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Original Article

Pharmacognostical, physico-chemical and HPTLC validation of Kabasura Kudineer - a Siddha polyherbal formulation

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ABSTRACT

The present work was aimed to study the Pharmacognostical features, physico-chemical investigations and HPTLC analysis of the Siddha poly herbal formulation, kabasura kudineer. Kabasura kudineer chooranam was not validated scientifically till date and is highly recommended for prophylatic and for treatment of COVID-19. Pharmacognostical study is the first step in the standardization of crude drugs, which provides the information regarding the identity, purity and quality of the ingredients. Physico-chemical investigation confirms the Shelf life of the formulation. HPTLC analysis reveals the presence of major phytoconstituents in the formulation. Microscopical examination of the formulations showed characteristic informations of the ingredients in kabasura kudineer which revealed the tested formulations are genuine. The results of the physico-chemical evaluation of Siddha formulation were in correlation with the WHO limits. All the formulations tested found to be free from microbial contamination, heavy metals and pesticide residues. The outcome of the present study will be the benchmark for the identification and authentication of the ingredients and the Siddha formulation kabasura kudineer chooranam which will be helpful for treatment of corona virus infections without any ambiguity.

Keywords: Siddha formulation, kabasura kudineer; pharmacognostic study; physico-chemical study; validation, HPTLC

INTRODUCTION

One of the indigenous and ancient system of medicine prevailed in Tamil Nadu is Siddha system of medicine [1]. The word "Siddha" in Tamil means perfection and the treatment is based on restoring the balance between mind and body**.** Raw/crude form of herbs, metals, minerals and animal origins were used in Siddha system of medicine. Kabasura kudineer a Siddha formulation has been recognized for the treatment swine flu five times a day for three to five days (adults should be

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given 60 ml and 30 ml for children) [2]. In Siddha literature, Kabam means cold, suram means fever and kudineer means concoction. Composition of the formulation consists of 6.66 % of 15 ingredients are detailed in Table 1. Kabasura kudineer is currently being used for preventive and treatment of COVID-19 patients having the symptoms of dry cough, sore throat, high fever and difficulty in breathing. Only two studies [3,4] are reported in literature for kabasura kudineer**.** Anitha John et al [3] in 2015 reported Loss on drying, total Ash, acid insoluble ash, water and alcohol soluble extractive values, preliminary phytochemical studies and HPTLC studies for only one Siddha product of kabasura kudineer. Thillaivanan et al in 2015 [4] reported anti-inflammatory, antipyretic, analgesic, anti-viral, anti-bacterial, anti-fungal, anti-oxidant, hepatoprotective, anti-diabetic, anti-asthmatic, anti-tussive, immunomodulatory, anti-diarrhoeal and anti-oxidant activities of kabasura kudineer choornam. Thus the present study was undertaken as significant investigations on Powder microscopy, Heavy metal analysis, Pesticide residue analysis and microbial contamination limits were not addressed by the reported literature for authenticity of this Siddha formulation.

MATERIALS AND METHODS

Fifteen ingredients of the formulation were separately procured from the local markets of Madurai and authenticated by the Professor of Pharmacognosy, KMCH College of Pharmacy, Coimbatore. Two marketed formulations of kabasura kudineer chooranam were procured from local market. Using the ingredients procured one formulation of kabasura kudineer chooranam was prepared in the institution. Standards of gallic acid, andrographolide, and quercetin were purchased from Sigma-Aldrich, India. Readymade HPTLC plates procured from M/s Merck, Mumbai. All other chemicals and solvents were of analytical grade obtained from Qualigens fine chemicals, Mumbai.

Organoleptic, microscopic and physico-chemical studies viz. presence of foreign matter, loss on drying, Ash value (Total ash), water soluble ash and acid Insoluble ash, Water Soluble Extractive, alcohol soluble extractive, and pH were carried out as per the WHO guidelines [5]. Bulk density, tapped density, angle of repose, Hausner ratio and Carr's Index were also determined. The fluorescence behaviour of the powdered plant material were observed after treating the powder in different reagents and viewed under day light and ultraviolet light at 254 and 366 nm [6]. TLC/HPTLC studies were performed on aluminum plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness (E. Merck) as adsorbent. The Ethyl acetate: toluene in the ratio of 3:5 was used as mobile phase. Fifteen ingredients and three Siddha formulations were prepared in methanol (10 % w/v solution) in a volumetric flask. Sample solutions were applied as 5 mm band using Linomat V automatic sampling device of Camag HPTLC at one end of 20 X 10 cm dimension plate with a gap of 5 mm between the bands. Allowed to dry the bands and developed the chromatogram up to 8 cm of the plate in twin trough chamber. Removed the plate, dried and scanned using CAMAG TLC Scanner with WINCATS 4.05 version software at a wavelength of UV 254 and 366 nm. Measured and recorded the distance of each spot from the point of its application and calculated the Rf from the ratio of the distance travelled by the spots from the origin with the mobile phase. The spots after spraying the visualizing agent vanillin-sulphuric acid were also measured and calculated the Rf value as mentioned above. The heavy metals and microbial load were determined as per WHO guidelines.

