



## Physicochemical characterization and HPTLC profiling of commercially available Kabasura Kudineer Chooranam

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The novel coronavirus disease-2019 (COVID-19) is an ongoing viral infection and currently, limited vaccines with no post-market safety data or repurposed drugs are available to combat this contagious viral disease. Kabasura Kudineer, an official polyherbal Siddha medicine containing numerous herbal ingredients, is being practiced primarily in southern parts of India in the management of COVID-19 pandemic for boosting the respiratory system and treating fever with flu-like symptoms. The present study is carried out to scientifically validate the traditional claim and to confirm identity, quality and purity of marketed Kabasura Kudineer polyherbal formulations in India. Kabasura Kudineer Chooranam manufactured by two different manufacturers was procured from New Delhi, India. The physicochemical characterization of the samples was carried out in accordance with the standards laid down by Indian Pharmacopoeia (IP) 2018. HPTLC profiling of key ingredients including Kalmegh (*Andrographis paniculata*), Guduchi (*Tinospora cordifolia*), Pippali (*Piper longum*), Vasaka (*Adhatoda vasica*) and Lavang (*Syzygium aromaticum*) were also carried out in accordance with IP 2018 monographs. The chromatographic analysis showed the presence of all major ingredients in both the marketed preparations and all the physicochemical properties were also found comparable among preparations. Our findings may enhance the global acceptance of Siddha medicines practiced widely in India and also used for laying down the pharmacopoeial standards of Kabasura Kudineer Chooranam.

**Keywords:** COVID-19, Guduchi, Kabasura Kudineer, Kalmegh, Lavang, Siddha medicine

**IPC Code:** Int Cl.<sup>21</sup>: AK61K 36/00, A61K 36/19, A61K 36/59, A61K 36/67, A61K 36/185, A61K 38/19

The novel corona virus disease (COVID-19), a pandemic affecting every continent, has caused significant distress around the globe. COVID-19, caused by highly pathogenic Severe Acute Respiratory Syndrome Corona-Virus 2 (SARS-CoV-2), has infected 29,439,989 Indians with confirmed mortality of 370,384 Indians as on June 13, 2021 (2133.3 cases and 26.8 deaths per 100 thousand population) and figure is still increasing briskly<sup>1</sup>. It is clear from available evidences and experiences, that quarantine measures and symptomatic treatment protocol for disease management is central to remain safe and delay the peak of this disease in an area where local transmission is ongoing<sup>2</sup>. Recently, few vaccines are invented for its cure and further prevention with no post-market safety data<sup>3</sup>, though repurposed approach

such as use of anti-retroviral drugs and hydroxychloroquine with limited efficacy have still been used in few centres across India<sup>4-6</sup>. Therefore, safe therapeutic agents and strategies to combat this deadly pandemic is the subject of investigation.

India is best known for its traditional systems of medicine-Ayurveda, Siddha, Unani, Homeopathy and management of epidemics is not new to our traditional health systems. Ayurveda, Siddha and Unani practitioners have significantly contributed to the management of epidemic and endemic i.e., dengue and chikungunya outbreaks, in southern parts of India<sup>7</sup>. Nilavembu Kudineer, a Siddha medicine, was widely dispensed using mobile units by healthcare professionals, state agencies and social organizations during the dengue outbreak in Tamil Nadu and observed to provide protection against infection and controlled morbidity<sup>8</sup>. The COVID-19 pandemic has brought another opportunity for Ayurveda, Siddha,

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Unani and Homeopathy practitioners to come up with indigenous preparations for the management of prevailing situations.

Kabasura Kudineer, reported in Siddha texts as “Citta Vaittiyattirattu”, is used for Aiyacuram (phlegmatic fevers) and is a potential medicine for cough, sore throat, high fever and difficulty in breathing. It consists of 15 distinct herbal ingredients, each of them having unique characteristic features of its own<sup>9</sup>. “Kabasuram Kudineer” in Siddha means fever due to immoderate accumulation of “Kapha” and this chooranam works by settling the “kapha dosha” which is infamous for infecting the respiratory system. The chooranam is highly effective for providing symptomatic relief in conditions associated with respiratory ailments such as fever, cough and cold, breathing difficulty and treating flu<sup>9</sup>. Kabasura Kudineer decoction may be given two times a day, after food to paediatrics (5 mL for <5 years, 10 mL for 5-12 years age group), adolescents (30 mL), adults (60-90 mL for 18-60 years age group) and geriatrics (60 mL for >60 years)<sup>8</sup>. The chooranam is generally available in powdered form (Fig. 1) and consumed after pouring it in lukewarm water and making a decoction or kadha like consistency.

