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Ingredient Elimination Effects on Bioactive Compound Extraction in *Rasam*: An HPTLC-based Quantitative Study

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Abstract

Background: *Rasam* is a traditional South Indian nutritious food that is made with *Tamarindus indica, Piper nigrum, Capsicum annuum, Curcuma longa* and other spices. The role of individual ingredients in maximising phytochemical extraction is not yet understood despite the standardisation and chemical characterisation of *Rasam*. **Aim:** To determine how the removal of specific ingredients affects the HPTLC-based quantitative analysis of the principal bioactive compounds in *Rasam.* **Method:** Four formulations were prepared, including control (all ingredients), T_1 (without tamarind and turmeric), T_2 (without tamarind), and T_3 (without chilli pepper). Validated HPTLC methods were employed to quantify piperine, capsaicin, curcumin, and ascorbic acid. **Results:** Control *Rasam* contained piperine (0.185 µg/mL), capsaicin (3.95 µg/mL), curcumin (1.143 µg/mL), and ascorbic acid (3.56 µg/mL). Removal of tamarind and turmeric reduced piperine (8.1%) and capsaicin (59.0%). Elimination of tamarind alone reduced curcumin extraction by 73.7%, while removal of chilli pepper decreased ascorbic acid by 16.0%. **Conclusion:** *Rasam* functions as a pH-mediated phytochemical extraction system, where tamarind-derived acidity and ingredient synergy significantly influence bioactive compound recovery.

Major Findings: Removal of tamarind significantly reduced curcumin extraction (73.7%), while elimination of tamarind and turmeric markedly decreased capsaicin (59.0%) and piperine (8.1%) levels. These findings highlight the critical role of tamarind-derived acidity in optimising phytochemical extraction in *Rasam*.

Keywords: Functional Food, HPTLC, Ingredient Synergy, Phytochemical Extraction, pH-Dependent Extraction, *Rasam*

Abbreviations: HPTLC - High-Performance Thin-Layer Chromatography, T1, T2, T3 - Selective elimination of ingredient formulations.

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1. Introduction

Rasam is a traditional functional food of South India, which is commonly used in Tamil Nadu, Karnataka, and Andhra Pradesh, traditionally known to have digestive, antimicrobial and therapeutic properties. It is made as an aqueous decoction of tamarind *(Tamarindus indica*) pulp, in a specific combination with spices, such as black pepper (*Piper nigrum*), cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), red chilli (*Capsicum annuum*) and turmeric (*Curcuma longa*)[1,2]. *Rasam* preparation over generations has been standardised empirically by cooking, and this suggests that it is an optimised formulation and not a random combination of ingredients. Previous

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studies have reported physicochemical properties, standardisation parameters, and phytochemical characterisation of *Rasam*, identifying major bioactive constituents such as piperine, capsaicin, curcumin, and ascorbic acid[1,3]. In addition, GC–MS-based identification has confirmed the presence of multiple volatile and non-volatile chemical constituents in *Rasam*[8]. Furthermore, simultaneous quantification of curcumin, piperine, and capsaicin using validated HPTLC methods has been established, confirming the suitability of chromatographic techniques for biomarker analysis in *Rasam*[13]. These substances have been reported to add to the functional properties of *Rasam,* such as antimicrobial and digestive-supportive effects[5]. Cytotoxic, antimitotic, and antiproliferative activities of *Rasam* have also been reported, supporting its broader therapeutic potential[9]. Anti-platelet aggregation activity has been documented, indicating potential cardiovascular relevance[11]. Moreover, *Rasam* has shown chemopreventive effects due to its ability to suppress the mammary tumour formation in the study animal models[7]. It was observed that the acidic conditions produced by tamarind pulp are important to increase the phytochemical extraction in the decoction process, and thus, to improve the extraction of alkaloids and curcuminoids[3,4]. High-Performance Thin-Layer Chromatography (HPTLC) analysis studies have also verified the appropriateness of the technique in making quantitative estimates of these bioactive markers in *Rasam* formulations[6,10]. Even though *Rasam* is chemically characterised and standardised, the role of each ingredient with regard to maximised phytochemical extraction has not been adequately investigated. Specifically, the literature on systematic studies to assess the impact of selective removal of ingredients in the recovery of bioactive compounds is very scarce[3,4]. The knowledge of the role of individual components to scientifically justify traditional preparation practices is also crucial for preserving formulation integrity. In silico screening studies have also explored the chemical constituents of *Rasam* as potential supplementary agents for viral infections[12]. Thus, the current research was designed to determine the impact of selective removal of ingredients on HPTLC-based quantitative extraction of major bioactive compounds in *Rasam*.

