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RESEARCH ARTICLE

# Simultaneous Quantification of Piperine, Vasicine and Eugenol in *Kabasura Kudineer* by HPTLC

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## Abstract

*Kabasura Kudineer* is a polyherbal decoction of the *Siddha* medical system (an Indian system of medicine), traditionally used to cure fever, colds, coughs, and respiratory ailments. The government of India had recommended *Kabasura Kudineer* as one of many preventive/treatment measures for COVID-19. *Kabasura Kudineer Choornam* is an admixed coarse powder of 15 herbs and its decoction is *Kabasura Kudineer*. The chemical constituents in the 15 herbs used for the preparation of the *Choornam* are known but the constituents present in the *Kabasura Kudineer* (decoction) are unidentified. Piperine, vasicine and eugenol are known for their potent activity against respiratory tract infections; hence, they were selected as marker compounds. The present work was planned to simultaneously quantify piperine, vasicine and eugenol in *Kabasura Kudineer* by the HPTLC method. The optimised mobile phase was toluene: ethyl acetate: methanol: ammonia (5:9:3:0.5, v/v/v/v), and the scanning was carried out at 287 nm. The $R_f$ values of piperine, vasicine and eugenol were found to be 0.70, 0.32 and 0.82, respectively. The linearity range of piperine and vasicine was 500-3000 ng spot$^{-1}$ and it was 10–60 ng spot$^{-1}$ for eugenol. The quantities of piperine, vasicine and eugenol in *Kabasura Kudineer* (100 mL) were 0.03, 0.056 and 0.035 % w/v, respectively. This developed method can be used to simultaneously quantify piperine, vasicine and eugenol in any polyherbal formulation.

**Keywords:** Cold, COVID-19, Decoction, Fever, *Kabasura Kudineer Choornam*, Respiratory Tract Infections

## 1. Introduction

*Siddha*, an Indian system of medicine has its origins in ancient Thamilakam (currently Tamil Nadu) and dates back to 10,000–4000 BCE[1]. The *Siddha* system is a collective practice of medicine, alchemy, spirituality and mysticism. *Siddha*, like another Indian system of medicine, has emphasised prevention and health rather than symptoms and diseases. Ministry of Ayurveda, Yoga and Naturopathy, *Unani, Siddha* and Homeopathy (AYUSH), Government of India had recommended many formulations for the prevention/ treatment of COVID-19; one such polyherbal formulation is *Kabasura Kudineer*. 'Kabam' means cold, 'Suram' means fever and 'Kudineer' means decoction; this medicine has been in practice for many hundred years. *Kabasura Kudineer* has traditionally been used to treat colds, coughs, fever and respiratory ailments[2]. It is available in the form of a coarse powder containing 15 herbs, known as *Kabasura Kudineer Choornam*. AYUSH recommends 5 g of *Choornam* (coarse powder) to be added to 240 mL of water, boiled and reduced to 60 mL. The filtrate obtained is referred to as *Kabasura Kudineer*[3]. In the past few decades, *Kabasura Kudineer* has been widely used as a prophylactic during viral epidemics[4]. It has also been used to combat the swine flu epidemic by the Government of Tamil Nadu, India, during the H1N1 influenza outbreak[5]. During an earlier outbreak of dengue, chikungunya and swine flu in Tamil Nadu, India, *Kabasura Kudineer* was used to control the febrile episodes.

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*Kabasura Kudineer Choornam* is prepared by mixing 15 herbs in equal proportions (Table 1). The chemical constituents in the 15 herbs used for the preparation of the *Choornam* are known but the constituents present in the *Kabasura Kudineer* (decoction) are unidentified. Earlier studies have only identified the major class of secondary metabolites of *Kabasura Kudineer,* like alkaloids, glycosides, phenols, saponins, etc[6]. When 15 herbs of different genera and families are added, it is obvious that the product mixture will contain almost all the class of major secondary metabolites. However, no detailed study has yet been carried out to identify the specific chemical constituent of an alkaloid or glycoside or phenol or saponin. The preparation process involves heating the *Choornam* in water (> 100°C) and the volume is reduced to 1/4$^{th}$. Higher temperatures may create a different chemical composition in the prepared decoction. The formation of New Chemical Entities (NCEs) is also possible.

Piperine, vasicine and eugenol are known for their potent activity against respiratory tract infections (both URTI and LRTI)[7–12] hence, they were selected as marker compounds. The present work was planned to simultaneously quantify piperine, vasicine and eugenol in *Kabasura Kudineer* by the HPTLC method.

