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ORIGINAL ARTICLE

Cytotoxic, Antimitotic, and Antiproliferation Studies on *Rasam*: A South Indian Traditional Functional Food

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ABSTRACT

Background: *Rasam* is a traditional South Indian food, prepared using tamarind juice as a base, with a variety of spices. *Rasam*, with all its ingredients medicinally claimed for various ailments, is a functional food. Systematic consumption of traditional functional food provides an excellent preventive measure to ward off many diseases. **Objective:** To study *rasam* for cytotoxic, antimitotic, and antiproliferation potential beyond its culinary and nutritional effect. **Materials and Methods:** Brine shrimp lethality assay, onion root tip inhibition assay, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in Calu-6, HeLa, MCF-7 cell lines for four stage-wise samples in the preparation of *rasam* (RS1, RS2, RS3, and RS4) were studied. **Results:** RS4, the end product of *rasam* showed high lethality with an LC_{50} value of 38.7 µL/mL. It showed maximum antimitotic activity in a dose-dependent manner compared to other samples with an IC_{50} value of 189.86 µL/mL. RS4 also showed an IC_{50} value of 350.22 and 410.15 µL/mL in MCF-7 and Calu-6 cell lines, respectively. **Conclusion:** From this study, we suggest that *rasam* is a classic example of traditional functional food and it can treat breast and lung cancer on chronic use.

Key words: Brine shrimp lethality, Calu-6, HeLa, MCF-7, onion root tip inhibition, *Saaru*

SUMMARY

- *Rasam*, a South Indian traditional functional food, showed high lethality (LC_{50} = 38.7 µL/mL) against brine shrimps
- *Rasam* also showed potential antimitotic activity (IC_{50} = 189.86 µL/mL) by inhibiting the onion root tips
- *Rasam* showed an IC_{50} value of 350.22 and 410.15 µL/mL against MCF-7 and Calu-6 cell lines respectively
- *Rasam*, when consumed on daily dietary basis, can treat breast and lung cancer.

Abbreviations used: SS 316: Stainless Steel 316 grade; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMEM: Dulbecco's modified Eagle medium; FBS: Fetal bovine serum media; TPVG: Trypsin phosphate versene glucose; EDTA: Ethylene diamine tetra acetic acid; PBS: Phosphate buffered saline; DMSO: Dimethyl sulfoxide.

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INTRODUCTION

The view that food can have an expanded role that goes well beyond providing a source of nutrients truly applies to traditional functional foods. The systematic consumption of traditional functional food provides an excellent preventive measure to ward off many diseases. Epidemiological randomized clinical trials carried out in different countries have demonstrated numerous health effects related to functional food consumption such as reduction of cancer risk, improvement of heart health, stimulation of immune system, decrease of menopause symptoms, improvement of gastrointestinal health, maintenance of urinary tract health, anti-inflammatory effects, reduction of blood pressure, maintenance of vision, antibacterial effect, antiviral effect, reduction of osteoporosis, and anti-obese effect.[1] Traditional functional foods can help prevent chronic disease or optimize health, therefore reducing health-care costs and improving the quality of life.

Spices play very important role in digestive function and the Indian tradition has a long history of use of spice in food as medicines to prevent and treat diseases.[2] Another epidemiological study suggested that curcumin, the bioactive compound of turmeric, as one of the most prevalent nutritional and medicinal compounds used by the Indian population, is responsible for the significantly reduced (4.4 times) prevalence of cardiovascular diseases, metabolic diseases, neurodegenerative diseases, and cancer in India compared to the United States of America.[3] It is also estimated that an adult in India consumes 80–200 mg/day of curcumin and 50 g of garlic in 1 week. Hence, there

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is a realistic possibility to reach a therapeutic dose by daily dietary consumption.[4,5] The whole world realized only in the 20th century that food plays a major role in disease prevention, but centuries before ancient India has realized the importance of food in health and wellness.