2. Materials and Methods

2.1 *Rasam* Preparation - Control Sample

The composition and quantity of ingredients used for the preparation of *Rasam* are presented in Table 1.

The *Rasam* control sample was prepared in five stages as per the reported literature:

Tamarind fruit pulp blend (T1): 6.88g of tamarind fruit pulp was blended with 450 mL of water for 10 min, then crushed manually 45 times and then filtered. Then, 5 mL of water, 4 g of sea salt, and 0.4 g of turmeric powder were put into the filtered liquid.

Tomato mixture (T2): Fresh tomato fruits (82.44 g) were mixed with water and crushed manually (60 times) and washed with 5 mL of water.

Spice blend (T3): The pepper drupes were ground in a mortar and pestle 85 times (m = 1.33 g). The cumin fruits (2.67 g) were added and crushed (100 times) with the crushed pepper. After that, 0.82 g of chilli pepper was put into it and crushed 50 times together with the mixture. Cloves of garlic were put into the mix and crushed 90 times, 9.63 g.

Combined mixture (T4): After rinsing, all the mixture T4 with tomato fruit mixture T2 and spice mixture T3 was washed with 10 mL of water and mixed with tamarind fruit pulp T1.

Final product (T5): Indian sesame oil (4.0 mL) was heated to 60°C in a stainless-steel vessel for 2 min.

Table 1. Quantity of ingredients used in *Rasam* preparation

Ingredients	Quantity
Tamarind	6.88 g
Turmeric	0.4 g
Sea salt	4 g
Tomato	82.44 g
Powdered chili	0.82 g
Cumin	2.67 g
Garlic	9.63 g
Black pepper	1.33 g
Indian sesame oil	4 mL
Black mustard	0.82 g
Whole chili	1.53 g
Curry leaves	0.61 g
Coriander	1.50 g
Asafoetida	0.05 g

Black mustard seeds (0.82 g) were added and allowed to splutter, followed by the addition of whole chilli peppers (1.53 g) and curry leaves (0.61 g). Subsequently, the combined mixture (T4) was added along with 20 mL of distilled water. The mixture was boiled for 5 min, after which fresh coriander leaves (1.50 g) and asafoetida (0.05 g) were added. Boiling was terminated upon frothing to obtain a standardised *Rasam*.

2.2 *Rasam* Test Samples Preparation

T1: Tamarind and turmeric were omitted.
T2: Tamarind omitted
T3: Chilli pepper omitted

Sample solution preparation control sample *Rasam*:

Fifty millilitres of *Rasam* were extracted with 50 mL of diethyl ether using a separating funnel. The ether layer was separated and re-extracted with an additional 50 mL of ether. The combined ether extracts were filtered and concentrated to 30 mL (CS1). The remaining aqueous layer was also concentrated to 30 mL (CS2).

2.3 Sample Preparation

Fifty millilitres of *Rasam* (control or test) were extracted with 50 mL of diethyl ether using a separating funnel. The ether layer was collected and re-extracted with an additional 50 mL of ether. The combined ether extracts were filtered and concentrated to 30 mL (organic extract), while the remaining aqueous layer was also concentrated to 30 mL (aqueous extract).

2.4 Piperine, Capsaicin, Curcumin, and Ascorbic Acid Standard Solution Preparation

Weighed 10 mg of the ingredients, piperine, capsaicin, curcumin, and ascorbic acid, and put them in 10 mL volumetric flasks. The volume was filled with methanol (HPLC grade) individually to obtain a concentration of 1000 µg/mL. The relevant dilution of the stock solution with the same solvent was done to prepare working solutions. The stock and working solutions were placed in a refrigerator at 2-4 °C temperature and kept away from light.

2.5 Preparation of Calibration Curve

Calibration curves of peak area versus concentration of standard piperine, capsaicin, curcumin and ascorbic acid solutions in concentrations of 1 to 5µg spot were prepared on TLC plates.

2.6 Piperine, Capsaicin, and Curcumin Determination in Control and Test *Rasam*

External standard Curcumin, capsaicin and piperine standards were used to identify the compounds by external standard. Sample solution was put on the TLC plate and developed in the toluene: ethyl acetate (7:3v/v) mobile phase. The scan was performed at 254 nm through absorption-reflection mode. Measurements on peak areas were taken, and the concentration of piperine, capsaicin, and curcumin was measured based on the calibration curve.

