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ANTI-MICROBIAL STUDIES ON *RASAM*: A SOUTH INDIAN TRADITIONAL FUNCTIONAL FOOD

Agilandeswari Devarajan[1,2]* and M. K. MohanMarugaRaja[2]

[1]Department of Pharmaceutics, Hillside College of Pharmacy and Research Centre, Bengaluru, Karnataka, India.

[2]Pharmacy Department, Centre for Research and Development, PRIST University, Thanjavur, Tamil Nadu, India.

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***Corresponding Author**
Prof. Agilandeswari Devarajan
Department of Pharmaceutics, Hillside College of Pharmacy and Research Centre, Bengaluru, Karnataka, India.

ABSTRACT

Rasam, is a traditional functional food as all of its ingredients are medicinally claimed for various ailments. *Rasam* is used as an effective home remedy for common cold, cough and an antidote for flu or fever. There have been no anti-microbial studies on *rasam* hence; this study was carried out to explore the anti-microbial potential of *rasam*. Stage wise samples in the preparation of *rasam* (RS1, RS2, RS3 and RS4) were studied for anti-microbial activity against *Bacillus cereus* (NCIM 5293), *Micrococcus luteus* (NCIM 5262), *Streptococcus pyogenes* (NCIM 5280), *Pseudomonas aeruginosa* (NCIM 5029), *Salmonella typhi* (NCIM 5255), *Escherichia coli* (NCIM 5347), *Candida albicans* (NCIM 3631) and *Penicillium digitatum* (NCIM 939). Zone of inhibition (ZoI) and minimum inhibitory (MIC) concentration were determined by agar well diffusion method and broth micro dilution method respectively. The final product of *rasam* (RS4) showed higher ZoI against *B. cereus* and *P. aeruginosa* with an MIC value of 50.07 and 201.69µL/mL respectively. *Rasam* is a traditional functional food with anti-bacterial activity. Daily dietary consumption of *rasam* can prevent bacteria associated illnesses. The potent anti-bacterial activity of *rasam* against *B. cereus* has scientifically justified its traditional consumption with boiled rice.

KEYWORDS: Anti-bacterial, *B. cereus*, *Chaaru*, *Saaru*, spices.

INTRODUCTION

The approach of traditional functional food is that, food can have an expanded role that goes well beyond providing a source of nutrients. Traditional functional foods can help to prevent chronic disease or optimize health, therefore reducing health care costs, improving the quality of life. Epidemiological randomized clinical trials carried out in different countries have demonstrated numerous health effects related to functional food consumption like 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, anti-bacterial effect, anti-viral effect, reduction of osteoporosis and anti-obese effect.[1]

Rasam, is a common South Indian traditional spice soup and consumed with rice. It is also called as *chaaru* or *saaru* in different South Indian languages. The main spices used in the preparation of *rasam* are tamarind, turmeric, chili pepper, cumin, garlic, black pepper, black mustard, curry leaves, coriander and asafoetida.[2] *Rasam*, is a traditional functional food as, its ingredients are medicinally claimed for various ailments. *Rasam* is used as an appetizer and also as medicinal formulation, based on the permutation and combination of its constituent spices.[3,4] In South India, *rasam* is considered an effective home remedy for common cold, cough and an antidote for flu or fever.[5,6,7] There have been no anti-microbial studies on *rasam* hence, this study was carried out to investigate the anti-microbial potential of *rasam* beyond its culinary and nutritional effects.

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). All other chemicals and solvents were obtained from SD Fine Chemicals (Mumbai, India) and were of analytical grade.

Test micro-organisms

Bacillus cereus (NCIM 5293), *Micrococcus luteus* (NCIM 5262), *Streptococcus pyogenes* (NCIM 5280), *Pseudomonas aeruginosa* (NCIM 5029), *Salmonella typhi* (NCIM 5255), *Escherichia coli* (NCIM 5347), *Candida albicans* (NCIM 3631) and *Penicillium digitatum* (NCIM 939) were procured from National Chemical Laboratory, Pune, India. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato

dextrose agar (PDA) medium (Sigma Aldrich, Bangalore, India), and stored in a refrigerator at 4°C. The different strains were grown in tryptic soy broth (TSB) (Sigma Aldrich, Bangalore, India), plates at 37°C maintained on nutrient agar slants at 4°C.

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, 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 was 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 a 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. To the above mixture 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), this 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 seconds 1.53 g of whole chili pepper was added. After 2 seconds 0.61 g of curry leaves was added, this 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 a 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 asafoetida was added and the heating was switched off to yield the final product, this was designated as sample RS4.