At present, limited standards are available for Siddha polyherbal preparations. Presently, it is very difficult to identify the presence of all the ingredients as claimed in a polyherbal formulation. Hence, the prime task is to evolve such parameters by which the presence of ingredients can be checked. Assessment of physicochemical properties and various chromatographic methods can be attempted to generate pattern for identifying the presence of different ingredients<sup>10</sup>. This study is carried out in the context of growing public interest

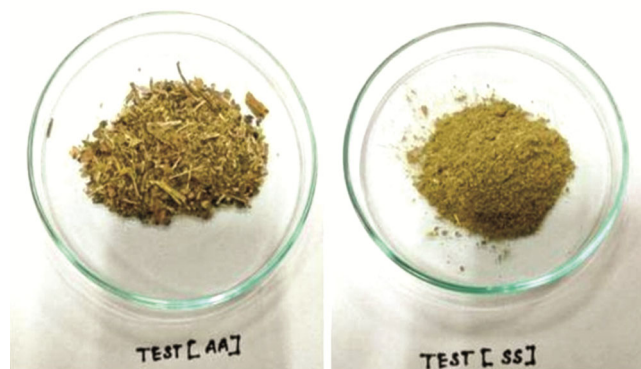


Fig. 1 — Two different Kabasura Kudineer Chooranam marketed preparations procured from New Delhi, India as shown in figure. For study purpose, preparations were named A A and S S.

for traditional systems of medicine. In present study, we characterized the commercially available Kabasura Kudineer Chooranam with respect to its physicochemical properties (organoleptic properties, pH, loss on drying, total ash, acid-insoluble ash, ethanol soluble extractives and water soluble extractives) and HPTLC profiling of key marker compounds as per pharmacopoeial methods.

## Materials & Methods

### Chemicals

Kabasura Kudineer Chooranam of two different manufacturers was procured from New Delhi, India and for study purpose formulations are coded as AA and SS (Compositional details of AA and SS formulations are tabulated in Table 1). The Botanical Reference Standards (BRS) of Kalmegh, Guduchi, Pippali, Vasaka and Lavang were obtained from the Indian Pharmacopoeia Commission (IPC), a standard setting autonomous organization of Ministry of Health & Family Welfare, Government of India, Ghaziabad. TLC aluminium plates (20 x 20 cm, 0.2 mm thickness) precoated with silica gel 60 F<sub>254</sub> were procured from Merck KGaA, Darmstadt, Germany. All other chemicals and reagents were of analytical grade until specifically indicated.

### Physicochemical characterization for polyherbal formulation

#### Organoleptic properties

Assessment of organoleptic properties refers to assessment of formulation for its colour, odour, taste, texture etc. Both the polyherbal chooranam were evaluated for their organoleptic characters as described by Siddiqui *et.al.*<sup>11</sup>.

#### pH

The pH was monitored using a calibrated digital pH meter (Mettler Toledo, Ohio, USA).

#### Loss on drying (LOD)

A glass-stoppered, shallow and dried weighing bottle weighed carefully. 1.0 g of sample was transferred to the bottle and the bottle along with the powder was accurately weighed. Sample was distributed evenly within practical limits by gentle sidewise shaking to a depth not more than 10 mm. Sample was dried by placing the loaded bottle in the drying chamber at 105°C for 3 h. Once drying is completed, the bottle was allowed to cool to room temperature in a desiccator before weighing. Weigh

Table 1 — Compositional details of Kabasura Kudineer Chooranam formulations from two different manufacturers

S. No.	Ingredients	AA sample (% w/w)	SS sample (% w/w)
1.	<i>Andrographis paniculata</i> Nees.	0.9	6.6
2.	<i>Syzygium aromaticum</i> (L.) Men. & L.M. Perry.	0.9	6.6
3.	<i>Piper longum</i> Linn.	0.9	6.6
4.	<i>Tinospora cordifolia</i> Willd.	0.9	6.6
5.	<i>Adhatoda vasica</i> Nees.	0.9	6.6
6.	<i>Zingiber officinale</i> Roscoe.	0.9	6.6
7.	<i>Tragia involucrata</i> Linn.	-	6.6
8.	<i>Anacyclus pyrethrum</i> Linn.	-	6.6
9.	<i>Hygrophila auriculata</i> Schumach.	-	6.6
10.	<i>Terminalia chebula</i> Retz.	-	6.6
11.	<i>Coleus amboinicus</i> Lour. ( <i>Plectranthus amboinicus</i> )	0.9	6.6
12.	<i>Saussurea lappa</i> Decne.	0.9	6.6
13.	<i>Clerodendrum serratum</i> (L.) Moon	0.9	6.6
14.	<i>Cissampelos pareira</i> Linn.	-	6.6
15.	<i>Cyperus rotundus</i> Linn.	0.9	6.6
16.	<i>Sphaeranthus indicus</i> Linn.	0.9	-

the bottle and the contents. Percentage LOD was calculated as follows-

$$\text{LOD (\% w / w)} = \frac{\text{Weight of sample (before drying)} - \text{Weight of sample (after drying)}}{\text{Weight of sample before drying (g)}} \times 100$$

#### Total Ash

2.0 g of the air-dried chooranam was weighed accurately in a tared silica crucible and incinerated, gently at first and the temperature was increased gradually up to 675±25°C until free from carbon. Sample was cooled in a desiccator, weighed the ash, and the percentage of ash with reference to the air-dried chooranam was calculated.

