Effect of other spices in the extraction of piperine from *Piper nigrum* in *rasam* formulation

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Abstract
Rasam is a South Indian traditional spice soup. Different types of *rasam*are made with spices in which their number, quantity and processes are different. Our earlier studies have standardized the ingredients used, their quantity, and process involved in the preparation of *rasam*. Traditional functional foods can maintain a healthy state of the body, prevent diseases, improve quality of life and thereby reducing healthcare cost. *Rasam*is a traditional functional food as the ingredients used in its preparation are medicinally used forvarious ailments. In view of all the above facts, the current research work was envisaged to study the effects of other spices (tamarind and cumin) in the extraction of piperine from *Piper nigrum* during the preparation process of *rasam*. Powdered black pepper was extracted with distilled water, cumin water and tamarind water to yield S1, S2 and S3 respectively. The ethereal layer of S1, S2 and S3 was subjected to HPTLC studies for the quantification of piperine. The piperine content in S1, S2 and S3 was found to be 0.002, 0.004 and 0.005 %w/w respectively. The concentration of piperine was in the order of S1>S2>S3. It is clear that the acidic pH of tamarind and cumin water were responsible for the higher extraction of piperine from black pepper. The processing of *rasam* traditionally involves heating black pepper in tamarind water and the current study proves the scientific fact that more piperine will be extracted with tamarind water rather than water.
Key words:*Rasam,*tamarind water, piperine, black pepper.

Introduction

Most spices are carminatives and play an important role in improving digestion hence, they are a vital ingredient in most of the Indian tradition foods. In India, spices have been used traditionally, since a long time to prevent and treat various diseases [1]. In South Indian traditional foods, coriander, black pepper, cumin, asafoetida, garlic,tamarind, black mustard, chili pepper, turmeric, curry leaves etc. are the frequently used. These spices are known for their digestive property, antimicrobial activity, antidiabetic activity, cholesterol-lowering activity, anti-lithogenic activity, antioxidant activities, anti-inflammatory activities and anticancer activity [2].

Rasam or *saaru*or *chaaru*in South Indian languages like Tamil, Malayalam, Telugu, and Kannada refers to a traditional spice soup. *Rasam* means “the essential products of digestion” in Sanskrit [3].*Rasam* can be considered as an ideal recipe as per the Indian system of medicines (ISM) especially, Ayurveda and Siddha as it delivers mixture of different tastes simultaneously [4].*Rasam* is prepared with different spices in tamarind water by boiling (Table 1). Different types of *rasam*are made with spices in which their number, quantity and processes are different [5].The ingredients used, their quantity, and process involved in the preparation of *rasam* has been standardizedin our earlier studies [6].Other studies reported on the standardized *rasam* includes, marker based analytical studies [7], antimicrobial studies

[8], cytotoxic, antimitotic, and antiproliferation studies [9], analytical studies by Gas Chromatography – Mass Spectrometry (GC-MS) [10] and breast cancer studies.[11]The physiochemical properties of *rasam* was evaluated and reported earlier [12].The chemical constituents of *rasam* was screened in silico as a beneficial supplementary treatment for COVID-19 [13,14].*Rasam*is a traditional functional food as the ingredients used in its preparation are medicinally used for various ailments. Traditional functional foods can maintain a healthy state of the body, prevent diseases, improve quality of life and thereby reducing healthcare cost. In view of all the above facts, the current research work was envisaged to study the effects of other spices (tamarind and cumin) in the extraction of piperine from *Piper nigrum* during the preparation process of *rasam*.

Methods

Black pepper, cumin and tamarind were obtained from local market in Vadodara, Gujarat, India. Piperinestandard was procured from Yucca enterprises, Mumbai, India. All reagents/solvents and chemicals used were of analytical grade. Black pepper, cumin and tamarind were passed through # 80 mesh sieve before use.Merck silica gel60F_{254}TLC plates were used for theHPTLC study. CamagTLC systemequippedwithCamagLinomatV was used for the current study. Evaluations of the plates was performed with Camag scanner4 (visionCATS3.1 integrationsoftware).

Preparation of spice solutions: Cumin (6 g) and tamarind (6 g) were added to 120 mL of distilled water separately and continuously stirred for 1 hour. The solutions were filtered to yield cumin water and tamarind water. The test spice solutions designated for black pepper extraction was cumin water and tamarind water while distilled water served as blank control. The pH of cumin water, tamarind waterand distilled water were measured before black pepper extraction using a digital pH meter.

Extraction of black pepper with spice solutions: 6 g of powdered black pepper was extracted with distilled water, cumin water and tamarind water (100 mL each) separately for 1 hour at 50 °C to yield the extracts S1, S2 and S3 respectively. About 25 mL of S1, S2 and S3 was extracted with 25 mL of diethyl ether separately. The ether layer of S1, S2 and S3 was separated and subjected to HPTLS study for the quantification of piperine.

Quantification of piperine by HPTLC method: A standard piperine solution was prepared with methanol (1 mg/mL). 2 and 4 µL of S1, S2 and S3 were applied on the TLC plate for analysis. Toluene: ethyl acetate(7:3, v/v) was used as the mobile phasefor the development of TLC plate. After development, the plate was dried and scanned at 254nm. The HPTLC chromatography estimation of piperine was carried out as per the reported method [1].