RESULTS AND DISCUSSION

Scientific documentation as per WHO guidelines of herbs or plant materials is very important for its identity and purity before designing a herbal formulation [7]. Hence the present work was undertaken to investigate poly herbal Siddha formulation Kabasura kudineer chooranam and its ingredients pharmacognostically and to standardize them using various physico-chemical evaluation methods. Powder analysis plays a significant role in identification of crude drug as it assist in the identification of right variety of the botanical source and identifies the presence of adulterants. Characteristic powder microscopical investigation of the formulations was in correlation with the microscopical examination of the fifteen individual ingredients present in the formulation 1 and 3 (Figure 1-4). Some of the characteristic microscopical identity is not present in the formulation 2. Thus genuineness and purity of the ingredients is confirmed in formulation 1 and 3 compared to formulation 2. Kabasura kudineer chooranam and its 15 ingredients were tested for the presence of foreign matters like sand, glass particles, dirt, insects, animal excreta and other species of plants. Foreign matter in the formulation and ingredients were found to be within the limits of WHO Guidelines [5]. Powder fineness analysis revealed almost all the ingredients and three formulations are coarse powder in nature. The two ingredients namely *Coleus ambonicus* and *Zingiber officinale* were found to be fine powder in nature. Loss on drying is one of the quality control parameter which determines the presence of excess of moisture in medicinal plant materials that may cause microbial growth. Percentage of loss due to

drying was found to be within the limit for the ingredients and for the formulations. Results of the fluorescence analysis of the formulations showed characteristic color under visible day light and under UV light at 254 and 366 nm which can be used for identity of the ingredients in the formulations. The Ash value normally designates the presence of inorganic residues present in herbs and pharmaceutical substances. The study results revealed that the ash values were within the limit as prescribed by Pharmacopoeia (Table 2). The extractive value of formulation in aqueous extract was found to be maximum (15.2%) as compare to alcohol extracts (5.42%). Thus this Siddha formulation can effectively dispensed as kabasura kudineer chooranam in aqueous medium as maximum constituents is getting extracted in aqueous medium. pH determination indicated the slight acidic nature of the formulation. Since there is no greater difference (less than 0.070) between the observed bulk and tapped density for the formulations, the flow properties of the kabasura kudineer was found to be good**.** Flowability of powders can be determined by direct (using kinetic or dynamic methods) or indirect (measurement on static bed) methods. Angle of repose is an indirect method for quantifying powder flowability where the range was found to be 33 to 37, clearly indicated that the flowability of the formulations were good. Hausner's ratio is a measure of both bulk volume and tapped volume of coarse powder of kabasura kudineer is 1.24 so it's found to be under fair category. Carr's index is an indirect measure of bulk density, size, shape, surface area, moisture content and cohesiveness of material. Since the kabasura kudineer formulations were in the range of 20-24 the flowablity was found to be in the passable category (Table 3).

The different mobile phase composition were attempted to elute the components in HPTLC for the kabasura kudineer choornam. The mobile phase comprising of Ethyl acetate: toluene: methanol: formic acid in the ratio of 6: 3:0.4:1.6 efficiently resolved the components present in the Siddha formulation. HPTLC photo documentation profile of methanol extract of Kabasura kudineer choornam at 254 nm, 366 nm and under white light was depicted in Figure 5, 6 and 7 respectively. The optimized chamber saturation time for mobile phase was 3 min at room temperature (25 ±1^{o}C). The densitometric analysis was performed at 254 nm in reflectance mode. Phytoconstituents viz. rutin, were found to be present in all the three methanol extract of kabasura kudineer chooranam. Gallic acid, quercetin, were present in Siddha formulations 2 and 3 (Figure 8,9). The presence of rutin and gallic acid were identified in aqueous extract of all the three Siddha formulations. Heavy metal analysis divulged that the formulation is not having any detectable limit of heavy metal contamination of like arsenic, cadmium, lead and mercury (Table 4).

Organo phosphorous analysis confirmed that the phosphate level is within the limit prescribed by WHO. Herbs and herbal materials normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. Microbial contamination may also occur during harvesting, production, transportation and storage process. Proliferation of microorganisms may result from failure to control the moisture levels of herbal medicines during transportation and storage. The presence of *Escherichia coli*, *Salmonella* spp. and moulds may indicate poor quality of production and harvesting practices. *Salmonella* and *Shigella* species should not be present in herbal medicines intended for internal use, at any stage. The test results revealed the absence of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* were absent (Figure 10,11,12) and the total fungal count and microbial plate count were within the WHO limits, indicating that the formulations are safe for internal use (Table 5).