Rasam is a very popular South Indian traditional spice soup. It is consumed on daily basis in every South Indian home. It is also called as *rasam* or *chaaru* or *saaru* in different South Indian languages. In a traditional South Indian meal, *rasam* is preceded by a *sambar* rice course and is followed by curd rice. *Rasam* is traditionally prepared using tamarind juice as a base, with a variety of spices which are considered to be good for health.[6] The main spices used in *rasam* preparation are coriander, garlic, curry leaves, tamarind, cumin, black pepper, mustard, turmeric, red chili, and asafetida.[7] *Rasam* is a functional food because all ingredients used in the preparation of *rasam* are medicinally claimed for various ailments. *Sambar*, another South Indian traditional dish, has shown preventive effect against colon cancer.[8] There is a need to understand traditional systems and visualize the future of medicine and health care. The linkage between "the past" and "the future" of medicine is much more important and can give us "new directions" for better understanding health, disease, and possible solutions.[9]

A study on *rasam*, which is being consumed from time immemorial, is only an approach of "drug rediscovery." In view of all the above facts, *rasam* was studied for cytotoxic, antimitotic, and antiproliferation potential beyond its culinary and nutritional effect.

MATERIALS AND METHODS

Materials

All ingredients of *rasam* were purchased from Arokya Organic Shop, Vellore, Tamil Nadu. All utensils used for the preparation of *rasam* were of Stainless Steel 316 grade (SS 316). Brine shrimp eggs were purchased from Ocean Star International Inc., Snowville, UT, USA. Onion bulbs were purchased from Nutrisiree Organics, Bengaluru, Karnataka. MCF-7 (ATCC HTB-22, passage number 11), HeLa (ATCC CCL2, passage number 13), CALU-6 (ATCC HTB-56, passage number 19) cell lines were procured. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) media, trypsin phosphate versene glucose (TPVG) solution, ethylene diamine tetra acetic acid (EDTA), phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO), trypsin, acetocarmine, and cedar wood oil were obtained from Sigma-Aldrich (Bengaluru, India) and HiMedia Ltd., (Mumbai, India). All other chemicals and solvents were obtained from SD Fine Chemicals (Mumbai, India) and were of analytical grade.

Preparation of *rasam*

Rasam was prepared in five stages as mentioned below.

1. Tamarind fruit pulp mixture (T1): 6.88 g of tamarind fruit pulp was immersed in 450 mL of water for 10 min, and then it was hand crushed for 45 times and strained. The strained liquid was rinsed with 5 mL water, to which 0.4 g of turmeric powder and 4 g of sea salt were added
2. Tomato fruit mixture (T2): 82.44 g of fresh tomato fruits were cut and hand crushed for 60 times. The crushed fruit was rinsed with 5 mL of water
3. Spice mixture (T3): 1.33 g of pepper drupes was crushed in an SS 316 mortar and pestle for 85 times. 2.67 g of cumin fruits was added over to the crushed pepper drupes and crushed for 100 times. To the above-crushed mixture, 0.82 g of chili pepper was added and crushed for 50 times; then, 9.63 g of garlic cloves was added and crushed for 90 times
4. All mixture (T4): Tomato fruit mixture (T2) was rinsed with 10 mL of water and spice mixture (T3) was rinsed with 10 mL of water. Both rinsing were added to tamarind fruit pulp mixture (T1), which was designated as sample RS1
5. Final product (T5): 4 ml of Indian sesame oil was heated at 60°C for 2 min. After 5 seconds 0.82 g of mustard seeds were added. After 3 s, 1.53 g of whole chili pepper was added. After 2 s, 0.61 g of curry leaves was added, which was designated as sample RS2. Immediately, all mixture (T4) was rinsed with 20 mL of water and added. The whole liquid was allowed to boil for 5 min. After 5 min, 1.50 g of coriander leaves was added; this was designated as sample RS3. When the liquid frothed, 0.05 g of asafetida was added and the heating was switched off to yield the final product, which was designated as sample RS4.

The stage-wise samples RS1, RS2, RS3, and RS4 in the preparation of *rasam* were studied to evaluate the significance of the traditional processing.

