients. It is indicated for *Iyyasuram* (4) and it used for fever with flu-like symptom (5). The previous studies on *Kudineer* aimed at analysing the physiochemical and pharmacological actions for particular diseases. Likewise Jaspreet jain et al in 2020 conducted study to *Nilavembu kudineer chooranam* against Dengue and Chikungunya virus infection via in vitro conditions (6). Harini.P et al in 2022 conducted study to KSK has good Anti-diabetic action at significant concentration (7). Kiran.G et al in 2020 conducted study to the results shown that Chrysoeriol and Luteolin from *KSK* and Quercetin from JACOM have high binding affinity and good binding interactions with spike protein(8). Rajasekaran. A et al in 2021 conducted study to analysis of KSK in Pharmacognostical, physico-chemical and HPTLC validation (9). Shree Devi M.S et al in 2020 conducted study KSK excellent neuraminidase inhibition activity (10) and Narrain shree et al in 2017 conducted study in antioxidant properties present in KSK (11).

The practitioners prepared decoctions by adding water to the coarse powder of the specified ingredients, heating and reducing the water extraction in different

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ratio like 1/4, 1/8, 1/16 and 1/24(12). The rational in terms of the presence of phytochemicals have not been studied so far. The present study aims in estimating the phytoconstituents present in KSK in 1/4, 1/8 and 1/16 ratio both qualitatively and quantitatively.

Materials and Methods

The polyherbal formulation of KSK by GMP certified manufacturer was purchased from IMPCOPS pharmacy. KSK contains equal proportion of 15 ingredients.

Table 1: Ingredients of *Kabasura Kudineer Choornam*

S. No.	Botanical name	Local name (Tamil)	Part
1	*Zingiber officinale* L,	Sukku	Rhizome
2	*Piper longum* L,	Thippili	Fruit
3	*Syzygium aromaticum* (L.)Merr. &L.M.Perry	Ilavankam	Flower bud
4	*Tragia involucrate* L.	Sirukanchori ver	Root
5	*Anacyclus pyrethrum* (L.) Link	Akkirakaram ver	Root
6	*Hygrophila auriculate* Schumach	Neermulli ver	Root
7	*Terminalia chebula* Retz.	Kadukkai	Fruit rind
8	*Justicia adhatoda* L.	Adathodai	Leaf
9	*Coleus aromaticus* Benth	Karpuravalli	Leaf
10	*Costus speciosus* (J.Koeing) Sm.	Koshtam	Root
11	*Tinospora cordifolia* (Thumb.) Miers	Seendhil	Stem
12	*Clerodendron serratum* (L.)Moon	Siruthekku	Root
13	*Andrographis paniculata* Burm.f.Nees	Nilavembu	Whole plant
14	*Sida acuta* Burm.f.	Vattatthiruppi ver	Root
15	*Cyperus rotundus* L.	Korai kilangu	Root tuber

Preparation of *Kabasura kudineer* at different concentrations:

The trial drug *(Kabasura kudineer chooranam*) was prepared by soaking 3.5gram of the *chooranam* added 268ml water (As the siddha literature, ratio of decoction powder and water is 35 grams for 2680ml.Since time duration for preparing large quantity of decoction in laboratory is long ,I took 1/10th of the above mentioned quantity and analysed) (4), then boiled at 90 degree Celsius for 2 hours on the water bath, cooled and filtered. The filtrate was concentrated to 67ml (sample A -1/4), 33.5ml (sample B-1/8) and 16.75ml (sample C-1/16). This extract was used for phytochemical analysis. Phytochemical analysis for phenol, flavonoid, alkaloid and tannin were carried out by standard procedures.

Table 2 : Preparation of *Kabasura Kudineer* at different concentrations

Quantity of KSK powder	Water added	Quantity obtained		
3.5 grams	268ml	¼	1/8	1/16
		67ml	33.5ml	16.75ml

Qualitative Phytochemical analysis (13)

- A. Test for Tannins: 1ml of the sample was added with 0.01grams of ferric chloride and 10ml of distilled water .The colour changed to brownish green was noted.
- B. Test for Alkaloid: 1ml of sample was added with Dragendorff reagent. The colour change to orange was noted.
- C. Test for flavonoids: 1ml of sample was added with 0.00133 gm lead acetate and 10ml distilled water. The colour changed to yellow colour precipitate formation was noted.
- D. Test for phenols: 1ml of sample was added with o.5gm and 10ml distilled water. The colour change to yellow colour precipitate formation was noted.

