ORIGINAL RESEARCH

Antimicrobial Effect of Ginger, Garlic, Honey, and Lemon Extracts on *Streptococcus mutans*

[1]Kiran Mathai, [2]Swathy Anand, [3]A Aravind, [4]Praveen Dinatius, [5]Anandhu V Krishnan, [6]Meera Mathai

ABSTRACT

Aim: The aim of this study was to evaluate the effect of lemon, ginger, garlic, and honey extracts on *Streptococcus mutans*.

Materials and methods: Commercially obtained honey, ginger, garlic, and lemon were included in the study to evaluate its efficacy in isolation and in combination against *S. mutans*. The efficacies of extracts were tested using well diffusion method, and its effect was evaluated by measuring the zone of inhibition around the well. Antimicrobial activity of the extracts was carried out individually and compared considering triplicates of all the extracts.

Results: When individual comparison of the extracts was made, garlic showed greatest antimicrobial activity with a mean zone of inhibition (34.9 ± 0.58 mm) and honey showed least antimicrobial activity (0.5 ± 0.6 mm). When combinations of extracts were tested against *S. mutans*, lemon and garlic combination showed the greatest zone of inhibition (27.6 ± 0.43 mm) compared with other combinations, and ginger + lemon combinations showed the least zone of inhibition (12.6 ± 0.43 mm).

Conclusion: This study concluded that garlic showed a greatest antimicrobial effect against *S. mutans* when compared with other preparations individually and garlic and lemon showed greatest zone of inhibition in combination than other preparations.

Clinical significance: Antibiotics and other chemical agents are mainly used to treat the common dental infections. However, due to the excessive use, it can result in antibiotic resistance. Hence, herbal medicines with medicinal values should be replaced with conventional methods.

Keywords: Dental caries, Garlic, *Streptococcus mutans*, Zone of inhibition.

[1]Department of Public Health Dentistry, St. Gregorios Dental College, Ernakulam, Kerala, India

[2]Department of Public Health Dentistry, Noorul Islam College of Dental Sciences, Thiruvananthapuram, Kerala, India

[3]Department of Public Health Dentistry, Government Dental College, Thrissur, Kerala, India

[4]Department of Public Health Dentistry, Century International Institute of Dental Sciences and Research Centre, Kasaragod Kerala, India

[5]Department of Public Health Dentistry, Azeezia College of Dental Sciences and Research, Kollam, Kerala, India

[6]Department of Oral Medicine & Radiology, PMS College of Dental Science & Research, Thiruvananthapuram, Kerala, India

Corresponding Author: Kiran Mathai, Department of Public Health Dentistry, St. Gregorios Dental College, Ernakulam Kerala, India, e-mail: drkiranmathai@gmail.com

INTRODUCTION

Dental caries and periodontal diseases are the major reasons for dental infections and tooth loss. Due to poor oral hygiene, dental plaque got accumulated on the tooth surface.[1] Bacteria present on the plaque ferment the dietary carbohydrates and result in acid production and dissolution of dental hard tissues.[2] Among the bacterial species, streptococci play significant role in the dental caries formation.[3]

After the eruption of teeth, within 6 to 9 months, oral cavity gets colonized by *Streptococcus mutans* and remains there till the teeth present.[1] Various chemical agents have found to reduce the plaque formation. Chlorhexidine is considered to have more antiplaque properties than other antimicrobial agents, but it causes brownish discoloration of the teeth and restorations.[4]

Antibiotics such as penicillin and vancomycin are found to have good anticaries effect, yet make changes in the oral and intestinal flora, and their regular use can result in antibiotic resistance.[5,6] For thousands of years, people are using herbal medicines for the prevention and treatment of various diseases.[7,8]

Garlic (*Allium sativum*) is used for treating various diseases. Allicin is the main component of garlic. It is obtained by crushing the garlic clove.[9] It has antioxidant properties and also protects the human body from various diseases.[10]

Ginger (Zingiber) is a member of Zingiberaceae family. It is used for treating various ailments and also it has anti-inflammatory, antioxidative, and antimicrobial properties.[11]

Honey is a natural product composed mainly of fructose and glucose. It is used as a traditional medicine for centuries. Honey was found to possess bactericidal activity against wide variety of microorganisms.[12]

Lemon belongs to the family of Rutaceae. It is used to treat infections, and it also has antioxidant and astringent properties.[13]

With the above background, the present study was undertaken to evaluate the antibacterial effect of lemon, ginger, garlic, and honey extracts on *S. mutans*.