2.7 Ascorbic Acid in Control and Test Samples of *Rasam* was Estimated

It was quantified by applying the sample solution (aqueous extract of *Rasam)* in triplicate to the TLC plate by applying 5 µL of the sample solution, which was developed using the mobile phase consisting of ethyl acetate: acetone: water: formic acid (10:6:2:2 v/v) as an external standard. Anisaldehyde-sulphuric acid was added to the dipping chamber to perform post-chromatographic derivatisation, after which it was heated in an oven at 105°C for a time between 5 and 10 minutes. Densitometric scanning was carried out by using the absorption reflection mode at 527 nm. Peak areas were obtained, and the quantity of ascorbic acid was determined by use of the calibration curve.

3. Results and Discussion

3.1 HPTLC Method Performance and System Suitability

The developed HPTLC methods provided clear and reproducible separation of piperine, capsaicin, curcumin, and ascorbic acid from *Rasam* extracts. Densitometric scanning was performed in absorption–reflection mode. Piperine, capsaicin, and curcumin were detected at 254 nm in the ether extract, while ascorbic acid was quantified at 527 nm in the aqueous extract after post-chromatographic derivatisation.

The R_f values of all biomarkers in control and test samples closely matched those of the corresponding

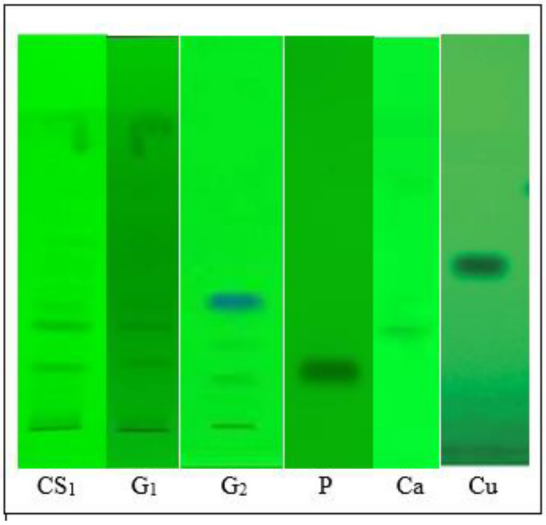


Figure 1. HPTLC chromatogram showing separation of piperine (Rf 0.44), capsaicin (Rf 0.24), and curcumin (Rf 0.36) in ether extracts (Mobile phase: toluene: ethyl acetate, 7:3 v/v; detection at 254 nm).

standards, confirming the specificity of the method. The representative HPTLC chromatogram showing separation of piperine, capsaicin, and curcumin is presented in Figure 1. Calibration curves showed good linearity within the concentration range of 1–5 µg/spot with correlation coefficients (r^2) ≥ 0.9757, demonstrating the suitability of the method for quantitative analysis of *Rasam* formulations.

3.2 Quantification of Bioactive Markers in Control and Test *Rasam*

The concentrations of piperine, capsaicin, curcumin, and ascorbic acid in control *Rasam* and ingredient-eliminated test formulations (T_1–T_3) are summarised in Table 2. Elimination of specific ingredients produced distinct and measurable reductions in biomarker extraction, indicating strong ingredient-dependent extraction behaviour.

Table 2. Concentration of bioactive markers in control and test *Rasam* formulations (µg/mL)

Sample	Piperine (µg/mL)	Capsaicin (µg/mL)	Curcumin (µg/mL)	Ascorbic acid (µg/mL)
Control (All ingredients)	0.185	3.95	1.143	3.56
T_1 (No tamarind + turmeric)	0.170	1.62	1.143	3.56
T_2 (No tamarind)	0.185	3.95	0.300	3.56
T_3 (No chilli pepper)	0.185	3.95	1.143	2.99