Cytotoxicity study

Cytotoxicity was studied by brine shrimp lethality bioassay as per the method of Meyer *et al.*, 1982.[10] Brine shrimps (*Artemia salina*) were hatched from eggs in a conical-shaped vessel (1 L), filled with artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) with constant aeration for 36 h at room temperature (20°C ± 5°C) under light. After hatching, active nauplii, free from egg shells, were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in vials each containing 4.5 mL of brine solution (24% of NaCl in water). In every vial, 0.5 mL of water or sample was added to the brine solution and maintained at room temperature (20°C ± 5°C) under light. The number of surviving nauplii after 24 h was counted. Experiments were conducted at different concentrations (20, 40, 80, 120, 160, 200, and 400 μL/mL) of the samples (RS1, RS2, RS3, and RS4) diluents being water. Each concentration of the sample was studied in six vials along with a control group.

The %lethality was determined from the number of surviving nauplii in control and sample using the below-mentioned formula.

$$\%\,\text{lethality} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Control is number of surviving nauplii in control, and sample is number of surviving nauplii in sample. LC_{50} values were calculated from percentage lethality versus concentration best-fit line graph.

Antimitotic study

Antimitotic study was studied by onion root tip inhibition assay as per the method of Rai *et al.*, 2007.[11] The old roots of *Allium cepa* bulbs (56.46 ± 4.14 g; values are expressed as mean ± standard deviation [SD]) were removed and grown in the dark over a small beaker containing 35 mL of tap water (the water was changed every 24 h) at 20°C ± 5°C until the root tips have grown to approximately 2–3 cm (in approximately 2–3 days). The bulbs with root tips grown up to 2–3 cm were selected. Ten root tips grown above 2 cm were kept as such, and rest of them were trimmed off. The bulbs with only 10 root tips were placed over water or sample solutions, and incubation was carried out at 20°C ± 5°C. Samples RS1, RS3, and RS4 each at a concentration of 1, 10, 100, and 200 μL/mL were studied in triplicate. The oil sample (RS3) would form a layer on the surface and never allow the growth of the root tip; hence, RS3 was omitted for this study.

In each bulb, root length and newly grown root tips were recorded at 0, 24, 48, and 72 h. One root in each bulb was cut using a scalpel between 8.00 to 13.00 h IST at 0, 24, 48, and 72 h. Extreme root tips (2–3 mm) of root were cut and put in a test tube containing water. The test tube was

treated with 1.5 mL of 0.1 N HCl and incubated at 60°C for 12 min. The 0.1 N HCl was drained and the root tips were washed with water 4 times to remove the acid traces. Then, five drops of acetocarmine was added and incubated at 60°C for 24 min. The red turned root tip was transferred to a clean glass slide and a cover slip was placed over. Very gentle pressure was applied with thumb over the cover slip to provide uniform spread of the cells. The number of mitotic and total meristematic cells was counted in 5–6 fields using high power (×100) light microscope. For control and all samples, 500 cells were counted and cells manifesting different stages of mitosis, i.e., interphase and prophase (P), metaphase (M), anaphase (A), and telophase (T), were recorded. The mitotic index at 0, 24, 48, and 72 h was calculated using the following formula.

Mitotic index = (P + M + A + T)/(Total number of cells) × 100

P, M, A, and T is prophase, metaphase, anaphase, and telophase, respectively.

IC_{50} values were calculated from the concentration versus percentage of inhibition best-fit line graph. The values are expressed as mean ± SD. GraphPad Instat Version 4 software (GraphPad Software, USA) was used. Data were subjected to the one-way analysis of variance to determine the significance of changes followed by Dunnett's multiple comparisons.