Development of TLC photo documentation and HPTLC fingerprinting profile:

Kabasura Kudineer extracted were applied in form of bands with Camagmicrolitre syringe on a precoated silica gel 60 F_{254} Plate (merck) with Automatic TLC sampler 4 (ATS4). Mobile phase used was Toluene: Ethyl acetate: Dichloromethene (3:1:6). Linear ascending development was done in twin through glass chamber saturated with mobile phase. The plate was air dried and then photo documented at UV 254 nm using Camag visualizer. The plate was scanned at UV 254 nm using TLC Scanner 4 with winCATS software and the finger print profiles were documented. After derivatisation using iodine reagent the plate was kept under red light and the TLC chromatograms were documented.

High performance thin lager chromatography (HPTLC)

HPTLC is very useful in quality assessment tool for the evaluation of herbal drugs.

Method of analysis:

- Instrument: CAMAG HPTLC Scanner
- Model: Scanner – III
- TLC plate: Aluminum coated silica gel $60F_{254}$
- Mobile phase: Toluene: Ethyl acetate: Dichloromethane (3:1:6)
- Sample preparation: Sample is taken
- Derivatization: Iodine.

Quantification of phenols and alkaloids in *Kabasura kudineer* by UV Spectrophotometric assay

UV Spectrophotometric assay

Simple and reproducible UV- spectrophotometric assay for the quantitative determination of phenol and alkaloid in *Kabasura kudineer* were developed and validated in the present work. The parameters linearity, precision, accuracy, and standard error were studied

according to Indian herbal pharmacopeia. This assay has been developed for the determination of phenol and alkaloid in herbal formulation of *Kabasura kudineer*.

Preparation of extract of *Kabasura kudineer* for alkaloid estimation

0.5ml of the sample was dissolved in the solvent and 5ml of this solution was taken and the pH is maintained at 2-2.5 using dil. HCl. 2ml of the Dragendorff reagent was added to it and the precipitate was collected by centrifugation. Supernatant was tested with Dragendorff reagent to ensure complete precipitation of Alkaloids. The precipitate collected with MeOH and the MeOH was discarded. The residue was treated with 2ml of disodium sulphide solution add and centrifuged. The brownish black precipitate formed is collected and the supernatant is tested for completion of precipitation. The collected residue was dissolved in con. HNO_3 and made up to 10ml using distilled water. 1ml from this was pipetted out and 5ml of thiourea is added. The absorbance of this was measured at 435nm using distilled water as blank.

Preparation of standard solution of alkaloid

1ml of Bismuth nitrate pentahydrate solution was taken and 5ml of thiourea was added to it. The absorbance of this solution was measured at 435nm against distilled water as blank.

Preparation of extract of *Kabasura kudineer* for phenol estimation

To 0.2ml of the sample, 0.8ml of freshly prepared Folin C reagent was added and 2 ml of 7.5 % of sodium carbonate was added. The final solution was made up to 10ml using distilled water and incubated at room temperature in darkness for 2 hours. The absorbance was measured at 700nm.

Preparation of standard solution of phenols

Gallic acid was used plot in standard curve. Into each of the standard flasks containing the Tannic acid solution, 0.5ml of Folin C and 1 ml of 35% sodium carbonate solution was added and total volume was made up to 10ml using distilled water. Absorbance was measured at 700nm against a blank (containing 0.5ml of Folin C and 1ml of 35% sodium carbonate solution made up to 10ml using distilled water).