MATERIALS AND METHODS

The present study was conducted in the Department of Oral Pathology and Microbiology, Coorg Institute of Dental Sciences, Virajpet. Ethical approval of the study was obtained from the Institutional Review Board.

Bacterial Strains and Culture Conditions

Streptococcus mutans Microbial type culture collection 497 culture was subcultured in mitis salivarius (MS) agar and was anaerobically incubated at 37°C for 48 hours.

Preparation of Solutions

Commercially available honey, ginger, garlic, and lemon were included in the study. Its efficiency is tested in isolation and in combination against *S. mutans*. Honey was used in its raw form, whereas ginger and garlic were crushed into a paste using mortar and pestle and the essence of the same was obtained by squeezing the paste using a sterilized mouth mask. The surface of the lemon was sterilized using 70% alcohol, and extract was collected using a sterile needle. The 100% concentrations of all the prepared extracts were used for the research purpose.

Antimicrobial Effect of Extracts against *S. mutans*

To test the efficacies of the extracts, well-diffusion method was used and its effect was assessed by measuring the zone of inhibition around the well. Mitis salivarius agar plates were made, and *S. mutans* colonies adjusted to McFarland standard (0.5) were swabbed onto the plates.
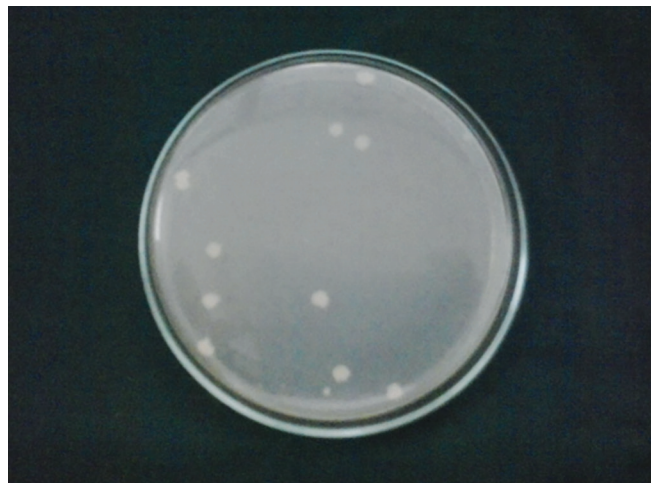

Fig. 1: Zone of inhibition of *S. mutans*

Using a cork borer, well of 8 mm was created. Each 50 µL freshly prepared extract was pipetted using a micropipette and was added to the well. Then, the plates were anaerobically incubated for 24 to 48 hours using an anaerobic jar, and the zone around the well was measured (Fig. 1).

Antimicrobial activity of the extracts was carried out individually and compared by considering triplicates of all the extracts. Depending on the results obtained, the effect of combination of the extracts was performed and analyzed.

RESULTS

Antimicrobial Activity of *S. mutans*

The effect of all extracts was evaluated individually against *S. mutans* using the well diffusion method and then compared.

When extracts were compared, individual garlic showed greatest antimicrobial activity with a mean zone of inhibition (34.9 ± 0.58 mm) and honey showed least antimicrobial activity (0.5 ± 0.6 mm; Table 1).