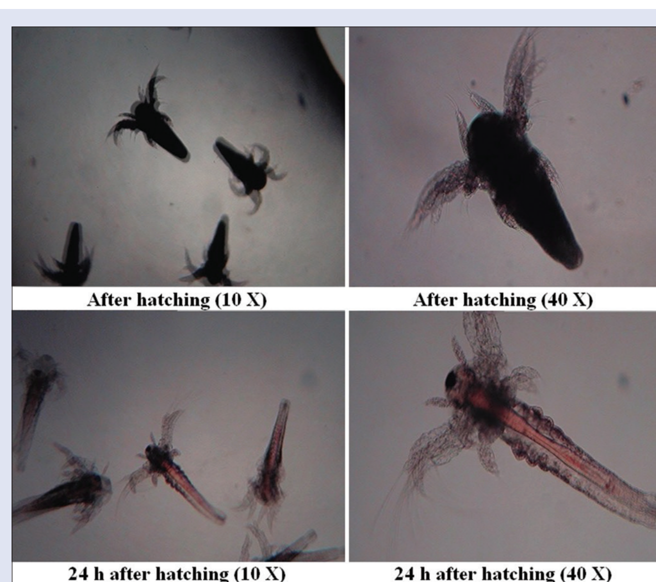

Figure 1: Brine shrimp nauplii after hatching at 0 and 24 h

Antiproliferation study

MCF-7, HeLa, and Calu-6 stock cells were cultured in DMEM supplemented with 10% inactivated FBS, penicillin (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (5 µg/mL) in humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells was checked and centrifuged.

MTT assay was carried out by the modified method of Lau *et al*., 2004.[12] The monolayer cell culture was trypsinized and the cell count was adjusted to 1 × 10^5 cells/mL using DMEM containing 10% FBS. To each well of the 96-well microtiter plate, 100 µL of the diluted cell suspension (5 × 10^4 cells/well) was added and incubated for 24 h at 37°C, 5% CO_2. After 24 h, the supernatant was flicked off and the monolayer was washed with the medium once.[13] All samples were mixed with DMEM supplemented with 2% inactivated FBS to obtain different concentrations, ranging from 166.67 to 500 µL/mL. Samples were added onto the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 72 h in 5% CO_2 atmosphere. After 72 h, the test solutions in the wells were discarded and 50 µL of MTT (5 mg/10 mL of MTT in PBS) was added to each well. The plates were gently shaken and incubated for 4 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed, 100 µl of DMSO was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a Tecan microplate reader at a wavelength of 590 nm. The percentage inhibition was calculated using the following formula.

Percentage inhibition = (ABS of control − ABS of samples)/(ABS of control) × 100

ABS is absorbance and IC_{50} values were calculated from the concentration versus percentage of inhibition best-fit line graph.

RESULTS AND DISCUSSION

Brine shrimp lethality assay is a rapid, reliable and has been used for over 30 years in cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition, and ion regulation activities.[14] The nauplii after hatching at 0 and 24 h are shown in Figure 1. RS4, the final product of *rasam*, showed high lethality (38.7 µL/mL) than RS1 (165.6 µL/mL), RS2 (387.6 µL/mL), and RS3 (124.2 µL/mL) [Table 1]. The percentage of lethality was found to be directly proportional to the concentration of the samples. Stagewise preparation analysis shows 3.2-fold increase of cytotoxicity in RS4 as compared to of RS1, RS2, and RS3. It is evident that the process in preparation of *rasam* plays an important role in increasing the physiological action of the final product (RS4). Brine shrimp lethality bioassay has good correlation with the human solid tumor cell lines;[15] hence, it can be suggested that the sample RS4 is bioactive, with cytotoxic and antitumor activity.