Results

The Analytical parameters along with Phytochemical analysis, TLC photo documentation, HPTLC fingerprinting profile and UV Spectro-photometer in various concentration of Kabasura Kudineeer are given below

Table 3: Phytochemical Analysis

Phytochemicals	Sample A	Sample B	Sample C
Alkaloid	+	+	+
Phenol	+	+	+
Tannin	+	+	+
Flavonoid	+	+	+

TLC and HPTLC analysis

Sample A TLC pattern showed three visible bands under UV at 254nm with Rf 0.02, 0.04 and 0.79 under given in figure 1. Sample B TLC pattern showed two visible bands under UV at 254nm with Rf 0.04 and 0.80 under given in figure 2 and Sample C TLC pattern showed four visible bands under UV at 254nm with Rf 0.04, 0.43, 0.51 and 0.71 under given in figure 3.The HPTLC fingerprinting profiles of the chloroform extract of KSK was recorded at 254nm.

Samples	Rf value
Sample A	0.02, 0.04, 0.79
Sample B	0.004, 0.80
Sample C	0.004 , 0.43, 0.51, 0.71

Fig 1: TLC Analysis of Kabasura Kudineer Sample A

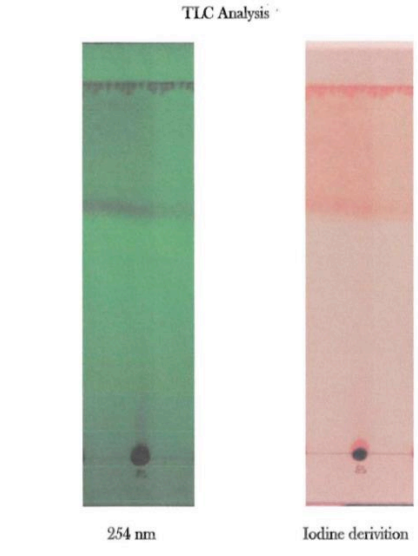


Fig 2: TLC Analysis of Kabasura Kudineer Sample B

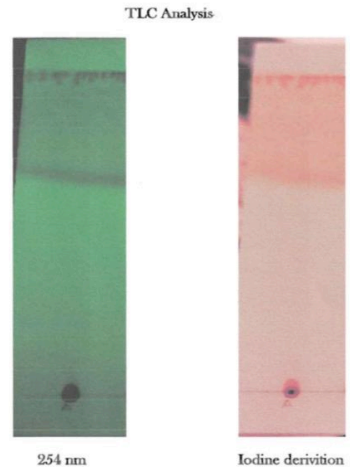


Fig 3: TLC Analysis of Kabasura Kudineer Sample C

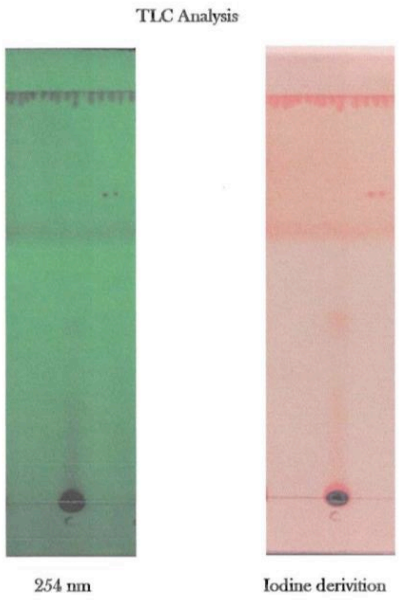


Fig 4: HPTLC fingerprint profile and Rf value of kabasura kudineer chooranam Sample A

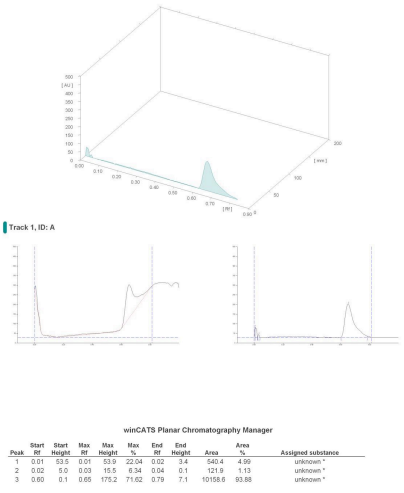


Fig 5: HPTLC fingerprint profile and Rf value of kabasura kudineer chooranam Sample B