CONCLUSION

Characteristic powder microscopical investigation of the formulations was in correlation with the microscopical examination of the fifteen individual ingredients present in the formulation 1 and 3. The presence of rutin and gallic acid were identified in aqueous extract of all the three Siddha formulations. Heavy metal analysis divulged that all the formulations are not having any detectable limit of heavy metal contamination of arsenic, cadmium, lead and mercury. The test results revealed the absence of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* Total fungal count and microbial plate count were within the WHO limits, indicating that the formulations are safe for internal use.

ACKNOWLEDGMENTS

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Table 1. Details of Ingredients of kabasura kudineer chooranam

SN	Kabasura Kudineer	Family	Tamil Name	Parts of the plant used
1	*Adathoda vasika* (L).Nees or *Justicia adhatoda* L.	*Acanthaceae*	*Adathodai Elai*	Leaf
2	*Anacyclus pyrethrum* (L.)Lag.	Asteraceae/ Compositae	Akkirakaram ver	Root
3	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	Nilavembu	Whole plant
4	*Cissampelos pareira L*	Menispermaceae	Vattathiruppi Ver	Root
5	*Clerodendron serratum* Roxb.	Lamiaceae	Siruthekku	Root
6	*Coleus amboinicus* Spreng	Lamiaceae	Karpoorvalli Elai	Leaf
7	*Cyperus rotundus* L.	Cyperaceae	Korai kizhangu	Rhizome
8	*Hygrophillia auriculata* (Schum.) Heine	Acanthaceae	Mulli ver	Root
9	*Piper longum* L.	Piperaceae	Thippili	Fruit
10	*Saussurea lappa* Clarke or *Saussurea costus* (Falc.) Lipsch.	Compositae	Kostam	Root
11	*Syzygium aromaticum* Merr & L.M. Perry	Myrtaceae	Ilavangam	Flower bud
12	*Terminalia chebula* Retz.	*Combretaceae*	Kadukkaithol	Fruit
13	*Tinospora cordifolia* Merr.	Menispermaceae	Seenthil Thandu	Stem
14	*Tragia involucrate* L.	Euphorbiaceae	Cirukancori ver	Root
15	*Zingiber officinale* Rosc	Zingiberaceae	Chukku	Rhizome

Table 2. Physico-Chemical analysis of kabasura kudineer chooranam

Parameters	Formulation 1	Formulation 2	Formulation 3
Foreign matter (%)	0.00002	0.00003	0.00002
LOD (%)	0.81 ± 0.14	0.97 ± 0.07	0.78 ± 0.14
Total Ash (%)	8.00 ± 0.50*****	7.67 ± 0.29*****	8.17 ± 0.58*****
Water soluble ash (%)	6.17*****	5.68*****	6.23*****
Acid insoluble ash (%)	1.62*****	1.60*****	1.60*****
Water extractive value (%)	15.00 ± 0.72*****	14.80 ± 0.72*****	14.80 ± 0.00*****
Alcohol extractive value (%)	5.31 ± 0.61*****	4.35 ± 0.40*****	4.40 ± 0.17*****
pH	5.4 ± 0.2*****	5.5 ± 0.2*****	5.8 ± 0.3*****

* average of three determinations

Table 3. Determination of flow property of the kabasura kudineer chooranam

Parameters	Formulation 1*	Formulation 3*	Formulation 3*
Bulk density	0.400	0.250	0.300
Tapped density	0.481	0.312	0.375
Angle of repose	37.56	33.40	34.50
Hausner Ratio	1.20	1.24	1.25
Carr's Index	20.0	24.0	23.3

* average of three determinations

Table 4. Determination of heavy metals in kabasura kudineer chooranam

Heavy metals	**Formulation 1** (in ppm)	**Formulation 2** (in ppm)	**Formulation 3** (in ppm)	**WHO permissible limit** (in ppm)
Arsenic	0.038	0.038	0.038	3
Cadmium	0.1	0.1	0.1	0.3
Lead	0.16	0.16	0.16	10
Mercury	0.009	0.009	0.009	1

Table 5. Determination of microbial contamination in kabasura kudineer chooranam

Parameters	**Results**			**WHO**
	Formulation 1	**Formulation 2**	**Formulation 3**	**Permissible limits**
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent	Absent
Escherichia coli	Absent	Absent	Absent	Absent
Total fungal count	2×10^2	4×10^2	4×10^2	10^3/g
Total microbial count	Absent	5×10^4	Absent	10^5/g

Adathoda vasika leaf showing (a) Fragments and trichomes (b) Crystals

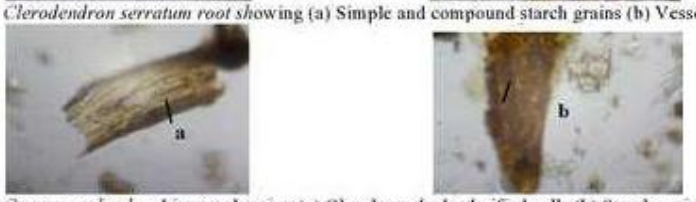
Anacyclus pyrethrum root showing (a) Scalariform pitted thickened vessels (b) Crystals