Results

The pH of distilled water, cumin water and tamarind water were found to be 6.9, 5.3 and 2.2respectively.Tamarind water is acidic in nature due to the presence of large quantities of tartaric acid in fruit pulp. The calibration curve of standard piperine is shown in Figure 1. Thepeakarea versus concentration graph for standard piperinewas found to be linear in the range of 500to3000 ng spot^{-1}. The R_f value of standard piperine was 0.86±0.01 and the R_f values of the corresponding bands in S1, S2 and S3 to that of standard piperinewas 0.87±0.01, 0.86±0.01, 0.86±0.01 respectively (Figure 2). The corresponding bands in S1, S2 and S3 to that of standard piperine was confirmed as piperine after spectral overlay study (Figure 3). The piperine content in S1, S2 and S3 was found to be 0.002, 0.004 and 0.005% w/w respectively.

Table 1: Botanical source of the ingredients used in the preparation of *rasam.*

Ingredients	Botanical source
Dried garlic cloves	*Allium sativum* L. (Amaryllidaceae)
Dried black mustard seeds	*Brassica nigra* L. (Brassicaceae)
Dried, crushed and whole fruits of long chili pepper	*Capsicum annuum* L. (Solanaceae)
Fresh coriander leaves	*Coriandrum sativum* L. (Apiaceae)
Dried ripped cumin fruits	*Cuminum cyminum* L. (Apiaceae)
Dried rhizome powder of turmeric	*Curcuma longa* L. (Zingiberaceae)
Dried powder of asafetida	*Ferulaassa-foetida* L. (Apiaceae)
Fresh curry leaves	*Murrayakoenigii* (L.) Sprengel (Rutaceae)
Dried unripped black pepper drupes	*Piper nigrum* L. (Piperaceae)
Indian sesame oil	*Sesamum indicum* L. (Pedaliaceae)
Fresh ripped tomato	*Solanum lycopersicum* L. (Solanaceae)
Dried ripped fruit pulp of tamarind	*Tamarindus indica* L. (Fabaceae)

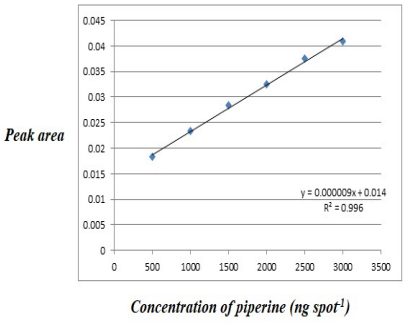

Figure 1: Calibration curve of standard piperine.

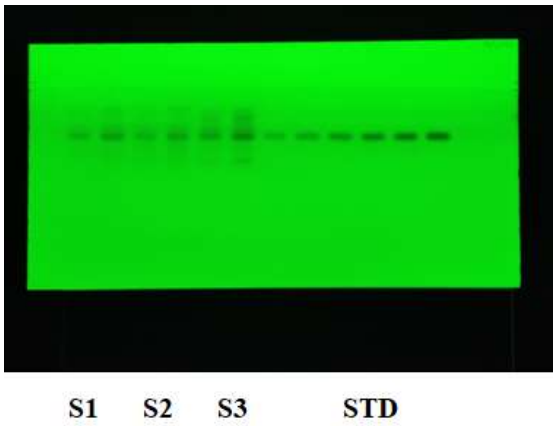

Figure 2:TLC plate showing standard piperine(STD) and the corresponding bands in S1, S2 and S3. (S1-distilled water extract; S2-cumin water extract; S3-tamarind water extract)

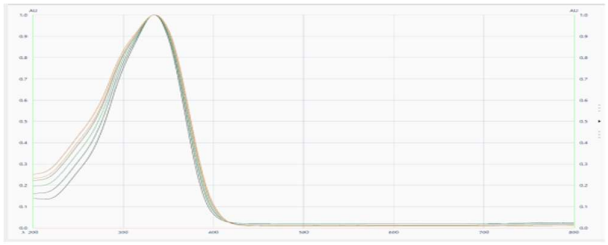

Figure 3: Spectral overlay of standard piperine and the corresponding bands in S1, S2 and S3.

Discussion and Conclusion

In the present investigation, black pepper extraction in the presence of different spices like cumin and tamarind was tested. The results clearly show that the quantity of piperine in S3 (black pepper extracted in tamarind water) was more than S2 (black pepper extracted in cumin water). The quantity of piperine in S2 was more than S1 (black pepper extracted in distilled water). The concentration of piperine was found to be S1>S2>S3 as per the present study. It is very clear that the acidic pH of tamarind and cumin water is responsible for the higher extraction of piperine from black pepper. Minimum quantity of piperine was detected

in distilled water due to its neutral pH. The processing of *rasam* traditionally involves heating black pepper powder in tamarind water. This traditional way of preparing *rasam* proves the scientific fact that more piperine will be extracted when heated with tamarind water rather than water. Further study is required to investigate the influence of tamarind water on other active ingredient of different spices used in *rasam*.

Conflict of interest: None

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