Most of the plant-derived anticancer drugs affect the microtubule dynamics of the cell and induce persistent modification of biological processes and signaling pathways that ultimately lead to apoptotic death.[16] Onion root tip inhibition assay is a quick, efficient, simple but sensitive antimitotic bioassay and also known to give a similar result to the *in vitro* animal cytotoxicity test.[17] The study showed that at 72 h, RS1, RS3, and RS4 at all concentrations (1, 10, 100, and 200 µL/mL) significantly inhibits root growth [Table 2]. Figure 2 shows the effect of RS1 (200 µL/mL), RS3 (200 µL/mL), and RS4 (200 µL/mL) on root tip growth at 72 h in comparison to the control group. It is evident that all samples affect cell division in long duration. There was no increase in root length for RS1 (200 µL/mL) and RS3 (200 µL/mL) after 24 h. The final product RS4 at 200 µL/mL showed no increase in root length even after 0 h, suggesting maximum root inhibition. RS3 (200 µL/mL) and RS4 (100 and 200 µL/mL) significantly prevented the growth of new root tips after 0 h [Table 2]. However, after 72 h, all samples at different concentrations also significantly inhibited the growth of new root tips. RS4 (200 µL/mL) not only significantly inhibits the root length but also prevents the growth of a new root tip after 0 h. The number of cells in prophase was nearly double the metaphase in RS1, RS3, and RS4 treated groups. The number of cells in telophase varied from 9 to 12 within the counted 500 cells of all sample-treated groups. The number of dividing cells affected and the mitotic index clearly confirms that RS4 (200 µL/mL) at 24 h significantly inhibits mitosis [Table 3]. RS3 (100 and 200 µL/mL) and RS4 (10 and 100 µL/mL) showed lesser number of dividing cells and lower mitotic index after 72 h. The IC_{50} value (189.86 µL/mL) of the final product of RS4 (*rasam*) only confirms maximum inhibition of mitosis in a dose-dependent manner compared to RS1 and RS3 [Table 3], and also, the IC_{50} value of RS4 was found to be 2.2 times lower than its constituents (RS1 and RS3). Antitumor drugs that interact with microtubules and tubulin are known to block mitosis and induce cell death by apoptosis.[18] Hence, it can be suggested that the

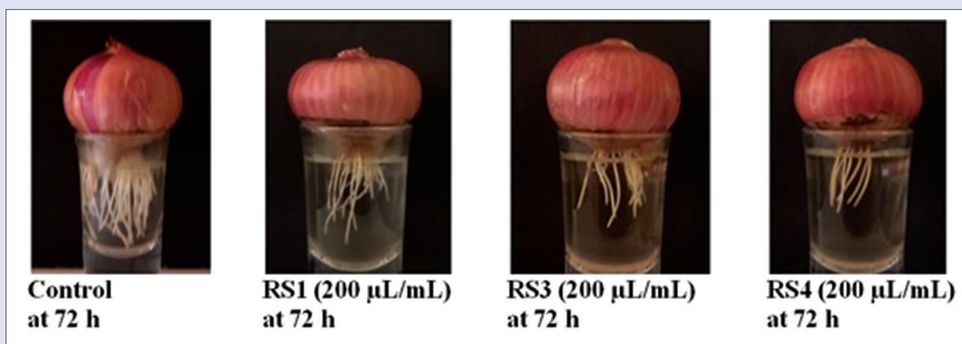

Figure 2: Effect of RS1, RS3, and RS4 on onion root tip growth at 72 h

Table 1: The lethality effect of RS1, RS2, RS3, and RS4 on brine shrimps

Control/sample	Concentration	Number of nauplii at 0 h	Number of surviving nauplii after 24 h	Percentage lethality	LC_{50} (µL/mL)
Water	0.1 mL/mL	60	60	0	NA
RS1	20 µL/mL	60	58	3.3	165.6
	40 µL/mL	60	51	15.0	
	80 µL/mL	60	41	31.7	
	120 µL/mL	60	38	36.7	
	160 µL/mL	60	31	48.3	
	200 µL/mL	60	27	55.0	
	400 µL/mL	60	16	73.3	
RS2	20 µL/mL	60	59	1.7	387.6
	40 µL/mL	60	56	6.7	
	80 µL/mL	60	53	11.7	
	120 µL/mL	60	51	15.0	
	160 µL/mL	60	47	21.7	
	200 µL/mL	60	44	26.7	
	400 µL/mL	60	29	51.6	
RS3	20 µL/mL	60	47	21.7	124.2
	40 µL/mL	60	43	28.3	
	80 µL/mL	60	37	38.3	
	120 µL/mL	60	31	48.3	
	160 µL/mL	60	26	56.7	
	200 µL/mL	60	20	66.7	
	400 µL/mL	60	11	81.7	
RS4	20 µL/mL	60	36	40.0	38.7
	40 µL/mL	60	29	51.7	
	80 µL/mL	60	20	66.7	
	120 µL/mL	60	13	78.3	
	160 µL/mL	60	9	85.0	
	200 µL/mL	60	4	93.3	
	400 µL/mL	60	0	100	