The stage wise samples RS1, RS2, RS3 and RS4 of *rasam* were used for anti-microbial studies.

Anti-microbial study[8,9,10]

***Determination of zone of inhibition* (ZoI)**

ZoI was determined by agar well diffusion method. The nutrient agar plate (90 mm) was inoculated by spreading 100 µL of the microbial inoculum (cell suspension prepared from cultures grown on TSB broth adjusted to $1X10^5$cells/mL) over the entire agar surface. Then, a hole with a diameter of 5 mm was punched aseptically with a sterile cork borer and 25 µL of the samples (RS1, RS2, RS3 and RS4) at desired concentration (100 to 1000µl/mL) was introduced into the well. Then, agar plates were incubated at 35 °C for 24-48 h and 22 °C for 48-72 h for bacterial and fungal organisms respectively. All experiments were conducted in triplicate. Post incubation the plates were observed for zone of inhibition around the well.

***Determination of minimum inhibitory concentration* (MIC)**

MIC was determined by broth micro dilution method. Two-fold dilutions of the samples of desired concentration (6.25, 12.5, 25, 50, 100 and 200 µL/mL) were prepared with TSB. 100 µL of the prepared samples along with 10 µL of the microbial inoculum (cell suspension prepared from cultures grown on TSB broth adjusted to $1X10^5$cells/mL) were added to a 96 well microplate in triplicate. A control in triplicate without samples was also maintained. The plate was incubated under the same conditions used for the determination of ZoI. After 24 or 48 h depending on the micro-organism, OD was measure at 590 nm in a Tecan plate reader. Percentage of inhibition was calculated based on the OD of samples compared with the control. MIC was calculated from the concentration Vs percentage of inhibition best-fit line graph.

RESULTS AND DISCUSSION

The biological source of the ingredients and its quantity used in the preparation of *rasam* is shown in Table 1.

Table: 1. Biological source of the ingredients and its quantity used in the preparation of *rasam*

Common names	Morphological part used	Nature of the material	Botanical name	Family	Quantity
Tamarind	Ripped fruit pulp	Dried	*Tamarindus indica* L.	Fabaceae	6.00 g
Turmeric	Rhizome powder	Dried	*Curcuma longa* L.	Zingiberaceae	0.40 g
Sea salt	NA	Solid	NA	NA	4 g
Tomato	Ripped fruit	Fresh	*Solanum lycopersicum* L.	Solanaceae	82.44 g
Chili pepper	Crushed fruit of long chilli pepper	Dried	*Capsicum annuum* L.	Solanaceae	0.82 g

Cumin	Ripped fruit	Dried	*Cuminum cyminum* L.	Apiaceae	2.67 g
Garlic	Cloves	Dried	*Allium sativum* L.	Amaryllidaceae	9.63 g
Black pepper	Unripe drupe	Dried	*Piper nigrum* L.	Piperaceae	1.33 g
Indian sesame oil	Seed	Oil	*Sesamum indicum* L.	Pedaliaceae	4 mL
Black mustard	Seed	Dried	*Brassica nigra* L.	Brassicaceae	0.82 g
Chili pepper	Whole fruit of long chili pepper	Dried	*Capsicum annuum* L.	Solanaceae	1.53 g
Curry leaves	Leaves	Fresh	*Murraya koenigii* (L.) Sprengel	Rutaceae	0.61 g
Portable water	NA	Liquid	NA	NA	500 mL
Coriander	Leaves	Fresh	*Coriandrum sativum* L.	Apiaceae	1.50 g
Asafoetida	Dried latex (oleogum resin) exuded from the rhizome or tap root	Powder	*Ferula assa-foetida* L.	Apiaceae	0.05 g

For the determination of anti-microbial activity of different stage wise samples of *rasam* (RS1, RS2, RS3 and RS4), three Gram-positive (*Bacillus cereus*, *Micrococcus luteus*, *Streptococcus pyogenes*), three Gram-negative (*Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*), and two fungal strains (*Candida albicans*, *Penicillium digitatum*) were selected. Samples with ZoI values <10 mm were considered as not active against micro-organisms. RS3 and RS4 showed a higher ZoI of 20.32±1.24 and 21.54±0.90 (mean±SD) against *Bacillus cereus* (Table 2). RS4 also showed moderate ZoI of 15.82±1.33 (mean±SD) against *Pseudomonas aeruginosa* (Table 2). The ZoI was found to be directly proportional to the concentration of samples RS3 and RS4. RS1 and RS2 did not show significant ZoI against all tested bacterial strains. Also, all samples of *rasam* (RS1, RS2, RS3 and RS4) at tested concentration did not show any significant ZoI against *Candida albicans* and *Penicillium digitatum* (Table 3). Samples which showed higher ZoI (RS3 and RS4) only were selected for MIC study.