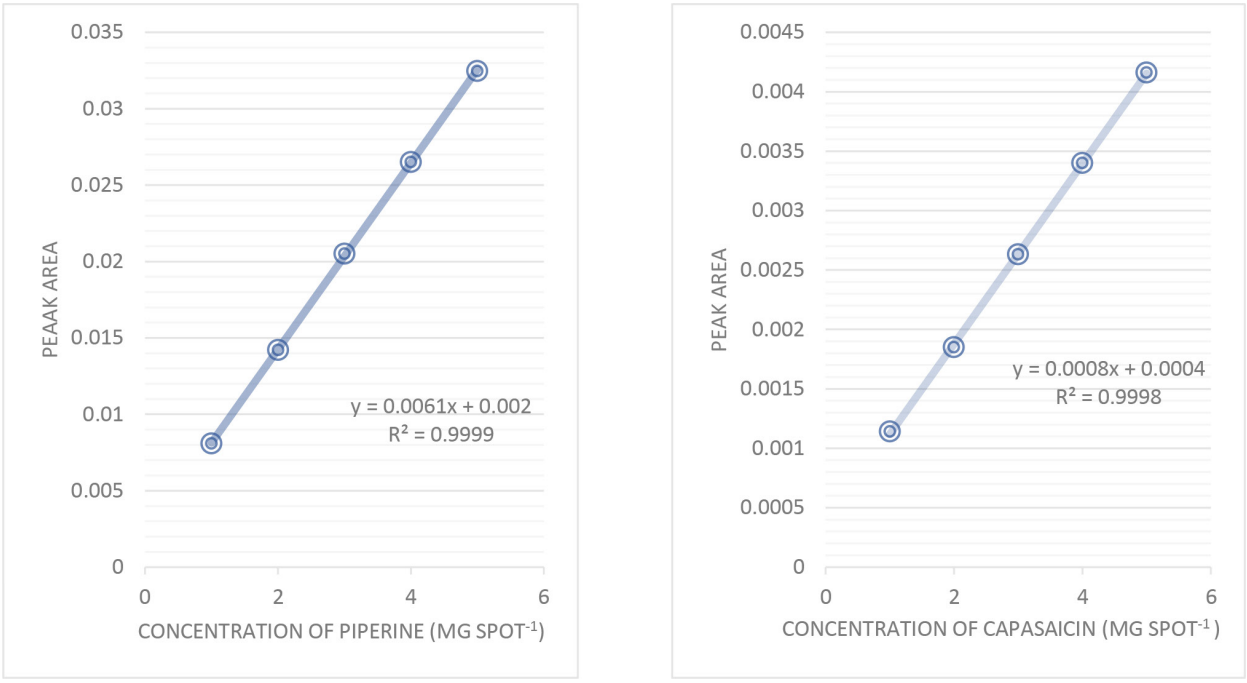


Figure 2. Effect of tamarind and turmeric elimination on piperine and capsaicin extraction in *Rasam* (T_1 formulation).

3.3 Effect of Ingredient Elimination on Biomarker Extraction

3.3.1 Effect of Tamarind and Turmeric Elimination (T_1)

Removal of tamarind and turmeric resulted in a significant reduction in alkaloid extraction. Piperine concentration decreased by 8.1% (0.185 → 0.170 µg/mL), while capsaicin showed a pronounced 59.0% reduction (3.95 → 1.62 µg/mL) compared to the control formulation. The comparative reduction in alkaloid content is illustrated in Figure 2.

This reduction highlights the role of tamarind-derived organic acids in creating an acidic environment that enhances the extraction of alkaloidal compounds from black pepper and chilli pepper.

3.3.2 Effect of Tamarind Elimination on Curcumin Extraction (T_2)

In the absence of tamarind, curcumin concentration decreased drastically by 73.7% (1.143 → 0.300 µg/mL). Tamarind contributes tartaric acid, which lowers the pH of the decoction, improving curcumin stability and solubility during extraction.

The substantial loss of curcumin in T_2 confirms that acidic pH is a critical determinant for efficient curcumin extraction in *Rasam*. The marked decrease in curcumin concentration is depicted in Figure 3.

3.3.3 Effect of Chilli Pepper Elimination on Ascorbic Acid (T_3)

Elimination of chilli pepper resulted in a 16.0% reduction in ascorbic acid concentration (3.56 → 2.99 µg/mL). The variation in ascorbic acid levels following ingredient elimination is shown in Figure 4. This suggests that chilli pepper may contribute directly or indirectly to ascorbic acid availability in *Rasam*, possibly through matrix interactions or synergistic extraction effects.

3.4 Mechanistic Interpretation and Traditional Validation

The results clearly demonstrate that *Rasam* preparation is a pH-mediated extraction system. Tamarind acidification (strongly acidic conditions) significantly enhances the extraction of alkaloids and curcuminoids by promoting protonation and improved partitioning into the extraction medium. Ingredient synergy, achieved through traditional culinary practices, maximises the recovery of therapeutic bioactive compounds.