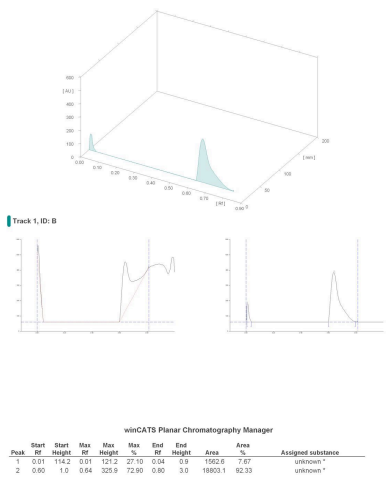
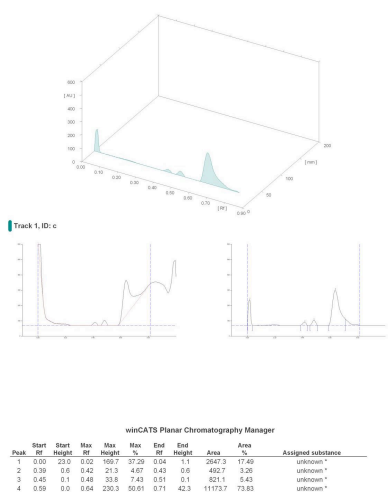


Fig 6: HPTLC fingerprint profile and Rf value of kabasura kudineer chooranam Sample C



Quantification of Alkaloid and Phenol by UV Spectrophotometer

Alkaloid concentration 1.61, 1.03 and 1.44 percent in Sample A,B,C and phenol concentration 17.62 ,19.71 and 18.60 percent in Sample A,B,C at 435 nm using in UV spectrophotometer.

Table 4 : Absorbance values of samples for Alkaloid estimation

S.No	Sample A (1/4)	Sample B (1/8)	Sample C (1/16)
1	0.1593	0.0969	0.1406
2	0.1596	0.0971	0.1406
3	0.1596	0.0974	0.1407

Table 5: Absorbance values of samples for Phenol estimation

ABS	Sample A (1/4)	Sample B (1/8)	Sample C (1/16)
1	0.7419	0.812	0.7755
2	0.7411	0.8080	0.7750
3	0.7405	0.8057	0.7746

Discussion

Kudineer (Decoction) is defined as either dry or wet crude drugs boiled in 16 parts of water which is reduced to 1/2 or 1/4 or 1/8 or 1/16 or 1/24 parts(1). *Kudineer* is easily prepared, cost effective and easily absorbed in our body. It is prepared in definite ratio such as 1/2, 1/4, 1/6, 1/8, 1/24 and so on which are indicated for *emesis, gargling, eye application* (drops), *enema, perspiration* respectively (3). It means that each definite ratio has its own significance in its administration of medications pertaining to its curative property. *Kabasura kudineer chooranam* contains 15 ingredients such as *Chukku, Milaku, Siruthekku, Nilavembu* etc (4)and its used for fever and respiratory illness(5).

Previous studies in *Kabasura kudineer* were aimed at analysing its physiochemical and pharmacological actions for particular disease. Likewise the studies on *Kabasura kudineer* is also done for its physiochemical and pharmacological activities like anti oxidative potential, Immuno-modulatory(11), H1N1 neuraminidase inhibitor(12), Anti-inflammatory, Antipyretic, Antibacterial actions (11) etc. No research have been conducted to validate the concept of reduction to different ratio in *kudineer* formulations.

Hence this study was conducted to analysis phytochemicals in KSK formulation in different reduction concentrations such as ¼ (Sample A), 1/8(Sample B), 1/16(Sample C). Since these concentrations are normally used they were included in the study. Phytochemical analysis of KSK revealed the presence of alkaloid, phenol, flavonoid and tannin in Sample A, B and C. Other phytochemicals present are carbohydrate, glycoside, cardiac glycosides, amino acid, saponins, hydrolysable tannin, phlobatanims, terpenoids, volatile oils, Vit C as per the previous study (10).