Table 2: Effect of *rasam* samples (RS1, RS2, RS3 and RS4) against bacterial test organisms

Samples	Conc. (µL/mL)	Zone of inhibition (mm)					
		B. cereus	***M. luteus***	***S. pyogenes***	***P. aeruginosa***	***S. typhi***	***E. coli***
RS1	100	NI	NI	NI	NI	NI	NI
	250	1.17±0.61	NI	NI	1.15±0.83	NI	NI
	500	3.28±0.74	1.21±0.52	1.04±0.53	2.61±0.73	1.91±0.62	1.50±0.61
	1000	6.82±0.80	4.56±0.84	4.27±1.10	3.10±1.24	3.72±1.05	4.03±0.91
RS2	100	NI	NI	NI	NI	NI	NI
	250	NI	NI	NI	NI	NI	NI
	500	2.34±0.63	1.91±0.64	2.27±0.54	2.72±0.46	1.22±0.55	2.62±1.01

	1000	7.39±1.05	4.52±0.52	5.06±0.83	4.12±0.91	3.71±0.84	4.84±0.88
RS3	100	11.22±1.12	NI	NI	NI	NI	NI
	250	13.58±0.94	NI	NI	3.38±1.39	NI	NI
	500	17.16±1.64	5.12±1.26	3.21±0.51	6.71±1.18	3.17±1.08	3.64±1.07
	1000	20.32±1.24	6.23±0.81	4.53±1.19	8.95±1.61	6.24±1.66	5.43±0.73
RS4	100	12.38±1.03	4.38±1.04	3.68±0.67	10.05±0.87	2.21±0.99	4.76±0.54
	250	14.27±1.51	5.65±0.91	5.04±1.20	11.86±1.17	4.13±1.11	6.87±1.21
	500	18.36±0.82	6.92±0.73	7.40±1.31	13.62±1.20	6.09±0.62	8.26±1.44
	1000	21.54±0.90	8.28±1.27	8.51±0.85	15.82±1.33	9.04±1.33	9.55±1.54

Conc. – concentration; NI – no inhibition; values are expressed as mean±SD; experiments were conducted in triplicate; statistical analysis was done by using Graphpad Instat Version 4 software.

Table: 3 Effect of *rasam* samples (RS1, RS2, RS3 and RS4) against fungal test organisms

Samples	Concentration (µL/mL)	Zone of inhibition (mm)	
		Candida albicans	***Penicillium digitatum***
RS1	100	NI	NI
	250	NI	NI
	500	NI	NI
	1000	3.21±0.84	2.83±0.60
RS2	100	NI	NI
	250	NI	NI
	500	3.07±1.07	2.43±1.06
	1000	5.88±0.64	6.19±1.37
RS3	100	NI	NI
	250	NI	NI
	500	3.62±1.20	3.17±0.80
	1000	6.54±0.86	5.91±1.41
RS4	100	3.27±0.58	3.60±0.59
	250	4.58±1.22	5.11±0.81
	500	6.87±0.76	7.05±1.31
	1000	8.21±0.88	8.50±1.22

NI – no inhibition; values are expressed as mean±SD; experiments were conducted in triplicate; statistical analysis was done by using Graphpad Instat Version 4 software.

The percentage inhibition of RS3 and RS4 against *B. cereus* and RS4 against *P. aeruginosa* are shown in Table 4. The percentage of inhibition was found to be directly proportional to the concentration of both samples, RS3 and RS4. MIC was calculated from the concentration Vs percentage of inhibition best-fit line graph. MIC of RS3 and RS4 against *B. cereus* was found to be 98.94 and 50.07 µL/mL respectively. RS4 showed a MIC of 201.69µL/mL against *P. aeruginosa*. The MIC values of *rasam* may not be very significant comparable to active pharmaceutical agents which, are administered in a fixed dose but, consuming *rasam*

as daily diet would reach its therapeutic dose in time to ensure preventive effect. Stage wise preparation analysis shows, two fold increase in anti-bacterial activity of RS4 compared to that of RS3 (Fig. 1). It is evident that, the process in preparation of *rasam* plays an important role in increasing the anti-bacterial activity of the final product (RS4) (Fig. 1 and 2).