The quantitative losses observed upon ingredient elimination scientifically validate the traditional *Rasam* formulation as an optimised functional food system rather than a random culinary mixture. These observations are in line with other past analytical studies

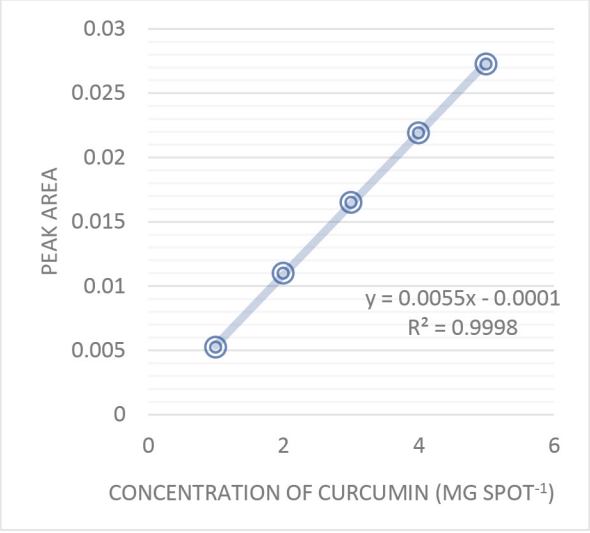


Figure 3. Effect of tamarind elimination on curcumin extraction in *Rasam* (T_2 formulation).

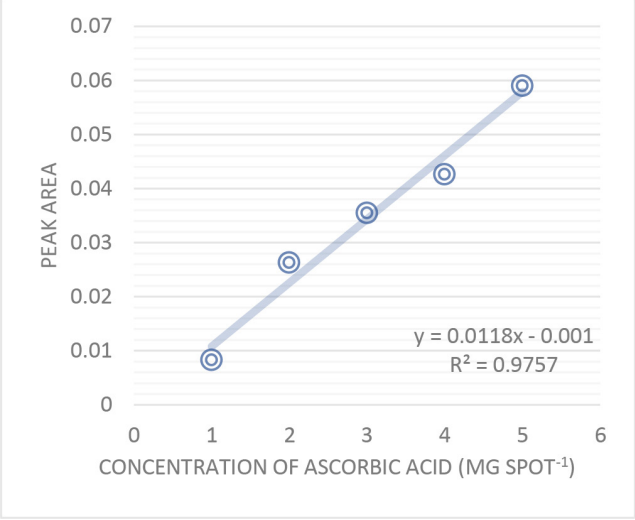


Figure 4. Effect of chilli pepper elimination on ascorbic acid content in *Rasam* (T_3 formulation).

that used markers to determine the phytochemical integrity and biomarker stability of *Rasam*[14].

4. Conclusion

The present study provides quantitative evidence that the traditional preparation of *Rasam* is an optimised, pH-mediated extraction system for bioactive phytochemicals. HPTLC analysis demonstrated that the elimination of key ingredients significantly reduced the extraction efficiency of major biomarkers. Removal of tamarind and turmeric resulted in marked reductions in alkaloid recovery, particularly capsaicin, while elimination of tamarind alone caused a substantial decrease in curcumin concentration. Similarly, removal of chilli pepper led to a measurable reduction in ascorbic acid content.

These findings confirm that tamarind-derived acidity plays a crucial role in enhancing the extraction and stability of alkaloids and curcuminoids, while ingredient synergy contributes to the overall bioactive profile of *Rasam*. The observed reductions in biomarker concentrations upon ingredient elimination scientifically validate the empirical knowledge underlying traditional *Rasam* preparation. Overall, this study reinforces *Rasam* as a functional food with optimised therapeutic potential and highlights the importance of preserving traditional formulation practices to maximise its bioactive benefits.

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Author Contribution

Rachit Patel: Conceptualization, methodology, experimental work, data collection, HPTLC analysis, drafting of manuscript. M. K. Mohan Maruga Raja: Study design, supervision, interpretation of results and critical revision of the manuscript. Thelma Zinzou: Experimental

support, data compilation and literature review. Sagar Singh: Analytical assistance, sample preparation and data validation. Akanchha Sonali: Literature survey, data analysis, manuscript editing. Dipesh Koradiya: Analytical support, interpreting statistics and reporting findings. Agilandeswari Devarajan: Supervision, scientific guidance, manuscript review and final approval of the manuscript. Vijayanandhan V: Conceptualization, coordination of research work, interpretation of the results, manuscript preparation, and author for correspondence. All authors have read and agreed to the published version of the manuscript.