#### Acid-insoluble Ash

The ash, obtained as described in total ash, was boiled for 5 min. with 25 mL of 2 M hydrochloric acid and filtered. The insoluble material collected on an ashless filter paper was washed with hot water, ignited, cooled upto room temperature in a desiccator and weighed accurately. The percentage of acid-insoluble ash with reference to the dried chooranam was calculated.

#### Ethanol-soluble extractives

2.0 g of the air-dried coarsely powdered chooranam was macerated with 40 mL of absolute ethanol in a closed flask for 24 h with frequent stirring during the initial 6 h and then allowed to stand for 18 h. After

indicated time, sample was filtered rapidly considering the precautions against loss of ethanol, evaporated 10 mL of the filtrate to dryness in a tared flat-bottomed shallow dish, dried at 105°C and weighed accurately. The percentage of ethanol-soluble extractive with reference to the air-dried chooranam was calculated.

#### Water-soluble extractives

Chloroform-water, prepared as per IP 2018<sup>12</sup>, was used instead of ethanol. Rest of the protocol was similar as described in ethanol-soluble extractives.

#### Identification of herbal ingredients by HPTLC analysis

Kabasura Kudineer Chooranam is a poly-herbal preparation, consisting of nearly fifteen herbal ingredients. Though it is not easy to identify/characterize all fifteen herbal ingredients by HPTLC, we chose 5 key herbal ingredients namely *Andrographis paniculata*, *Syzygium aromaticum*, *Piper longum*, *Tinospora cordifolia* and *Adhatoda vasica* and HPTLC was performed using BRS of each selected herbal ingredients.

#### *Andrographis paniculata*

Mobile phase- Mixture of 7 volumes of chloroform and 1 volume of methanol

Test solution- 1.0 g of coarsely powdered chooranam was refluxed with 50 mL methanol for 15 min; cooled and filtered. The residue was further refluxed for two times with 50 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated to 10 mL.

Reference solution- 0.5 g of kalmegh BRS was refluxed for two times with 50 ml of methanol for 15 min, cooled and filtered. The residue was further refluxed for two times with 50 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated to 5 mL.

10 µL of each solution as bands of 8 mm by 2 mm was applied to the plate. The mobile phase was allowed to rise by 8 cm. The plate was dried in air and examined under ultraviolet light at 254 nm and 366 nm. The plate was sprayed with methanolic sulphuric acid (20%, v/v) and heated at 120°C for 5-10 min and examined in day light<sup>12</sup>.

#### *Syzygium aromaticum*

Mobile phase- Mixture of 93 volumes of toluene and 7 volumes of ethyl acetate

Test solution- 2.0 g of the coarsely powdered chooranam was refluxed with 50 mL of dichloromethane for 15 min, cooled and filtered. The residue was further refluxed for two times with 50 mL of dichloromethane, cooled and filtered. All the filtrates were combined and concentrated under vacuum to 25 mL.

Reference solution- 1.0 g of the coarsely powdered *Syzygium aromaticum* BRS was refluxed with 25 mL dichloromethane for 15 min, cooled and filtered. The residue was further refluxed for two times with 50 mL dichloromethane, cooled and filtered. All the filtrates were combined and concentrated under vacuum to 10 mL.

10 µL of each solution as bands of 8 mm by 2 mm was applied to the plate. The mobile phase was allowed to rise by 8 cm. The plate was dried in air and examined under ultraviolet light at 254 nm and 366 nm. The plate was sprayed with vanillin sulphuric acid reagent and heated at 105°C for 5 min and examined in day light<sup>12</sup>.

#### *Piper longum*

Mobile phase- Mixture of 60 volumes of benzene, 30 volumes of ethyl acetate and 10 volumes of diethyl ether

Test solution- 2.0 g of the coarsely powdered chooranam was refluxed with 50-75 mL of methanol for 15 min, cooled and filtered. The residue was further refluxed for two times with 75 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated under vacuum to 50 mL.

Reference solution- 0.4 g of the coarsely powdered *Piper longum* BRS was refluxed with 50-75 mL of

methanol for 15 min, cooled and filtered. The residue was further refluxed for two times with 75 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated under vacuum to 10 mL.