Table 4: Percentage inhibition of samples RS3 and RS4 against bacterial test organisms

Concentration (µL/mL)	% inhibition of *B. cereus*		% inhibition of *P. aeruginosa*
	RS3	RS4	RS4
6.25	33.65	31.25	22.65
12.5	43.56	44.65	37.21
25	52.35	63.87	50.28
50	86.33	99.87	73.61
100	101.07	138.92	92.86
200	-	-	110.87

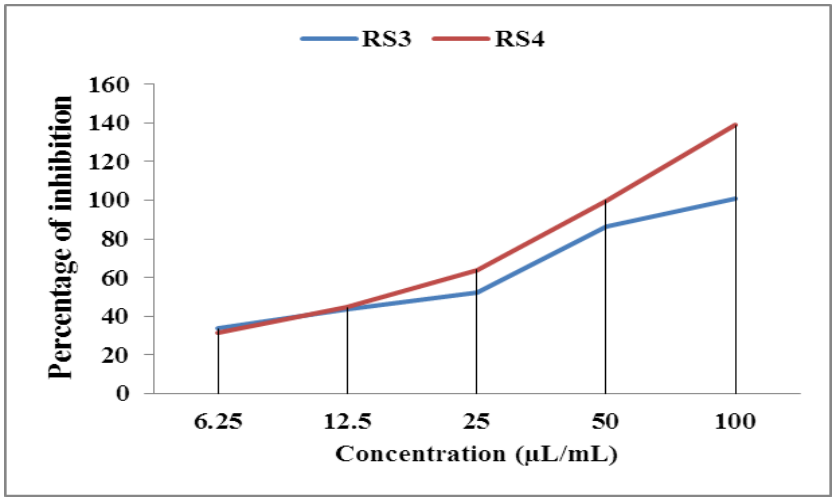

Fig: 1: Percentage inhibition of RS3 and RS4 against *Bacillus cereus* at different concentrations

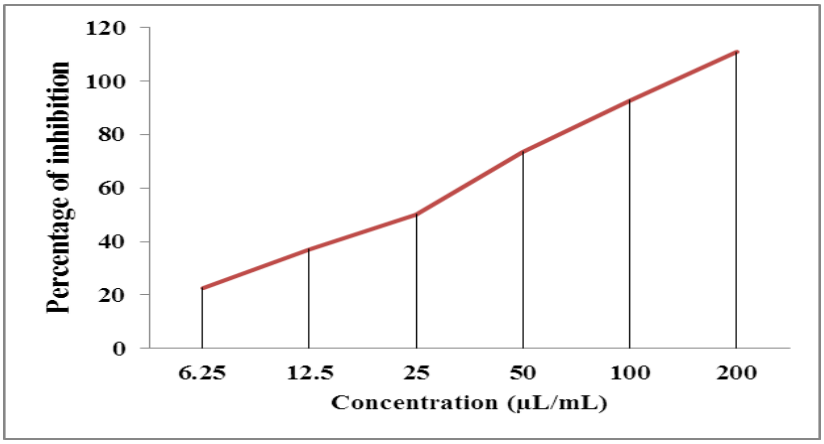

Fig. 2: Percentage inhibition of RS4 against *Pseudomonas aeruginosa* at different concentrations

Bacillus cereus, a Gram-positive, rod-shaped, aerobic, motile, beta hemolytic bacterium causes food borne illness, especially, "fried rice syndrome". The spores of *B. cereus* survive cooking in boiled and fried rice and are capable of germination and outgrowth.[11] Interestingly, *rasam* has shown potent activity against *B. cereus* with an MIC of 50.07 µL/mL. It is evident that, consuming boiled rice with *rasam* will prevent any food borne disease associated with *B. cereus*. The traditional practice of consuming boiled rice with *rasam* from time immemorial is scientifically justified.

The exhibited anti-bacterial activity of *rasam* may be due to the effects of turmeric,[12] cumin, [5] garlic,[13] pepper,[14] curry leaves,[15] and asafoetida.[16] The processing in the formulation of *rasam* involves heating the spices in water and oil. This processing provides tremendous opportunity for a completely altered/different chemical composition of the finally formulated *rasam*. Loss of active principles or synergetic effect or breakdown of inactive metabolite to an active one or formation of new chemical entities (NCEs) is a real possibility. Hence, accurate mechanism for the anti-bacterial activity of *rasam* needs further evaluation.

CONCLUSION

Rasam is a traditional functional food with anti-bacterial activity. Daily dietary consumption of *rasam* can prevent bacteria associated illnesses. The potent anti-bacterial activity of *rasam* against *B. cereus* has scientifically justified its traditional consumption with boiled rice. The real challenge lies not in proving whether *rasam* is a traditional functional food having health benefits, but in defining the benefits and developing the methods to expose them by scientific means.

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