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Article

Antibacterial activity of homemade Indian tomato tamarind soup (rasam) against common pathogens

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SUMMARY

Systematic consumption of traditional foods is a popular way of treating diseases in India. Rasam, a soup of spices and tomato with a tamarind base, is a home remedy for viral infections such as the common cold. While its ingredients have been shown to possess antibacterial properties, the antibacterial activity of rasam itself has not been widely studied. Here, we investigate if rasam, prepared under household conditions, exhibits antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, two common pathogenic bacteria. Due to the antibacterial properties of its ingredients, we hypothesized that rasam would also exhibit antibacterial activity. We observed the growth of each of the bacteria in nutrient broth media with different concentrations of rasam and found that rasam did not exhibit any antibacterial activity. Our results show rasam prepared under household conditions lacks antibacterial activity despite its ingredients possessing such properties. Future studies may aim to understand the reason for the lack of antibacterial properties of spices in the dish when prepared under household conditions and may use alternate methods of preparation and experimentation to further investigate the dish's antibacterial potential.

INTRODUCTION

Traditional Indian food has been shown to possess various beneficial properties, such as being hypoglycemic and reducing hypertension (1). Rasam, an integral part of South Indian cuisine, is no exception. Rasam is a soup that is usually drunk or eaten with rice after a meal. Its antipyretic properties make it a home remedy for the common cold and flu (2). Rasam consists of various spices and herbs that, in previous studies, have been shown to exhibit antibacterial activity (3–9). Asafoetida, curcumin (a terpenoid present in turmeric), piperine (an alkaloid present in black pepper), capsaicin (a terpenoid present in chili peppers), allicin (a sulfoxide compound present in garlic), curry leaves and black mustard seeds have all been shown to exhibit significant antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) at different concentrations (3–9). For example, curcumin and piperine had lower minimum inhibitory concentrations (MIC) of 163 µg/mL and 200 µg/mL respectively while capsaicin had a higher MIC of 512 mg/L against *E. coli* (4–6). Although the antibacterial activity of its ingredients has been shown, the antibacterial properties of rasam have not been properly investigated or quantified.

We aimed to investigate the antibacterial properties of rasam prepared at home rather than in a laboratory setting to get a result that would be applicable in practical conditions. The discovery of rasam's antibacterial properties could pave the way for the investigation of rasam's therapeutic potential for treating bacterial infections.

Due to the antibacterial activity of its ingredients, we hypothesized that rasam would show antibacterial activity against *E. coli* and *S. aureus*. We tested our hypothesis by incubating the bacteria with different concentrations of our homemade rasam sample. However, our data showed bacterial growth in all test tubes. Thus, rasam does not appear to exhibit antibacterial properties. This could be due to a couple of reasons. The concentration of ingredients in rasam may not have been high enough to result in antibacterial activity. As rasam involves cooking the ingredients at a high temperature, the antibacterial activity of these ingredients may have been reduced. Curcumin and capsaicin are unstable at higher temperatures and reduce in content on heating (10–11). The antimicrobial activity of garlic has also been shown to decrease with increase in temperature (12). Extracting the antibacterial phytochemicals from rasam and testing their antibacterial activity at their respective concentrations in the original mixture would be helpful in determining whether the cause of rasam showing no antibacterial activity is due to the concentration of antibacterial phytochemicals not being high enough. Therefore, our result suggests that rasam cannot be an effective home remedy for bacterial infections as it is for the common cold or flu.

RESULTS

To test our hypothesis that rasam will show antibacterial activity against *E. coli* and *S. aureus*, we conducted a separate experiment for each species where we inoculated an equal amount of media with the test organism and added rasam in increasing volume to each of the test tubes. One test tube in each experiment inoculated with bacteria and without rasam acted as the positive control. As rasam itself is colored and turbid, the initial absorbances of each of the tubes were taken using pure media as the blank. Then, after incubating the tubes for 24 hours, the final absorbances were taken to identify bacterial growth and compared with the initial absorbances.

In our first experiment where we evaluated *S. aureus* growth in rasam-containing media, we found that the positive control showed bacterial growth and the samples with the rasam showed greater growth than the control with a sharp increase at 50 µL and a sharp decrease at 100 µL. Following this, there was a gradual increase in the absorbance until 250 µL (**Figure 1**). Overall, there was a moderately positive correlation between the volume of rasam and absorbance, with the Pearson correlation coefficient r(4) = 0.38 and p =

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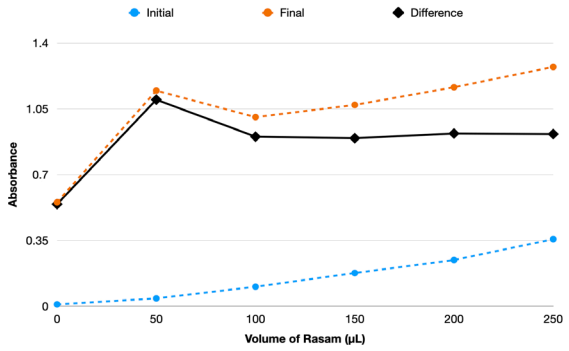

Figure 1: Dose response curve of *S. aureus* grown in nutrient broth with different volumes of rasam. Line graph showing absorbance (at 546 nm) values of samples containing each volume of rasam. The positive control and the five media treated with rasam were measured before (initial) incubation at 37°C for 24 hours (final). The difference between the final and initial measurements is also shown (difference). Pearson r = 0.39, p = 0.23. Samples were run in singular.