10 µL of each solution as bands of 8 mm by 2 mm was applied to the plate. The mobile phase was allowed to rise by 8 cm. The plate was dried in air and examined under ultraviolet light at 254 nm and 366 nm. The plate was sprayed with vanillin sulphuric acid reagent, heated at 100°C for 5-10 min and examined in day light<sup>12</sup>.

#### *Tinospora cordifolia*

Mobile phase- Mixture of 85 volumes of chloroform and 15 volumes of methanol

Test solution- 2.0 g of the coarsely powdered chooranam was refluxed with 25 mL of methanol for 15 min, cooled and filtered. The residue was further refluxed for two times with 25 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated under vacuum to 5 mL.

Reference solution- 2.0 g of the *Tinospora cordifolia* BRS was refluxed with 25 mL of methanol for 15 min, cooled and filtered. The residue was further refluxed for two times with 25 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated under vacuum to 5 mL.

10 µL of each solution as bands of 8 mm by 2 mm was applied to the plate. The mobile phase was allowed to rise by 8 cm. The plate was dried in air and examined under ultraviolet light at 254 nm and 366 nm. The plate was sprayed with anisaldehyde sulphuric acid reagent, heated at 110°C for 10 min and examined in day light<sup>12</sup>.

#### *Adhatoda vasica*

Mobile phase- Mixture of 8 volumes of ethyl acetate, 2 volumes of methanol and 0.2 volume of strong ammonia solution

Test solution- 1.0 g of coarsely powdered chooranam was refluxed with 50 mL methanol for 15 min, cooled and filtered. The residue was further refluxed for two times with 50 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated to 10 mL.

Reference solution- 0.5 g of *Adhatoda vasica* BRS was refluxed with 50 mL methanol for 15 min, cooled and filtered. The residue was further refluxed for two times with 50 mL of methanol, cooled and filtered. All the filtrates were combined and concentrated to 5 mL.

10 µL of each solution as bands of 8 mm by 2 mm

was applied to the plate. The mobile phase was allowed to rise by 8 cm. The plate was dried in air and examined under ultraviolet light at 254 nm and 366 nm. The plate was sprayed with Dragendorff's reagent, heated at 100°C for 5-10 min and examined in day light<sup>12</sup>.

A CAMAG TLC system equipped with CAMAG Automatic TLC Sample spotter 4 (ATS4), CAMAG glass twin trough chamber (20×10 cm), CAMAG TLC visualizer, CAMAG TLC scanner 4 and integrated win CATS software were used for the analysis. TLC was performed on 20×10 cm, Silica gel 60 F<sub>254</sub> precoated aluminium plates. Samples and standards were applied on the plate as 8 mm wide bands with an automatic TLC sampler 4 under a flow of nitrogen gas, 8 mm from the bottom and 15 mm from the side of the plate. The linear ascending development was carried out in a CAMAG twin trough chamber (20×10 cm) which was presaturated with 20 mL mobile phase for 20 min at room temperature (25±2°C and 40-60% relative humidity). The length of the chromatogram run was 8 cm. Subsequent to chromatographic development; TLC plates were dried in current air with the help of a dryer and visualized in CAMAG TLC visualizer at specified wavelengths.

#### Statistical analysis

Each experiment was repeatedly carried out three times and for all the quantitative parameters represented in table, the data are presented as mean value ± SD.

## Results

### Physicochemical characterization

Organoleptic characteristics and physicochemical parameters of both the marketed polyherbal preparation of Kabasura Kudineer Chooranam i.e., colour, texture, taste, odour, loss on drying, total ash, acid-insoluble ash, ethanol soluble extractive, water soluble extractive and pH are summarized in Table 2.

Sensorial characters of both the formulations (AA and SS) were same apart from appearance and texture. Formulation AA was observed as coarse, fibrous and uneven powder while SS was fine and smooth powder (Fig. 1). Loss on drying at 105°C for AA and SS were 8.63±0.021 and 6.06±0.021, respectively. The ash values (% w/w), viz., total ash and acid insoluble ash for AA and SS formulation were 7.55±0.071, 1.10±0.424 and 6.15±0.212, 1.55±0.354, respectively. Extractive values (%) viz., water soluble and ethanol soluble for AA and SS formulation were 2.97±0.007, 5.75±0.071 and 4.01±0.686, 6.14±0.02, respectively. pH of AA and SS formulation was recorded 6.35±0.255 and 5.92±0.042, respectively.