Depending on the above-obtained results, in which honey did not show significant antimicrobial activity, combinations were made between lemon, garlic, and

Table 1: Mean zone of inhibition observed by the different extracts at 48 hours

Extracts	n	Mean ± standard deviation	Standard error
Garlic	9	34.9444 ± 0.58333	0.19444
Honey	9	0.5556 ± 0.58333	0.19444
Ginger	9	11.7222 ± 0.61802	0.20601
Lemon	9	27.8889 ± 1.19315	0.39772
Ginger + garlic + lemon	9	17.9444 ± 0.46398	0.15466
Garlic + ginger	9	23.8889 ± 0.33333	0.11111
Garlic + lemon	9	27.6667 ± 0.43301	0.14434
Ginger + lemon	9	12.6667 ± 0.43301	0.14434

Table 2: Comparison of zone of inhibition among extracts using ANOVA

	Sum of squares	Df	Mean square	f-value	Significance
Between groups	7,768.413	7	1,109.773	2,785.313	0
Within groups	25.500	64	0.398		
Total	7,793.913	71			
Df: Degree of freedom					

Table 3: Within-group comparison between different extracts using Tukey's *post hoc* test

Extract	Compared with	Mean difference (I–J)	Standard error	Significance
Garlic	Honey	34.38889*	0.29756	0
	Ginger	23.22222*	0.29756	0
	Lemon	7.05556*	0.29756	0
	Gin + gar + lem	17.00000*	0.29756	0
	Gin + gar	11.05556*	0.29756	0
	Gar + lem	7.27778*	0.29756	0
	Gin + lem	22.27778*	0.29756	0
Honey	Garlic	−34.38889*	0.29756	0
	Ginger	−11.16667*	0.29756	0
	Lemon	−27.33333*	0.29756	0
	Gin + gar + lem	−17.38889*	0.29756	0
	Gin + gar	−23.33333*	0.29756	0
	Gar + lem	−27.11111*	0.29756	0
	Gin + lem	−12.11111*	0.29756	0
Ginger	Garlic	−23.22222*	0.29756	0
	Honey	11.16667*	0.29756	0
	Lemon	−16.16667*	0.29756	0
	Gin + gar + lem	−6.22222*	0.29756	0
	Gin + gar	−12.16667*	0.29756	0
	Gar + lem	−15.94444*	0.29756	0
	Gin + lem	−0.94444*	0.29756	0.045
Lemon	Garlic	−7.05556*	0.29756	0
	Honey	27.33333*	0.29756	0
	Ginger	16.16667*	0.29756	0
	Gin + gar + lem	9.94444*	0.29756	0
	Gin + gar	4.00000*	0.29756	0
	Gar + lem	0.22222	0.29756	0.995
	Gin + lem	15.22222*	0.29756	0

*Statistically significant

Table 4: Comparison between combinations using Tukey's *post hoc* test

Extract	Compared with	Mean difference (I–J)	Standard error	Significance
Ginger + garlic + lemon	Garlic	−17.00000*	0.29756	0
	Honey	17.38889*	0.29756	0
	Ginger	6.22222*	0.29756	0
	Lemon	−9.94444*	0.29756	0
	Gin + gar	−5.94444*	0.29756	0
	Gar + lem	−9.72222*	0.29756	0
	Gin + lem	5.27778*	0.29756	0
Garlic + ginger	Garlic	−11.05556*	0.29756	0
	Honey	23.33333*	0.29756	0
	Ginger	12.16667*	0.29756	0
	Lemon	−4.00000*	0.29756	0
	Gin + gar + lem	5.94444*	0.29756	0
	Gar + lem	−3.77778*	0.29756	0
	Gin + lem	11.22222*	0.29756	0
Garlic + lemon	Garlic	−7.27778*	0.29756	0
	Honey	27.11111*	0.29756	0
	Ginger	15.94444*	0.29756	0
	Lemon	−0.22222	0.29756	0.995
	Gin + gar + lem	9.72222*	0.29756	0
	Gin + gar	3.77778*	0.29756	0
	Gin + lem	15.00000*	0.29756	0
Ginger + lemon	Garlic	−22.27778*	0.29756	0
	Honey	12.11111*	0.29756	0
	Ginger	0.94444*	0.29756	0.045
	Lemon	−15.22222*	0.29756	0
	Gin + gar + lem	−5.27778*	0.29756	0
	Gin + gar	−11.22222*	0.29756	0
	Gar + lem	−15.00000*	0.29756	0

*Statistically significant

ginger and its effect was tested against *S. mutans* using well diffusion method.

