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Original Article

Microbiology Section

Comparison of Antimicrobial Efficacy of Garlic, Ginger, Cardamom Oil and Chlorhexidine against *Streptococcus mutans*: An In-vitro Study

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ABSTRACT

Introduction: Essential herbs such as garlic, ginger, and cardamom have shown antimicrobial activity with no potential adverse effects and are cost-effective. Despite these advantages, the efficacy of these agents needs to be tested against common oral pathogenic microorganisms to ensure that if found effective, they can be used in clinical settings.

Aim: To evaluate and compare the effectiveness of garlic, ginger, and cardamom oils on *Streptococcus mutans* (*S. mutans*) against chlorhexidine.

Materials and Methods: An in-vitro study was conducted at Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, in the Department of Microbiology and Bharati Vidyapeeth College of Pharmacy, Navi Mumbai, Maharashtra, India from January 2023 to September 2023. Oil extracts of garlic, ginger, and cardamom were loaded onto sterile filter paper discs measuring 6 mm in diameter in concentrations of 50 μL, 100 μL, and 200 μL. Additionally, 2% chlorhexidine (control group) was loaded onto similar sterile paper discs. The discs were dried and placed aseptically on culture media plates inoculated with *S. mutans*, and the plates were then incubated at 37°C overnight. Subsequently, the zones of inhibition were measured in millimeters.

Results: Ginger oil exhibited the highest zone of inhibition, measuring 25 mm, followed by cardamom oil and garlic oil measuring 18 mm and 12 mm, respectively, at a concentration of 200 μL. The zone of inhibition measured for 100 μL concentrations of garlic, ginger, and cardamom oils were 7 mm, 16 mm, and 13 mm, respectively. For 50 μL concentrations of the oils, the zones of inhibition for garlic, ginger, and cardamom were 0 mm, 13 mm, and 9 mm, respectively. The zone of inhibition shown by chlorhexidine was 22 mm for concentrations of 50 μL, 100 μL, and 200 μL.

Conclusion: Essential oil extracts from ginger, cardamom, and garlic were found to have antimicrobial activity against *S. mutans*, with ginger oil showing the highest effectiveness, followed by cardamom oil and garlic oil.

Keywords: Dental caries, Early childhood caries, Herb, Mouthwash, Oil extraction

INTRODUCTION

Dental caries is an infectious disease that leads to demineralisation and destruction of tooth structure [1]. It is a multifactorial disease involving intrinsic factors (host susceptibility, age, immunity, and behaviour) and extrinsic factors (time, diet, and microbiota). There are more than 700 species of microorganisms present in the oral cavity, of which *S. mutans* and *Lactobacillus* species are most commonly involved in the development and progression of caries [2]. *S. mutans* has both acidogenic and aciduric properties and the ability to form colonies on the tooth surface even at low pH [3]. They can utilise sucrose at a faster rate compared to other oral microorganisms, resulting in acid production and creating a suitable environment for the initiation of dental caries [2,3].

There are various possible ways to prevent the colonisation of *S. mutans*, including antimicrobial agents that affect bacterial growth by inhibiting microbial adhesion, colonisation, and metabolic activity [4]. Among various topical antimicrobial agents, chlorhexidine has been considered the gold standard due to its higher antimicrobial activity compared to other agents [4]. Chlorhexidine digluconate is a broad-spectrum antiseptic with both bacteriostatic and bactericidal properties [5]. However, there are various adverse effects such as staining of teeth (brown pigmentation), an unpleasant taste, and desquamation of oral tissues [5].