### Identification of herbal ingredients by HPTLC

The well resolved HPTLC profile of key ingredients of Kabasura Kudineer Chooranam and corresponding BRS at different wavelengths (254 nm & 366 nm) before and after derivatization as specified in individual IP-2018 monograph are shown in Fig. 2-6 to authenticate the presence of key ingredients in marketed Kabasura Kudineer Chooranam. Chromatogram of *Andrographis paniculata* BRS showed 3 clear bands at 254 nm (Rf values 0.29, 0.50 and 0.66) and 3 distinctive bands at 366 nm (Rf values 0.34, 0.38 and 0.62) before derivatization and 3 bands after derivatization in day light (Rf values 0.34, 0.53 and 0.67), which was also clearly evident in both the marketed AA and SS preparations (Fig. 2). HPTLC chromatogram of *Syzygium aromaticum* BRS shows 2 sharp bands at 254 nm (Rf values 0.51 and 0.74) and 2 bands at 366 nm (Rf values 0.37 and 0.60) before derivatization and 4 bands after derivatization in day light (Rf values 0.12, 0.32, 0.52 and 0.82) after derivatization in day light (Fig. 3). Marketed preparations of Kabasura Kudineer Chooranam, AA and SS showed characteristic bands at 254 nm and 366 nm before derivatization and after derivatization in day

Table 2 — Physicochemical characterization of two different commercially available kaba sura kudineer chooranam

S. No.	Parameter	AA*	SS*
1.	Organoleptic properties	Brownish yellow coarse powder, bitter in taste with aromatic odour	Brownish yellow fine powder, bitter in taste with strong aromatic odour
2.	Loss on Drying at 105°C (% w/w)	8.63 ± 0.021	6.06 ± 0.021
3.	Total Ash Content (% w/w)	7.55 ± 0.071	6.15 ± 0.212
4.	Acid Insoluble Ash (% w/w)	1.10 ± 0.424	1.55 ± 0.354
5.	Water Soluble Extractive (%)	2.97 ± 0.007	4.01 ± 0.686
6.	Ethanol Soluble Extractive (%)	5.75 ± 0.071	6.14 ± 0.021
7.	pH	6.35 ± 0.255	5.92 ± 0.042

mean ± RSD, n= 3

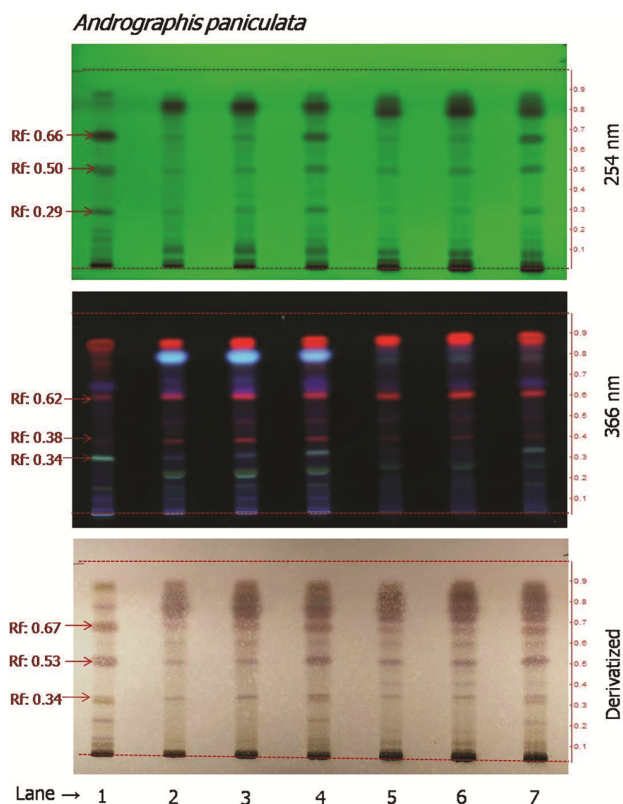


Fig. 2 — Photographs of HPTLC plates of Kabasura Kudineer Chooranam marketed preparations identified for *Andrographis paniculata* at 254 nm & 366 nm before derivatization and in day light after derivatization. Lane 1- 10 µL of BRS, Lane 2- 10 µL of AA sample, Lane 3- 15 µL of AA sample, Lane 4- 10 µL of AA sample spiked with 5 µL of BRS, Lane 5-10 µL of SS sample, Lane 6- 15 µL of SS sample, Lane 7- 10 µL of SS sample spiked with 5 µL of BRS.

light as shown by *Syzygium aromaticum* BRS chromatogram (Fig. 3). HPTLC chromatogram of *Piper longum* BRS showed 3 clear bands at 254 nm (Rf values 0.47, 0.53 and 0.62) and 3 bands at 366 nm (Rf values 0.47, 0.69 and 0.87) before derivatization (Fig. 4) which was also observed in AA formulation but respective bands in SS formulation were either absent or poorly visible. Chromatogram of *Tinospora cordifolia* BRS showed 4 clear bands at 254 nm (Rf values 0.39, 0.51, 0.69 and 0.77) and 4 bands at 366 nm (Rf values 0.16, 0.39, 0.77 and 0.87) before derivatization (Fig. 5) which was also clearly visible in AA formulation but respective bands in SS formulation were mostly absent (only 1 band at 254 nm and 2 bands at 366 nm were visible). HPTLC chromatogram of *Adhatoda vasica* BRS showed 2 clear bands at 254 nm (Rf values 0.23 and 0.83) and 3 distinctive bands at 366 nm (Rf values 0.09, 0.55 and 0.83) before derivatization which is equally marked in respective chromatograms of both the AA and SS formulations (Fig. 6).