When combinations of extracts were tested against *S. mutans,* lemon and garlic combination showed greatest zone of inhibition (27.6 ± 0.43 mm) compared with other combinations, and ginger + lemon combinations showed least zone of inhibition (12.6 ± 0.43 mm; Table 1).

Analysis of variance (ANOVA) was used to compare the difference in the antimicrobial effect between and within the groups, and the result was found to be statistically highly significant (p = 0.000; Table 2).

Table 3 shows the comparison of individual preparations with other groups. The individual preparations of garlic, ginger, and honey showed a statistically highly significant (p = 0.000) difference between the groups, whereas lemon did not show any statistical difference (p = 0.995) when compared with combinations of garlic and lemon. When the combinations were compared, all the combinations showed a statistically highly significance (p = 0.000) except garlic + lemon combinations which did not showed a significant difference (p = 0.995) when compared with lemon (Table 4).

DISCUSSION

Streptococcus mutans, a member of viridans streptococci, is the main etiological agent in the causation of dental caries.[1] The studies have shown that during the period of "window of infectivity," these *S. mutans* get transferred from mother to child resulting in more caries-prone teeth in children.[14]

Antibiotic therapy is the conventional method of treatment for bacterial infections. However, few *S. mutans* have started to show resistance to these antibiotics.[1]

Herbal medicines have been used for the treatment of various ailments. According to the World Health Organization, majority of human population depends on herbal medicines for disease treatment and health maintenance.[15]

The present study was done to assess the antimicrobial effect of extracts of lemon, ginger, garlic, and honey on *S. mutans*.

In the present study, garlic showed the greatest zone of inhibition (34.9 ± 0.58 mm) compared with the other extracts. This finding is almost similar to a study done by Jain et al[16] which showed a zone of inhibition (22–44 mm) compared with the study done by Karuppiah and Rajaram.[17]

Honey showed the least zone of inhibition (0.5 ± 0.6 mm). This may be due to the fact that we used the 100% concentration of honey and did not use any dilution. This might have prevented the penetration of honey into the agar plates. However, this result is in contrast to a study done by Ghabanchi et al[18] which showed an inhibition of 13 mm with undiluted honey.

In the present study, ginger showed a zone of inhibition (11.7 ± 0.6 mm), which is in contrast to a study done by Premkishore et al[19] which showed a zone of inhibition (20 ± 0.7 mm).

Our study showed a zone of inhibition (27 mm) for lemon which is in contrast to a study done by Krumina et al[20] which showed a zone of inhibition of 14 mm for lemon extract.

Among the extracts, garlic showed the greater zone of inhibition against *S. mutans*.

Allicin is the main antibacterial agent present in the garlic. It is effective against wide variety of Gram-positive and Gram-negative bacteria. Medicinal effect of garlic is due to its sulfur compounds.[21]

Among the combinations, lemon and garlic combination showed the greatest zone of inhibition (27.6 ± 0.43 mm) which was slightly higher to a study done by Owhe-Ureghe et al[22] which showed a zone of inhibition of 23 mm.

CONCLUSION

Our study concluded that garlic showed a greatest antimicrobial effect against *S. mutans* when compared with other preparations individually and garlic and lemon showed the greatest zone of inhibition in combinations than other preparations. Thus, this study strongly suggests the use of garlic and lemon extracts on preparations of mouthwashes and tooth paste for the prevention of dental caries. To strengthen the use of such agents, further *in vivo* studies and clinical trials are recommended.

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