TLC and HPTLC fingerprinting of KSK at different concentrations revealed different fractions and different R_f values. The R_f value obtained by HPTLC for Sample A is 0.02, 0.04 and 0.79 .The R_f value obtained by HPTLC for Sample B is 0.04 and 0.80. The R_f value obtained by HPTLC for Sample C is 0.04, 0.43, 0.051 and 0.71. The R_f value of 0.43 in sample C indicates the presence of *Andrographis paniculata, Syzgium aromaticum* and *Tinospora cordifolia* whose R_f corresponds to 0.38, 0.37 and 0.39 respectively. The R_f 0.71 may be the fraction corresponding to *Syzygium aromaticum, Tinospora cordifolia* and *Piper longum* whose R_f is 0.74, 0.77 and 0.89 as per the previous studies. The R_f of 0.80 corresponds to 0.83 fraction of *Adathoda vasica* as per the previous study (14).

The quantification of alkaloids and phenols was done by UV Spectrometry method. The concentration of alkaloid present in the different concentrations of

Sample A, Sample B and Sample C are 1.61, 1.03 and 1.44 percent respectively. The present study showed variation in the presence of amount of alkaloid and phenols content in various reduction concentrations. Alkaloid content was highest in Sample A and phenol content was highest in Sample B .Since 3 reduction concentrations, Sample A (1/4 reduction) has the maximum concentration of alkaloids and phenols. This reduction concentration is mostly advised to patients. The alkaloids possess anti oxidation, inhibition of phospholipase A2, strengthening of myocardial contractility, inhibition of angiogenesis, and induction of apoptosis in cancer cell (15-16) .

Many alkaloids are present in KSK formulation were Piperdine possess Bactericidal, Anti-histaminic, Anticancer, Central Nervous System stimulant and Depressant, Herbicidal, Insecticidal and Fungicidal properties (16). Piperine has Antioxidant, Antimicrobial, Anti-tumour, Cytotoxicity properties (17). Piperine significantly suppressed the tumour growth of both androgen-dependent and androgen-independent prostate cancer cells (17). Berberine is an alkaloid isolated from *Berberis vulgaris* L.,Its protective effect in Alzheimer's, cerebral ischemia, mental depression, schizophrenia and anxiety(18).

Some important phenols present in KSK are Vasicine, Gingerone A, Gingerol and Zingirone. Flavonoids and phenols are nontoxic and have been reported as interesting candidate for pharmaceutical and medical application because of their effective antioxidant, anticancer, antibacterial, cardio protective, anti-inflammatory, immune stimulant, anti-allergic and antineoplastic activity. Vasicine and vasinone possess bronchodilator activity respiratory stimulants activity, cardiac–depressant effect, while vasicinone is a weak cardiac stimulant. It increases the production of serous mucus in the respiratory tract which makes the phlegm thinner and less viscous, which allows the cilia to more easily transport the phlegm out of the lungs. This property of vasicine which is present in KSK may be helpful in SARS COV -2 infections (19).

Gingerenone A has been decreased production of the SASP proinflammatory cytokine IL-6 , enhanced cleaved caspase-3, reduced senescent cell viability, decreased levels of the anti-apoptotic protein Bcl-XL (20). Gingerol has Anticancer, Anti-inflammatory, Anti-fungal,Antioxidant, Neuroprotective and Gastroprotective properties , Gingerols facilitate healthy glucose regulation for diabetics (21).

From the analysis 1/4th concentrations seems to have all phytochemical and most of the phytochemicals got extracted in this ratio. More over the alkaloid and phenol content is also maximum in this reduction concentration. This concentration is being practised widely in the Siddha hospitals. Hence by this study it is clear that the phytochemicals concentrations vary with different reduction concentrations. Further specific chemical markers can be quantified and the therapeutic efficacy can be as studied in further studies.

Conclusion

Analysis of various concentration of *Kabasura kudineer* has been carried out with evaluating its quality and quantity of phytochemicals present in it . The Analytical parameters along with TLC photo documentation, HPTLC fingerprinting profile and UV Spectrometer in various concentration of *Kabasura kudineer* are different in different reduction concentration ratio. Further specific chemical markers can be quantified using HPTLC and the therapeutic efficacy can be explored in further studies.

Competing interests: Nil
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