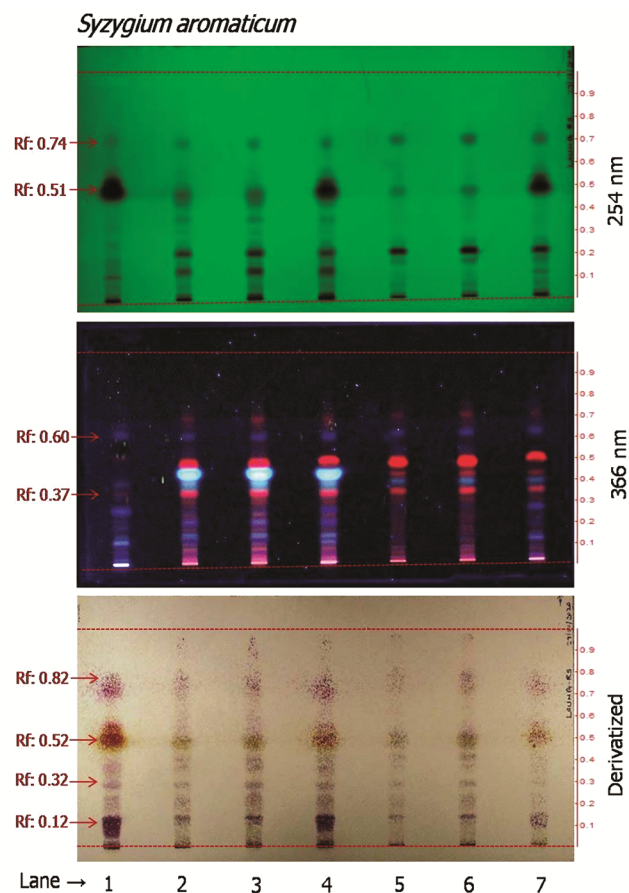


Fig. 3 — Photographs of HPTLC plates of Kabasura Kudineer Chooranam marketed preparations identified for *Syzygium aromaticum* at 254 nm & 366 nm before derivatization and in day light after derivatization. Lane 1- 10 µL of BRS, Lane 2- 10 µL of AA sample, Lane 3- 15 µL of AA sample, Lane 4- 10 µL of AA sample spiked with 5 µL of BRS, Lane 5-10 µL of SS sample, Lane 6- 15 µL of SS sample, Lane 7- 10 µL of SS sample spiked with 5 µL of BRS.

## Discussion

Indian systems of traditional medicine are known for the use of various polyherbal formulations and multi-ingredients therapeutics for the management of health and diseases. The present work dealt with detailed evaluation of marketed preparations of Kabasura Kudineer Chooranam, a polyherbal Siddha medicine, widely used in southern parts of India in symptomatic management of COVID-19, following the specifications prescribed by Indian Pharmacopoeia 2018. As public interest is growing enormously towards traditional systems of medicine during current pandemic outbreak, both the marketed preparations were tested for relevant physicochemical and analytical properties for its quality and consistent efficacy through various quality control measures as per