NA: Not available

Table 2: Effect of RS1, RS3, and RS4 on root length and newly grown root tips at 0, 24, 48, and 72 h

Control/ sample	Concentration	Root length (cm)				Total number of newly grown root tip at			
		0 h (*n*=30)	24 h (*n*=27)	48 h (*n*=24)	72 h (*n*=21)	0 h	24 h	48 h	72 h
Water	1 mL/mL	2.46±0.20	3.82±0.33	5.02±0.32	6.41±0.43	0	10.33±1.53	14.33±0.58	22.67±0.58
RS1	1 µL/mL	2.31±0.17	3.62±0.28*	4.78±0.35*	5.51±0.30**	0	9.67±1.53 (NS)	13.67±0.58 (NS)	21.67±1.16 (NS)
	10 µL/mL	2.43±0.18	3.51±0.24**	4.36±0.28**	5.12±0.32**	0	9.33±0.58 (NS)	12.67±1.16 (NS)	20.33±0.58**
	100 µL/mL	2.54±0.17	3.14±0.24**	3.67±0.38**	4.02±0.27**	0	8.67±0.58 (NS)	11.33±1.16**	18.67±0.58**
	200 µL/mL	2.44±0.17	2.91±0.29**	2.90±0.30**	2.92±0.25**	0	8.33±0.58 (NS)	8.67±1.16**	8.67±1.16**
RS3	1 µL/mL	2.41±0.16	3.52±0.23**	4.29±0.35**	5.11±0.18**	0	9.33±1.53 (NS)	12.33±0.58 (NS)	20.67±0.58*
	10 µL/mL	2.31±0.16	2.71±0.36**	3.28±0.20**	3.85±0.20**	0	9.33±0.58 (NS)	11.33±0.58**	18.67±0.58**
	100 µL/mL	2.35±0.18	2.41±0.13**	2.49±0.18**	2.46±0.15**	0	8.33±0.58 (NS)	8.33±0.58**	8.33±0.58**
	200 µL/mL	2.59±0.25	2.60±0.26**	2.58±0.26**	2.58±0.26**	0	7.33±1.16**	7.33±1.16**	7.33±1.16**
RS4	1 µL/mL	2.57±0.22	3.46±0.20**	4.53±0.35**	4.93±0.16**	0	8.33±1.16 (NS)	9.67±1.53**	12.33±0.58**
	10 µL/mL	2.46±0.19	3.25±0.28**	3.83±0.28**	4.02±0.26**	0	8.33±0.58 (NS)	8.33±0.58**	8.33±0.58**
	100 µL/mL	2.53±0.25	2.61±0.27**	2.60±0.27**	2.58±0.28**	0	1.67±1.58**	1.67±0.58**	1.67±0.58**
	200 µL/mL	2.48±0.18	2.48±0.19**	2.48±0.20**	2.46±0.20**	0	0.33±0.58**	0.33±0.58**	0.33±0.58**

Values are expressed as mean±SD; *P<0.05; **P<0.01; NS (P>0.05). NS: Not significant; SD: Standard deviation

Table 3: Effect of RS1, RS3, and RS4 on total number of dividing cells, mitotic index at 0, 24, 48, and 72 h, and their IC_{50} values after 72 h