## 2. Experimental

### 2.1 Ingredients and Chemicals

*Kabasura Kudineer Choornam* was procured from *Lakshmi Siddha Ayurveda Marundhagam,* Coimbatore, Tamil Nadu. Silica gel 60$F_{254}$ TLC plates were procured from Merck. Standard piperine, vasicine and eugenol were procured from Yucca Enterprises, Mumbai, Maharashtra, India. All solvents used were of analytical grade.

### 2.2 Method Development

Camag TLC system equipped with Linomat V, glass twin trough chamber (Camag, 20 X 10 cm) and visions CATS 3.1 software installed Camag scanner 4 was used

**Table 1.** Ingredients used in the preparation of *Kabasura Kudineer Choornam*

| S. No. | Binomial Name | Family | Parts Used |
|---|---|---|---|
| 1 | *Zingiber officinale* Roscoe | Zingiberaceae | Rhizome |
| 2 | *Syzygium aromaticum* (L.) Merr. & L.M.Perry | Myrtaceae | Flower bud |
| 3 | *Hygrophila auriculata* Schumach. | Acanthaceae | Root |
| 4 | *Justicia adhatoda* L. | Acanthaceae | leaf |
| 5 | *Piper longum* L. | Piperaceae | Fruit |
| 6 | *Tragia involucrate* L. | Euphorbiaceae | Root |
| 7 | *Sida acuta* Burm.f. | Malvaceae | Root |
| 8 | *Coleus amboinicus* Lour. | Lamiaceae | Leaf |
| 9 | *Tinospora cordifolia* (Thunb.) Miers | Menispermaceae | Stem |
| 10 | *Rotheca serrata* (L.) Steane & Mabb. | Lamiaceae | Root |
| 11 | *Cyperus rotundus* L. | Cyperaceae | Rhizome |
| 12 | *Dolomiaea costus* (Falc.) Kasana & A.K.Pandey | Asteraceae | Root |
| 13 | *Anacyclus pyrethrum* (L.) Link | Asteraceae | Root |
| 14 | *Andrographis paniculate* (Burm.f.) Nees | Acanthaceae | Whole plant |
| 15 | *Terminalia chebula* Retz. | Combretaceae | Fruit |

for the analysis. All basic protocol was carried out as per earlier reports[13]. TLC plates were densitometrically scanned in absorption-reflection mode with a slit width of 6 X 0.45 mm a scanning speed of 100 mm/s and a resolution of 100 µm.

### 2.3 Method Validation

The TLC method was validated for parameters like linearity, precision, accuracy, LOD, LOQ and robustness as per ICH guidelines[13,14]. Moreover, specificity and peak purity were also assessed. $R_f$ values and spectral overlay were compared with the marker compound for identification.

### 2.4 Preparation of *Kabasura Kudineer*

5 g of *Kabasura Kudineer Choornam* (coarse powder) was added to 240 mL of distilled water in a conical flask. The contents of the flask were heated until the volume was reduced to 60 mL. It was filtered and designated as *Kabasura Kudineer*[3]. 60 mL of *Kabasura Kudineer* were extracted with 40 mL of chloroform using a separating funnel. A rotary evaporator in vacuum condition was used to reduce the chloroform layer to 5 mL.

### 2.5 Preparation of Standard Solution

One mg of piperine and vasicine were dissolved separately in methanol to make 1 mg/mL concentrated stock solutions. Eugenol, 0.1 mL was diluted with 5 mL of diethyl ether to obtain the stock solution (0.02 mg/mL). By mixing all three standard solutions, a standard mixture solution was prepared for further processing.

### 2.6 Preparation of the Calibration Curve

Piperine and vasicine solutions were applied in the range of 500 to 3000 ng spot$^{-1}$ and eugenol in the range of 10 to 60 ng spot$^{-1}$ on the TLC plate. A calibration curve with peak area versus concentration was plotted for piperine, vasicine and eugenol.

### 2.7 Identification and Quantification of Piperine, Vasicine and Eugenol in *Kabasura Kudineer*

The external method was used for the identification of piperine, vasicine and eugenol in the sample. The sample volume applied was 5 µL and the optimised mobile phase was toluene: ethyl acetate: methanol: ammonia (5:9:3:0.5, v/v/v/v). The peak areas were recorded by scanning at 287 nm in absorption-reflection mode.

## 3. Results and Discussion

### 3.1 Identification of Piperine, Vasicine and Eugenol in *Kabasura Kudineer*

The $R_f$ values of the samples were in a match with piperine, vasicine and eugenol ($R_f$ values of piperine, vasicine and eugenol were 0.70, 0.32 and 0.82, respectively) (Figure 1). The spectral overlay confirmed the presence of piperine, vasicine and eugenol in the sample. The same procedure was repeated with the sample and standard mixture for the simultaneous estimation.