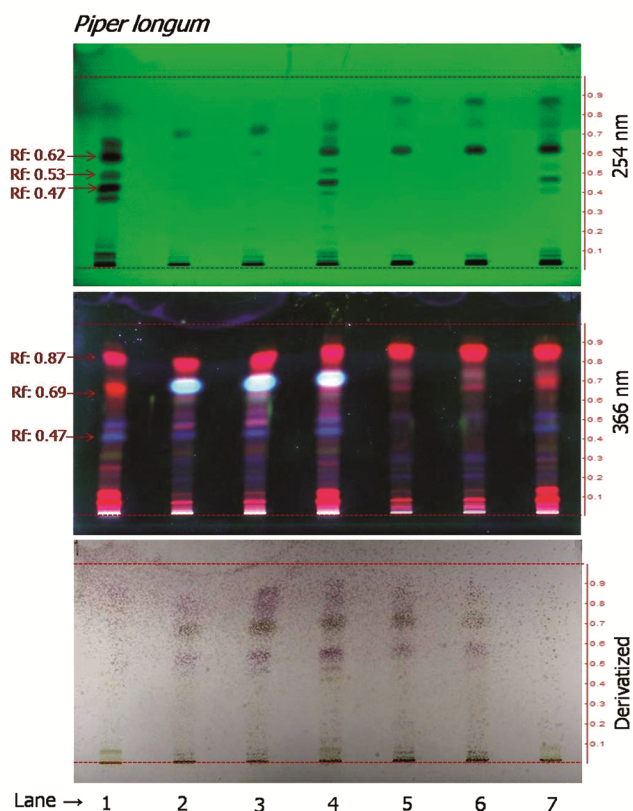


Fig. 4 — Photographs of HPTLC plates of Kabasura Kudineer Chooranam marketed preparations identified for *Piper longum* at 254 nm & 366 nm before derivatization and in day light after derivatization. Lane 1- 10 µL of BRS, Lane 2- 10 µL of AA sample, Lane 3- 15 µL of AA sample, Lane 4- 10 µL of AA sample spiked with 5 µL of BRS, Lane 5-10 µL of SS sample, Lane 6- 15 µL of SS sample, Lane 7- 10 µL of SS sample spiked with 5 µL of BRS.

pharmacopoeial specifications. Organoleptic properties are the characteristics of herbs/food/supplements as experienced by the sensory organs, including taste, appearance, smell, and touch. Even slight variation in organoleptic properties gives a primary indication about quality variation<sup>13</sup>. Hence, the characteristic organoleptic features of Kabasura Kudineer Chooranam formulations are reported in our work and a difference in appearance and texture was observed (Fig. 1). Well established physicochemical standards are crucial for assurance of uniformity of the quality of formulations and provide key information for further assessments and facilitate the identification of formulations in routine industrial production. The test for percentage of moisture content (loss on drying), one of the most widely used measurements in the processing and testing of foods and herbs, measures both water content and amount of volatile matter present in sample. Total ash denotes the amount of

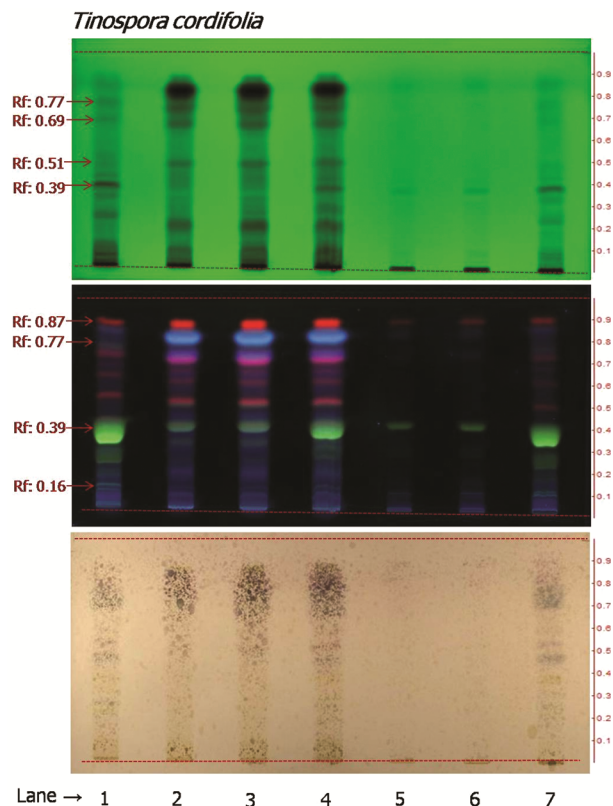


Fig. 5 — Photographs of HPTLC plates of Kabasura Kudineer Chooranam marketed preparations identified for *Tinospora cordifolia* at 254 nm & 366 nm before derivatization and in day light after derivatization. Lane 1- 10 µL of BRS, Lane 2- 10 µL of AA sample, Lane 3- 15 µL of AA sample, Lane 4- 10 µL of AA sample spiked with 5 µL of BRS, Lane 5-10 µL of SS sample, Lane 6- 15 µL of SS sample, Lane 7- 10 µL of SS sample spiked with 5 µL of BRS.

materials remaining after ignition. Acid insoluble ash assesses the amount of silica present especially, sand and siliceous matter in the samples. Extractive values are useful in particular for assessing consistency of nature and amount and type of chemical constituents present in the herbal drug. Considering the importance of these physicochemical parameters, Kabasura Kudineer Chooranam preparations were characterised by evaluating loss on drying at 105°C for 3 h, total ash, acid insoluble ash, ethanol soluble extractive, water soluble extractive and pH (Table 2). Both the formulations showed difference in their physicochemical properties and stimulated us to perform further investigation.