Andrographis paniculata whole plant powder showing (a) Scalariform vessels (b) Crystals (c) Leaf fragments (d) Fibers

Cissampelose pareira root showing (a) Bunched Fibers (b) Grains

Figure 1.A Microscopical examination of *fifteen ingredients* under 10 X magnification

Clerodendron serratum root showing (a) Simple and compound starch grains (b) Vessels



Cyperus rodundus rhizome showing (a) Closely packed sclerified cells (b) Starch grains

Hygrophillia auriculata root showing (a) Vessels (b) Crystals (c) Pitted fragments

Piper longum fruit showing (a) Stone cell (b) Oleoresin

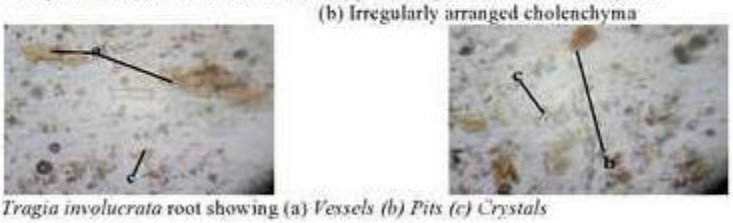
Saussurea lappa root showing (a) unstained phloem fibers (b) Resin and oil glands

Figure 1.B Microscopical examination of *fifteen ingredients* under 10 X magnification

Syzygium aromaticum flower bud showing (a) Syzygium oil cavity (b) Fibers

Terminalia chebula fruit showing (a) simple pits vessels and group of sclerides (b) Fibers

Tinospora cordifolia stem shows (a) Polygonal shaped cells with starch grains (b) Irregularly arranged cholenchyma



Tragia involucrata root showing (a) *Vessels (b) Pits (c) Crystals*

Zingiber officinale rhizome showing (a) Oblique pits

Figure 1.C Microscopical examination of *fifteen ingredients* under 10 X magnification