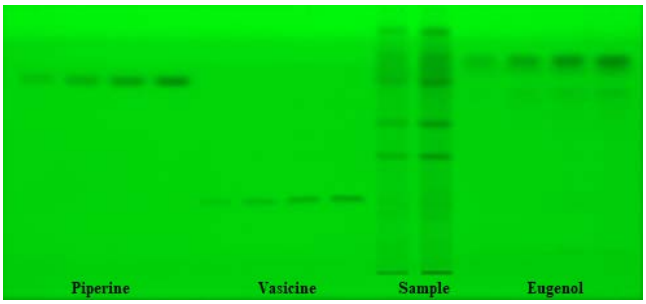


**Figure 1.** Identification of piperine, vasicine and eugenol in *Kabasura Kudineer.*

### 3.2 System Suitability

%RSD was < 2%, which shows that system suitability was excellent for the developed method.

### 3.3 Specificity

High specificity was shown when the $R_f$ values, spectra of standards and samples were compared.

### 3.4 Linearity and Detection Limit

The details of the linearity and detection limits are shown in Table 2. The linearity from 500 to 3000 ng spot$^{-1}$ was better for both piperine and vasicine. The calibration graph of eugenol showed better linearity from 10 to 60 ng spot$^{-1}$. LOD (limit of detection) for piperine, vasicine and eugenol was 578.41, 711.33 and 7.755 ng respectively. The limit of quantification was 1752.76, 2155.55 and 23.5 ng for piperine, vasicine and eugenol, respectively (Table 2).

**Table 2.** Linearity regression data for estimation of piperine, vasicine and eugenol

| Parameter | Piperine | Vasicine | Eugenol |
|---|---|---|---|
| $R_f$ | 0.70 | 0.32 | 0.82 |
| Linearity range (ng spot$^{-1}$) | 500-3000 | 500-3000 | 10-60 |
| Equation | y = 0.00001x + 0.021 | y = 0.000001x + 0.000 | y = 0.00004x + 0.005 |
| Correlation coefficient | 0.994 | 0.994 | 0.996 |
| Specificity | Specific | Specific | Specific |
| LOD (ng) | 578.41 | 711.33 | 7.755 |
| LOQ (ng) | 1752.76 | 2155.55 | 23.5 |

LOD - Limit of Detection; LOQ - Limit of Quantification

**Table 3.** Precision data for piperine, vasicine and eugenol

| Parameter | Standard | % RSD* |
|---|---|---|
| **Instrumental precision** | Piperine | 1.500237 |
| | Vasicine | 1.258356 |
| | Eugenol | 1.336359 |
| **Interday precision** | Piperine | 0.756984 |
| | Vasicine | 1.438277 |
| | Eugenol | 1.278448 |
| **Intraday precision** | Piperine | 0.816159 |
| | Vasicine | 1.399573 |
| | Eugenol | 1.083676 |

*n = 3

**Table 4.** Recovery studies data of piperine, vasicine and eugenol

| % Level | Standard | Amount of Standard in Sample (µg) | Amount Spiked (µg) | Mean % Recovery* |
|---|---|---|---|---|
| 80% | Piperine | 1.93 | 1.5 | 99 |
| | Vasicine | 1.8 | 1.4 | 98.97 |
| | Eugenol | 0.046 | 0.036 | 98.88 |
| 100% | Piperine | 1.93 | 1.9 | 101 |
| | Vasicine | 1.8 | 1.8 | 99.57 |
| | Eugenol | 0.046 | 0.046 | 99.53 |
| 120% | Piperine | 1.93 | 2.3 | 100.37 |
| | Vasicine | 1.8 | 2.1 | 102.33 |
| | Eugenol | 0.046 | 0.056 | 101.53 |

*n = 3

### 3.5 Precision and Robustness Studies

Precision on intraday and interday for consecutive three days was determined and expressed as % RSD (Table 3). % RSD was found to be within limits indicating better robustness of the method.

### 3.6 Simultaneous Quantification and Recovery Studies

The quantities of piperine, vasicine and eugenol in *Kabasura Kudineer* (100 mL) were 0.03, 0.056 and 0.035% w/v, respectively. Recovery studies were carried out at 80, 100 and 120 % and the data is shown in Table 4.

## 4. Conclusion

The currently developed HPTLC method was found to be simple, specific, precise, robust and accurate for the simultaneous quantification of piperine, vasicine and eugenol. This method can be used effectively for the identification and quantification of piperine, vasicine and eugenol in *Kabasura Kudineer.* The amounts of piperine, vasicine and eugenol in 100 mL of *Kabasura Kudineer* was found to be 0.03, 0.056 and 0.035 % w/v respectively. The developed TLC method can be used to simultaneously quantity piperine, vasicine and eugenol in any polyherbal formulation.

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