0.23. Due to p > 0.05, this result is not statistically significant.

Then, in our next experiment using *E. coli*, we found that the positive control showed bacterial growth and the samples with the rasam showed greater growth than the control with an increase from 50 µL to 250 µL (**Figure 2**). Overall, there was a strong positive correlation between the volume of rasam and absorbance, with the Pearson correlation coefficient r(4) = 0.97 and p < 0.05.

Hence, we found that there was growth of bacteria in both experiments at all concentrations of rasam.

DISCUSSION

We conducted experiments to investigate the antibacterial activity of homemade rasam. While we hypothesized that rasam would show antibacterial activity against *E. coli* and *S. aureus* because of its ingredients possessing antibacterial properties, the results showed that rasam had neither inhibitory nor bactericidal effects. Rather, there was additional growth in the media containing rasam compared to the positive control in both cases.

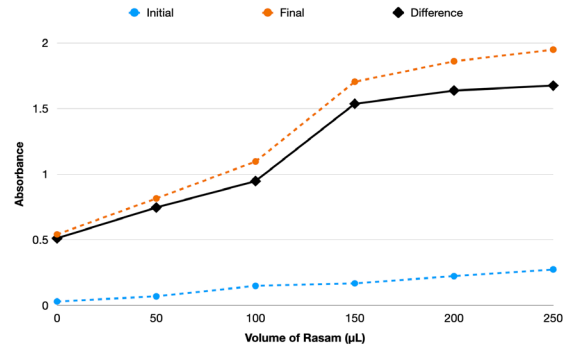

Figure 2: Dose response curve of *E. Coli* grown in nutrient broth with different volumes of rasam. Line graph showing absorbance (at 546 nm) values of samples containing each volume of rasam. The positive control and the five media treated with rasam were measured before (initial) incubation at 37°C for 24 hours (final). The difference between the final and initial measurements is also shown (difference). Pearson r = 0.96, p < 0.05. Samples were run in singular.

Common Name	Morphological Part Used	Botanical Name	Family Name	Quantity Used
Asafoetida	Dried latex exuded from rhizome or tap root	*Ferula assa-foetida* L.	Apiaceae	0.65 g
Black mustard	Seed	*Brassica nigra* L.	Brassicaceae	1.67 g
Black pepper	Unripe drupe	*Piper nigrum* L.	Piperaceae	4.17 g
Chili pepper	Unripe whole fruit (fresh)	*Capsicum annuum* L.	Solanaceae	2.25 g
Chili pepper	Ripe whole fruit (dried)	*Capsicum annuum* L.	Solanaceae	4.52 g
Coriander	Leaves	*Coriandrum sativum* L.	Apiaceae	7.27 g
Cumin	Ripped fruit	*Cuminum cyminum* L.	Apiaceae	4.53 g
Curry leaves	Leaves	*Murraya koenigii* (L.) Sprengel	Rutaceae	1.59 g
Garlic	Bulb	*Allium sativum* L.	Amaryllidaceae	6.18 g
Sesame oil	Seed (oil)	*Sesamum indica* L.	Pedaliaceae	10 mL
Tamarind	Ripped fruit pulp	*Tamarindus indica* L.	Fabaceae	7.62 g
Tomato	Ripped fruit	*Solanum lycopersicum* L.	Solanaceae	49.70 g
Turmeric	Rhizome powder	*Curcuma longa* L.	Zingiberaceae	1.22 g
Salt	NA	NA	NA	3.90 g
Water	NA	NA	NA	200 mL

Table 1: Ingredients used in rasam, biological sources, and quantities.

The additional growth of bacteria could be attributed to the presence of sugars and nutrients in the rasam that promote bacterial growth (**Table 1**). For the ingredients' antibacterial properties to be exhibited, their antibacterial compounds must be present in sufficient concentration. However, as seen in the ingredients list, the amount of antibacterial ingredients used is much less than the amount of nutrient sources such as tomato. Tomatoes have high water content and high solute concentration which enhances microbial growth (13). This idea is supported by previous studies which show the growth of a variety of microbes in tomatoes under storage and in pureed form (14–16). In summary, the lack of antibacterial activity observed could be due to the relatively lower concentrations of antibacterial compounds and conditions suitable for bacterial growth.