HPTLC is an important tool by which the key quality control procedures including fingerprints of herbs/phytopharmaceuticals can be assessed. HPTLC in comparison with TLC shows very good resolution and therefore, allows simultaneous identification of

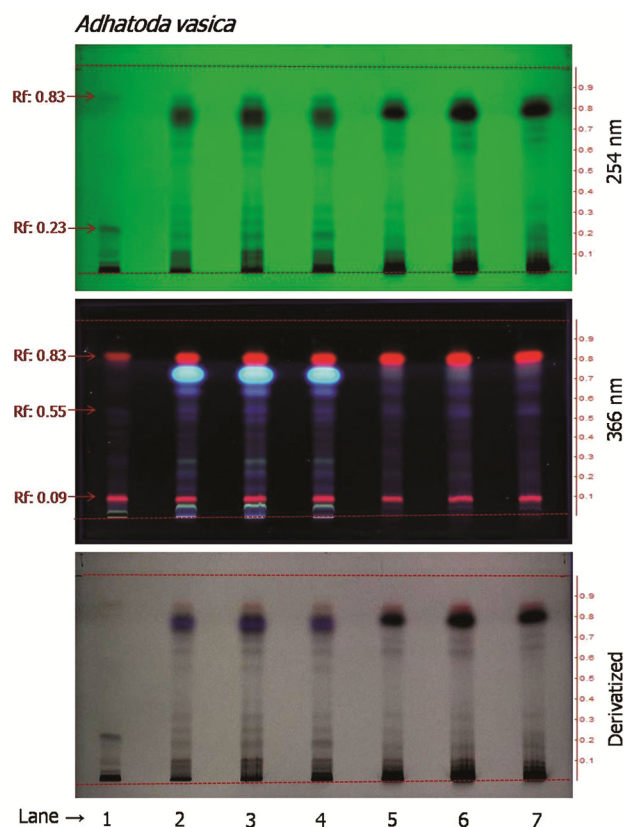


Fig. 6 — Photographs of HPTLC plates of Kabasura Kudineer Chooranam marketed preparations identified for *Adhatoda vasica* at 254 nm & 366 nm before derivatization and in day light after derivatization. Lane 1- 10  $\mu$ L of BRS, Lane 2- 10  $\mu$ L of AA sample, Lane 3- 15  $\mu$ L of AA sample, Lane 4- 10  $\mu$ L of AA sample spiked with 5  $\mu$ L of BRS, Lane 5-10  $\mu$ L of SS sample, Lane 6- 15  $\mu$ L of SS sample, Lane 7- 10  $\mu$ L of SS sample spiked with 5  $\mu$ L of BRS.

various compounds in a single run. It also helps to notify the individual herbs in herbal formulations. One of the main objectives of the HPTLC study of Kabasura Kudineer Chooranam was to develop a unique HPTLC chromatogram with their BRS as identifier of its key ingredients in formulations. HPTLC chromatograms of all the selected herbal ingredients with respect to their standards are indicative of their presence in both the marketed Siddha formulations AA and SS. Though, the presence of *Piper longum* and *Tinospora cordifolia* specific bands were less noticeable in SS formulation as compared to AA formulation. However, it might be a result of different source, variety or quality of herbal ingredients used in the formulation.

Tests and analytical procedures adopted in this study are as specified in Indian Pharmacopoeia 2018 and addenda. Pharmacopoeias i.e., IP, Ayurvedic

Pharmacopoeia of India, Siddha Pharmacopoeia of India etc. develop authoritative standards for herbs, their products and formulations containing herbal/natural ingredients by providing standard specifications and test methods. Further, there is a scope for harmonization in analytical methods and specifications adopted for herbs, their products and formulations containing herbal/natural ingredients as differences were observed in different pharmacopoeial requirements (for identification of *Piper longum* by HPTLC, IP 2018 recommend mixture of 60 volumes of benzene, 30 volumes of ethyl acetate and 10 volumes of diethyl ether as mobile phase while Ayurvedic Pharmacopoeia of India recommend 90 volumes of toluene and 10 volumes of ethyl acetate). Here, we are in agreement that adoption of a harmonized monograph could increase further the interest of the global stakeholders in alternative systems of medicine.

### Conclusion

Kabasura Kudineer Chooranam formulations are distinguished on the basis of various physicochemical and chromatographic parameters. The analytical specifications are established for the chooranam with respect to the procedures as mentioned in Indian Pharmacopoeia 2018. The chromatographic analysis showed the presence of all selected herbal ingredients in the AA chooranam while *Piper longum* and *Tinospora cordifolia* specific bands were less noticeable in SS chooranam as compared to AA. This study may serve as standard reference for assessing quality as well as quantity for selected herbs and quality control analysis of various Kabasura Kudineer Chooranam formulations available in the market.

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### Conflict of interest

All authors declare that no potential conflicts of interest exist regarding research, authorship and publication of article. The views, thoughts and opinions expressed in the text belong solely to the authors, and not necessarily to the authors' employer, organization, committee or other group or individual.



**Authors' contributions**

VKS and SS conceived and designed the study plan. SS, MUR completed the literature search, acquired the data and drafted the initial report. SS, RT, MS and VKS analysed and interpreted the data. VKS, EK and JP critically reviewed and finalized the manuscript. All authors have approved the final version and are in agreement to submit the manuscript.

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