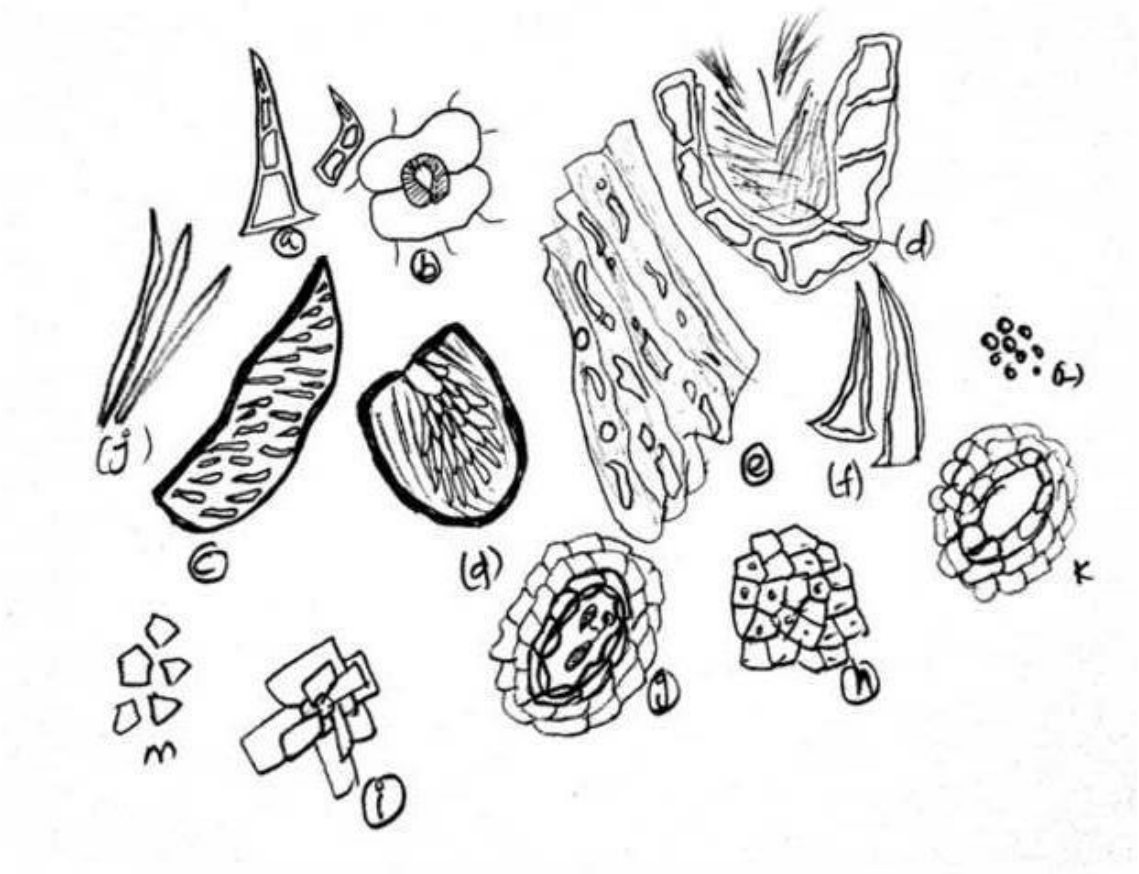


Figure 2. Microscopical examination of formulation 1 under 10 X magnification

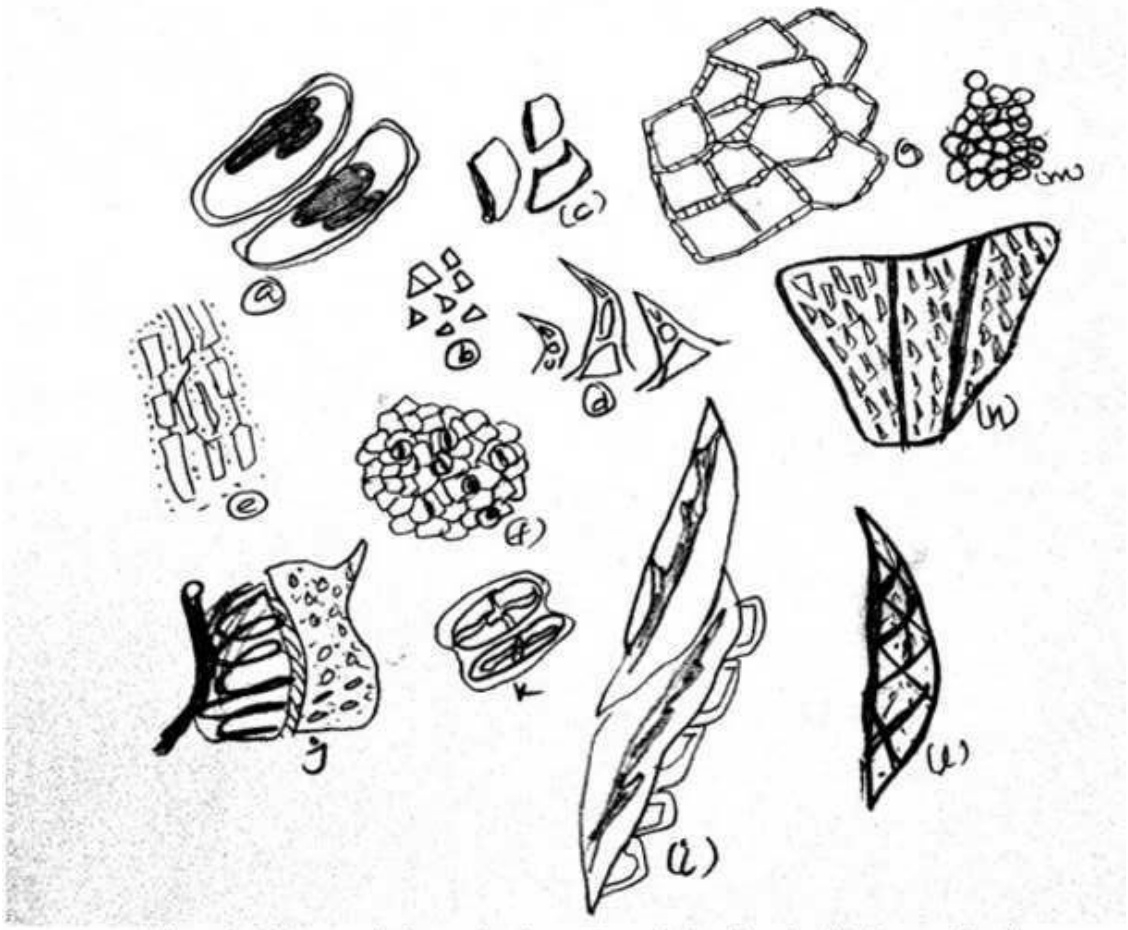


Figure 3. Microscopical examination of formulation 2 under 10 X magnification