Control/ sample	Concentration	Cells manifesting different stages of mitosis				Mitotic index				Percentage of inhibition at 72 h	IC_{50} (µL/mL)
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h		
Water	1 mL/mL	252.33±11.02	247.67±10.26	248.67±17.50	243.67±14.30	50.47±2.20	50.13±2.08	49.73±3.5	48.73±2.86	0	NA
RS1	1 µL/mL	248.33±13.05	250.33±11.06 (NS)	244.67±9.45 (NS)	239.33±9.07 (NS)	49.67±2.61	50.07±2.21 (NS)	48.93±1.89 (NS)	47.87±1.82 (NS)	1.76	380.22
	10 µL/mL	242.33±6.51	252.67±20.79 (NS)	238.67±10.50 (NS)	238.67±10.50 (NS)	48.47±1.30	50.53±4.16 (NS)	47.73±2.10 (NS)	47.33±2.10 (NS)	2.87	
	100 µL/mL	248.67±7.02	250.67±13.58 (NS)	228.67±9.29 (NS)	228.67±9.29 (NS)	49.73±1.41	50.13±2.72 (NS)	45.73±1.86 (NS)	45.73±1.86 (NS)	6.16	
	200 µL/mL	251.67±13.01	247.33±5.03 (NS)	226.33±21.50 (NS)	216.33±10.60*	50.33±2.60	49.47±1.01 (NS)	45.27±4.3 (NS)	43.27±2.12*	11.2	
RS3	1 µL/mL	252.33±6.51	252.67±7.64 (NS)	246.33±14.50 (NS)	244.67±14.57 (NS)	50.47±1.30	50.53±1.53 (NS)	46.27±3.0 (NS)	48.93±2.91 (NS)	0.41	440.64
	10 µL/mL	244.67±11.72	225.33±13.58 (NS)	231.33±5.86 (NS)	227.67±8.33 (NS)	48.93±2.34	45.07±2.72 (NS)	46.27±1.17 (NS)	45.53±1.67 (NS)	6.57	
	100 µL/mL	256.67±10.02	223.67±9.29 (NS)	216.33±4.73*	214.33±5.03**	51.33±2.0	44.73±1.86 (NS)	43.27±0.95*	42.87±1.01**	12.02	
	200 µL/mL	242.33±14.05	219.67±14.98 (NS)	206.33±6.66**	206.33±4.51**	48.47±2.81	43.93±3.0*	41.27±1.33**	41.27±0.90**	15.31	
RS4	1 µL/mL	251.33±12.01	247.67±12.66 (NS)	236.33±13.66 (NS)	232.67±8.08 (NS)	50.27±2.4	49.53±2.53 (NS)	47.27±2.73 (NS)	46.53±1.62 (NS)	4.51	189.86
	10 µL/mL	235.67±6.43	234.33±10.79 (NS)	214.33±6.81*	208.67±3.51**	47.13±1.29	46.87±2.16 (NS)	42.87±1.36*	41.73±0.70**	14.36	
	100 µL/mL	254.67±7.37	231.33±11.72 (NS)	164.33±7.64**	142.67±10.41**	50.93±1.47	46.27±2.34 (NS)	32.87±1.53**	28.53±2.08**	41.45	
	200 µL/mL	237.33±11.68	203.33±8.51**	152.67±9.71**	113.33±6.81**	47.47±2.34	40.67±1.7**	30.53±1.94**	22.67±1.36**	53.48	

Total number of cells counted was 500; values are expressed as mean±SD; $^{*}P<0.05$; $^{**}P<0.01$; NS ($P>0.05$). NS: Not significant; SD: Standard deviation

Table 4: Percentage of inhibition and IC_{50} values of RS1, RS2, RS3, and RS4 in MCF-7, HeLa, and Calu-6 cell lines