The rasam could have been contaminated during its preparation as it was prepared under household conditions and not under sterile conditions with properly sterilized equipment. As the aseptic technique was followed during all procedures except the preparation of the formulation, it is unlikely that contamination could have occurred during the bacterial growth measurement portion of the experiment. Hence, rasam prepared under sterile conditions could be used to get an alternate result. In future experiments, the bacteria from the incubated rasam samples could be grown in agar plates (in addition to turbidimetric quantification) to determine growth of any other species due to contamination.

The lack of antibacterial activity could be because the rasam used in the experiments was a dilute solution of its ingredients, not containing a uniformly fixed proportion of its ingredients. Therefore, a mixture of the ingredients (in proper proportions) in powdered form could be used to investigate its antibacterial properties. This method was used in a similar experiment by Nithya et al., where ethanolic and aqueous extracts of the mixture of dried and powdered ingredients of a similar polyherbal formulation called 'Thaaleesadhi Chooranam' were used (17). The result of this experiment showed that the polyherbal formulation exhibited antibacterial activity against *S. aureus* and *E. coli* (17).

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As rasam itself is a thick and colored mixture, the absorbance values might not be a proper indication of bacterial growth (as it might have been affected by the suspended particles present in the rasam itself). Alternatively, methods such as disk diffusion in which the antibacterial effect of compounds is tested using bacteria-coated petri dishes could be used to test growth inhibition (18).

For *S. aureus*, the results were not statistically significant ($p = 0.22$). However, *E. coli* growth in rasam was statistically significant ($p = 0.001$). The growth trends of *E. coli* in rasam were opposite of the expected result. We found that *E. coli* growth had a strong positive correlation with the increasing volume of rasam. This could be due to the presence of growth-promoting nutrients in the rasam or potential contamination, as previously mentioned. However, as the experiment was conducted without replicates, it is not possible to draw any statistical conclusions. To confirm these results and minimize experimental errors, future experiments should be conducted with more replicates.

The results of our experiment failed to disprove our null hypothesis that liquid homemade rasam does not show any antibacterial activity against *E. coli* or *S. aureus* despite its ingredients expressing such properties. This suggests that rasam is not an effective home remedy for bacterial infections. However, a more refined formulation containing the antibacterial phytochemicals in proper proportions can be tested for antibacterial properties to determine if rasam could be effective in treating bacterial infections under different conditions.

MATERIALS AND METHODS

The experiments were conducted in the microbiology lab of Al Hoty-Stanger Laboratories using rasam that was prepared at home. The ingredients for the rasam were sourced from a local grocery store.

Preparation of Rasam

The rasam mixture was prepared under household conditions. While there are many variations of this dish, the ingredients used in this formulation form a common base for all others. To prepare rasam, 7.62 g of tamarind fruit pulp was immersed in 200 mL of water and crushed. This liquid was strained, and 3.90 g of sea salt and 1.22 g of turmeric were added to it. Then, 49.70 g of fresh tomato was hand-crushed into this mixture. 4.17 g of pepper, 4.53 g of cumin fruit, 6.18 g of garlic cloves and 4.52 g of green chillies were crushed together in a mortar and pestle. This spice mixture was then added to the tamarind-tomato liquid and stirred. 10 mL of sesame oil was heated in a pan for 1 minute after which 1.67 g of mustard seeds was added. After a few seconds, 2.25 g of dry chili peppers and 1.59 g of curry leaves were added to the oil. A few seconds later, the tamarind-tomato liquid containing the spice mixture was added to the oil and allowed to boil for 5 minutes after which 7.27 g of coriander leaves and 0.65 g of asafetida were added to the mixture. The rasam was then refrigerated at 2 °C for 8 hours.

Bacterial Growth and Quantification

Staphylococcus aureus (NCTC 6571) and *Escherichia coli* (NCTC 10418) were grown in nutrient broth (Himedia) for 24 hours at 37 °C in an incubator. Then, 20 µL of this inoculum was added to glass tubes containing 5 ml nutrient broth using a micropipette. For each of the two species, one tube acted as the positive control containing only the media and the inoculum. 50 µL, 100 µL, 150 µL, 200 µL and 250 µL of the rasam mixture was respectively added to each of the remaining test tubes in each of the experiments using a micropipette. The initial absorbance of these samples was taken at 546 nm wavelength in a Hach spectrophotometer using pure media as the blank. The tubes were then incubated at 37 °C for 24 hours. Then, the final absorbance of these samples was taken at 546 nm wavelength using pure media as the blank and compared to the initial values.

Statistics

The difference between the final and initial absorbance values for each volume of rasam was found. Then, the Pearson correlation coefficient (r) was found between these differences and the volumes of rasam. The p-value was calculated from the Pearson correlation coefficient to determine if the results were statistically significant.

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