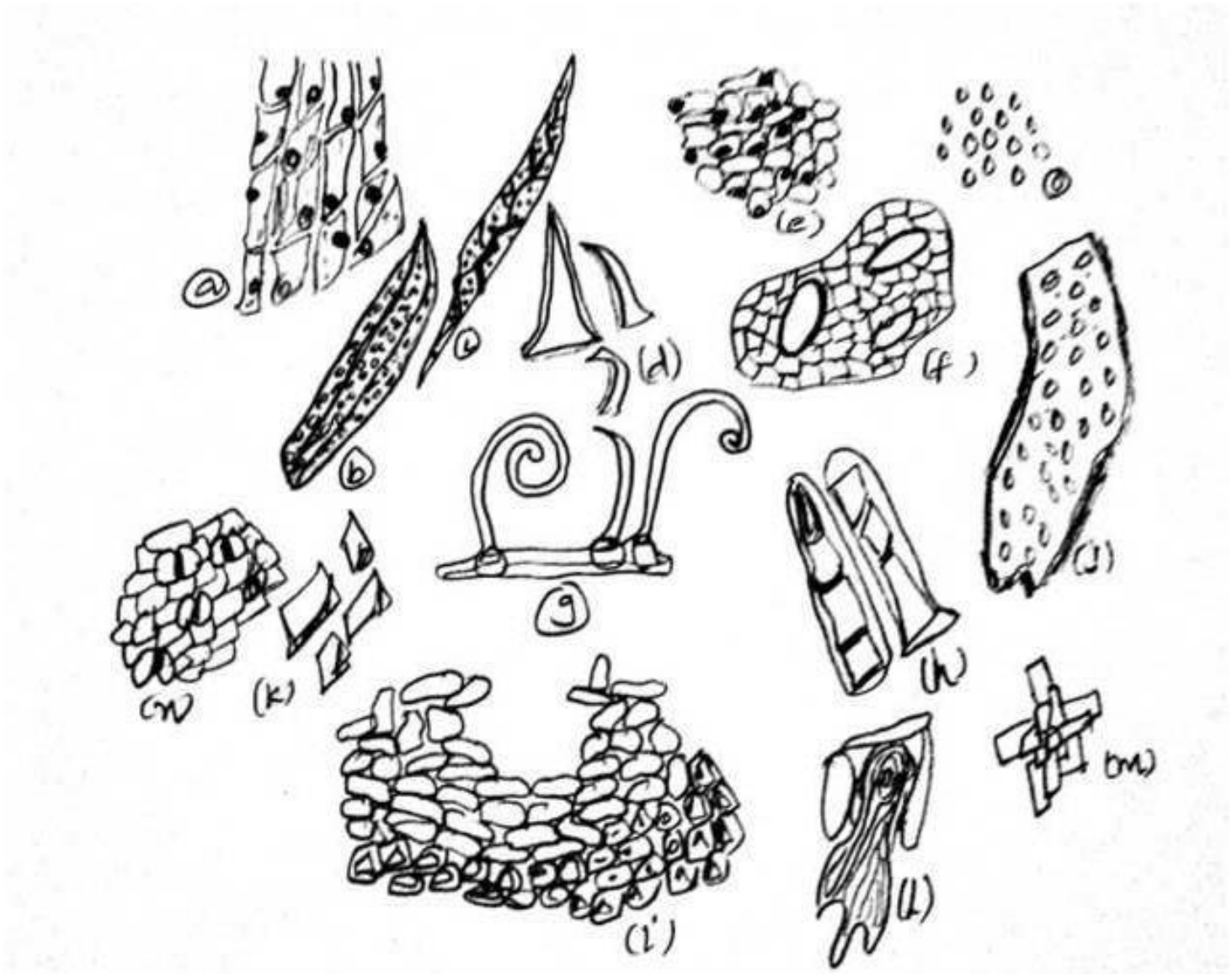


Figure 4. Microscopical examination of formulation 3 under 10 X magnification

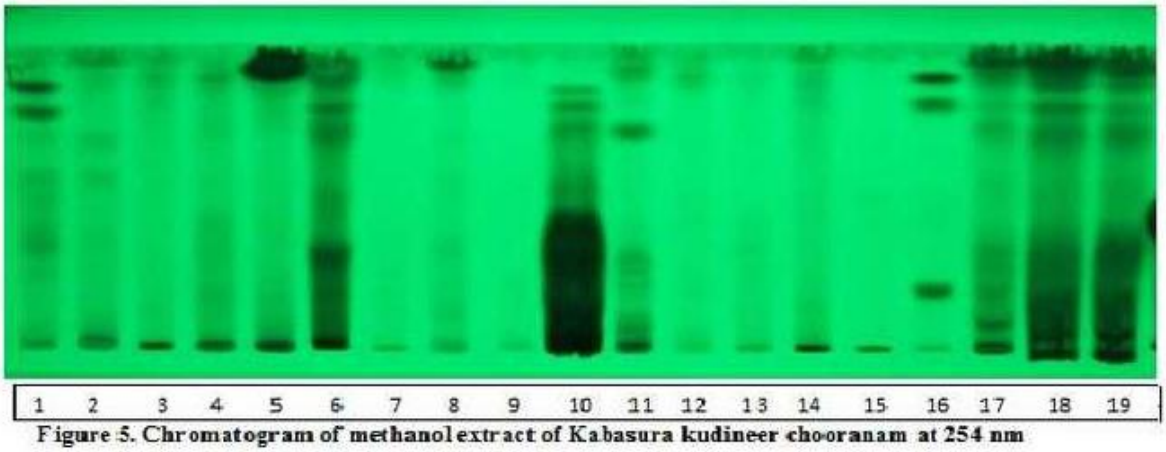


Figure 5. Chromatogram of methanol extract of Kabasura kudineer chooranam at 254 nm