Samples	Concentration (µL/mL)	MCF-7 cell lines			HeLa cell lines			Calu-6 cell lines		
		ABS at 590 nm	Percentage of inhibition	IC_{50} (µL/mL)	ABS at 590 nm	Percentage of inhibition	IC_{50} (µL/mL)	ABS at 590 nm	Percentage of inhibition	IC_{50} (µL/mL)
Control	-	0.7368	0	0	0.5568	0	0	0.7632	0	0
RS1	166.67	0.6079	17.50	>500	0.4912	11.78	>500	0.6574	13.86	>500
	200	0.5897	19.97		0.4717	15.28		0.6203	18.72	
	250	0.5468	25.79		0.4543	18.41		0.5558	27.18	
	333.33	0.5121	30.50		0.3957	28.93		0.5343	29.99	
	500	0.4826	34.50		0.3727	33.06		0.5012	34.33	
RS2	166.67	0.5961	19.10	>500	0.4779	14.17	>500	0.6687	12.38	>500
	200	0.5758	21.85		0.4488	19.40		0.6427	15.79	
	250	0.5517	25.12		0.4072	26.87		0.6198	18.79	
	333.33	0.5281	28.33		0.3631	34.79		0.5958	21.93	
	500	0.5017	31.91		0.3245	41.72		0.5524	27.62	
RS3	166.67	0.5996	18.62	>500	0.4687	15.82	>500	0.5779	24.28	>500
	200	0.5682	22.88		0.4427	20.49		0.5488	28.09	
	250	0.5417	26.48		0.4198	24.60		0.5072	33.54	
	333.33	0.5101	30.77		0.3958	28.92		0.4631	39.32	
	500	0.4814	34.66		0.3524	36.71		0.4245	44.38	
RS4	166.67	0.5562	24.51	350.22	0.4892	12.14	>500	0.6452	15.46	410.15
	200	0.5303	28.03		0.4565	18.01		0.6050	20.73	
	250	0.4982	32.38		0.4224	24.14		0.5894	22.77	
	333.33	0.3843	47.84		0.4020	27.80		0.4663	38.90	
	500	0.3125	57.59		0.3786	32.00		0.3611	52.69	

ABS: Absorbance

exhibited antimitotic activity of RS4 may be due to its interaction with microtubules.

RS4 showed an IC_{50} value of 350.22 and 410.15 μL/mL in MCF-7 and Calu-6 cell lines, respectively [Table 4]. RS1, RS2, and RS3 showed an IC_{50} value more than 500 μL/mL in MCF-7, HeLa, and Calu-6 cell lines, respectively. The results showed that the final product RS4 may be active against breast and lung cancers. However, the mechanism for such an effect needs further evaluation.

The postmodern "preventive medicine" concept of the Western medicine has absolutely recognized that food plays an important role in the incidence of many diseases. Dietary choice remains the basis for maintaining a healthy lifestyle and well-being, especially relating to cardiovascular disease, diabetes, obesity, hypertension, some cancers, circulatory diseases, and stroke, despite remarkable advances in medicine and pharmaceutical drug development.[19,20] According to the National Cancer Registry Programme of the India Council of Medical Research, more than 1300 Indians die every day due to cancer. Between 2012 and 2014, the mortality rate due to cancer increased by approximately 6%. The risk of cancer incidence may also be due to the deviation from traditional functional food toward fast, junk, and westernized foods.

The different ingredients used in *rasam* have been individually attributed to various pharmacological effects in preclinical and clinical studies. It is trouble-free to ascertain that the *rasam*'s effects are due to the antioxidant effect of tamarind fruit pulp;[21,22] antioxidant and anticarcinogenic effect of turmeric;[23] antioxidant and anticancer activity of chili pepper;[21] antioxidant activity of cumin;[21] anticancer and antioxidant effects of garlic bulbs;[21,24-27] antioxidant and bioavailability enhancing effect of black pepper;[28,29] and antioxidant activity of coriander leaves.[30] However, if all ingredients and/or active constituents of *rasam* were scientifically formulated together using available technology, it may not yield the desired physiological result; however, somehow in the preparation of *rasam*, the traditional processing naturally ensures higher cytotoxic, antimitotic, and antiproliferation activity in the final product. The LC_{50} and IC_{50} values of *rasam* may not be very significant compared to active pharmaceutical agents that are administered in a fixed dose but consuming *rasam* as daily diet can ensure healing effect. The real challenge lies not in proving whether *rasam* is functional foods having health benefits, but in defining what these benefits are and developing the methods to expose them by scientific means.

CONCLUSION

Rasam is a South Indian traditional functional food that can treat breast and lung cancer on chronic use.

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Conflicts of interest

There are no conflicts of interest.

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