1)Andrographis paniculata 2) Saussurea lappa 3) Cyperus rotundus 4) Zingiber officinale 5) Piper longum 6) Syzygium aromaticum 7) Tragia involucrata 8) Anacyclus pyrethrum 9) Hygrophillia auriculata 10) Terminalia chebula 11) Adathoda vasica 12) Coleus amboinicus 13) Tinospora cordifolia 14) Clerodendron serratum 15) Cissampelos pareira 16) Standards rutin, gallic acid and quercetin 17) Formulation 1 18) Formulation 2 19) Formulation 3

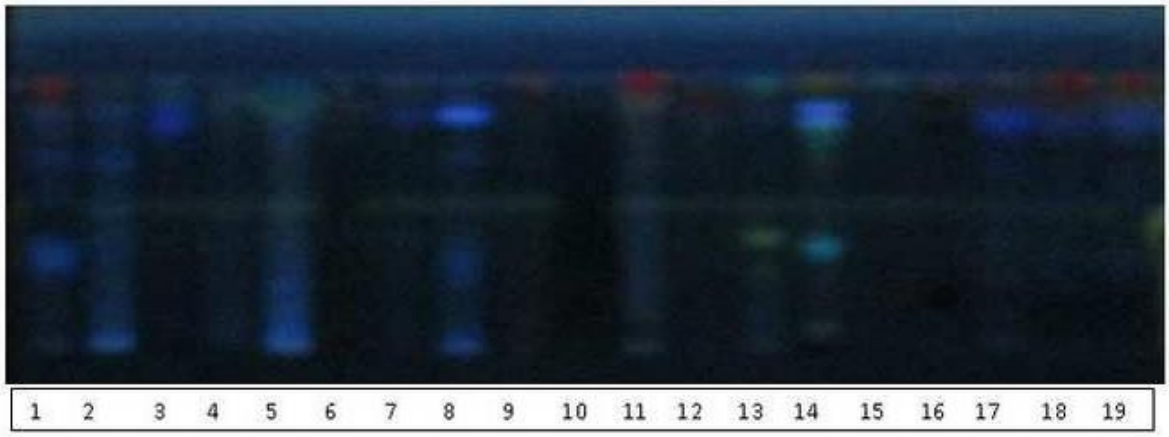


Figure 6. Chromatogram of methanol extract of Kabasura kudineer chooranam at 366 nm

1)Andrographis paniculata 2) Saussurea lappa 3) Cyperus rotundus 4) Zingiber officinale 5) Piper longum 6) Syzygium aromaticum 7) Tragia involucrata 8) Anacyclus pyrethrum 9) Hygrophillia auriculata 10) Terminalia chebula 11) Adathoda vasica 12) Coleus amboinicus 13) Tinospora cordifolia 14) Clerodendron serratum 15) Cissampelos pareira 16) Standards rutin, gallic acid and quercetin 17) Formulation 1 18) Formulation 2 19) Formulation 3

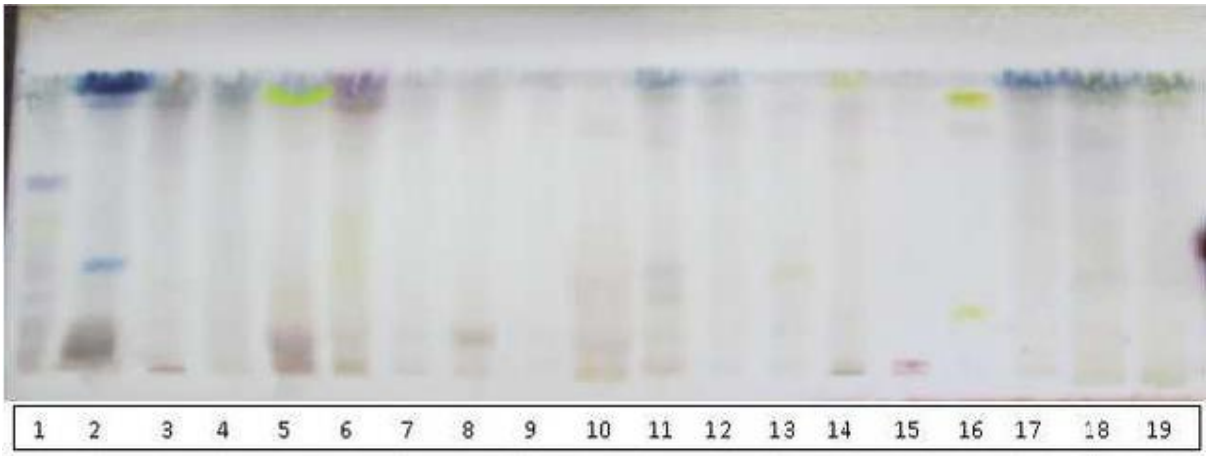


Figure 7.Chromatogram of methanol extract of Kabasura kudineer after dipping with vanillin sulphuric acid

1)Andrographis paniculata 2) Saussurea lappa 3) Cyperus rotundus 4) Zingiber officinale 5) Piper longum 6) Syzygium aromaticum 7) Tragia involucrata 8) Anacyclus pyrethrum 9) Hygrophillia auriculata 10) Terminalia chebula 11) Adathoda vasica 12) Coleus amboinicus 13) Tinospora cordifolia 14) Clerodendron serratum 15) Cissampelos pareira 16) Standards rutin, gallic acid and quercetin 17) Formulation 1 18) Formulation 2 19) Formulation 3

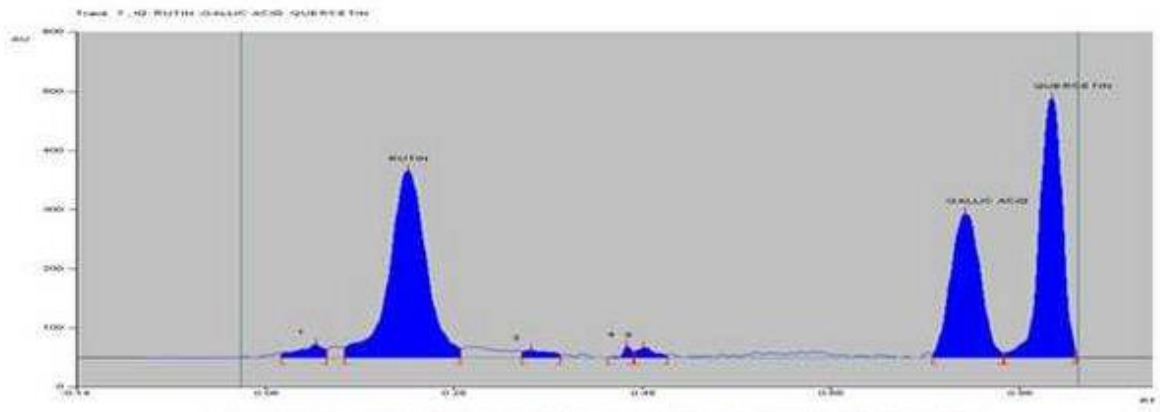


Figure 8. Densitogram of markers rutin, gallic acid and quercetin at 254 nm

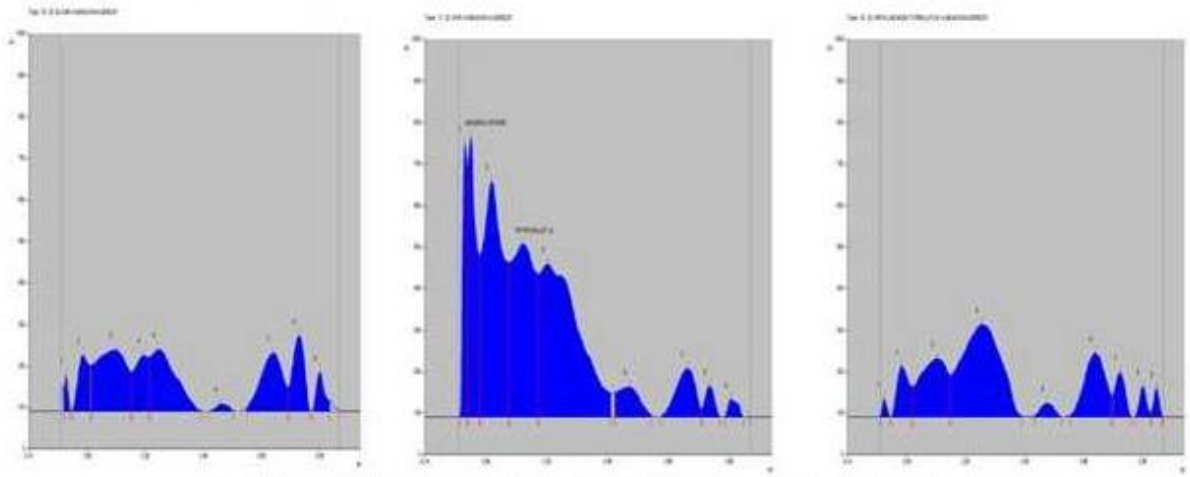


Figure 9. Densitogram of kabasura kudineer formulation 1, 2 and 3 at 254 nm

Figure 10. Photograph showing the total fungal growth in three formulation

Figure 11. Photograph showing the total microbial growth in three formulation

Figure 12. Photograph showing absence of *E.coli, P.aeruginosa , S. aureus* in formulation

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