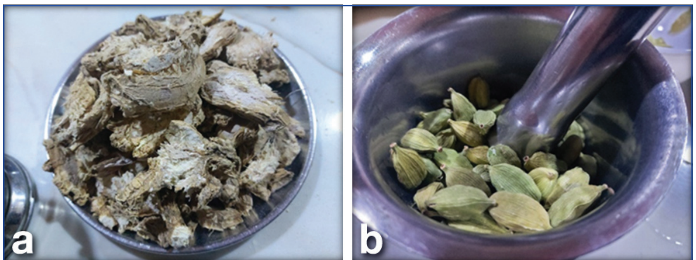
Various essential herbs or medicinal plants have been explored for their properties such as antiseptic, antibacterial, antimicrobial, antifungal, antioxidant, and analgesic properties [6]. In addition to these properties, they are easily available, safe, and cost-effective [5]. The antimicrobial activity is predominantly due to the disruption of the cell membrane, causing cell leakage and eventually cell death [7]. They have the ability to exert biological effects because of their high volatile and lipophilic nature [7]. Blockage of the cell membrane and inhibition of cellular respiration are the secondary actions of essential oils on microorganisms. The need for the study was to explore the antibacterial nature of these oils against commonly found pathogenic microorganisms like *S. mutans*. Not only do these oils possess antibacterial properties, but also, due to their oily nature, they will prevent mechanical adhesion to the teeth. Thus, the rationale of this study was to check their effectiveness in caries prevention by directly evaluating the action of these oils at different concentrations against *S. mutans*. The medium was carefully chosen as oil rather than an aqueous solution because oils have the property to decrease wettability, which is a strong prerequisite for adhesion. Therefore, the aim of the study was to comparatively evaluate the antimicrobial effectiveness of garlic, ginger, and cardamom oils on *S. mutans* against chlorhexidine mouthwash.

MATERIALS AND METHODS

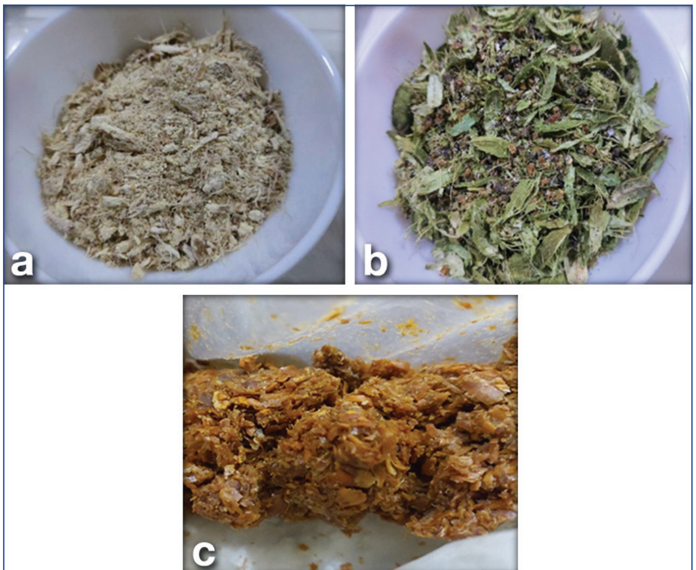
The study was an in-vitro study planned in the Department of Paediatric and Preventive Dentistry and carried out in the Department of Microbiology at Dental College and Hospital (BVDUDCH), Bharati Vidyapeeth (deemed to be University) in collaboration with the Department of M.Pharm., Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, Maharashtra, India. Since this was an in-vitro study, it does did not had any specific inclusion and exclusion criteria as the bacterium chosen was the pioneer bacteria specifically targeted due to its unquestionable role in caries aetiology. The study was conducted from January 2023 to September 2023. The study was approved by the Scientific Research Committee of Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, Maharashtra, India.

Variables of the study: The independent variables of the study were the different concentrations of garlic, ginger, and cardamom oils categorised as categorical variables, while the dependent variable was the zone of inhibition of *S. mutans* categorised as quantitative continuous variables. This pilot study was conducted to determine the antimicrobial efficacy of garlic, ginger, and cardamom oils at three different concentrations.

Conduction of the study: The study was conducted in two parts: the first part involved extracting oils from garlic *(Allium sativum)*, ginger *(Zingiber officinale)*, and cardamom *(Elettaria cardamomum)* using a steam distillator in the Pharmacy College, while the second part included screening the antimicrobial activity of these oils against *S. mutans* using the disc diffusion test in the Department of Microbiology. The dried form of ginger and cardamom, and ground form of garlic, were obtained from a registered herbal shop (S.V. Ayurvedic Bhandar) as depicted in [Table/Fig-1]. Ginger and cardamom were ground into coarse particle sizes as shown in [Table/Fig-2]. Among various oil extraction methods, steam distillation was chosen as it is one of the best methods to obtain the pure form of oil, including their active ingredients [8]. Approximately, 10 mL of oil was extracted from each of the mentioned herbs using a steam distillator. The entire oil extraction process took around six days, with each oil being extracted over two days, as illustrated in [Table/Fig-3,4].

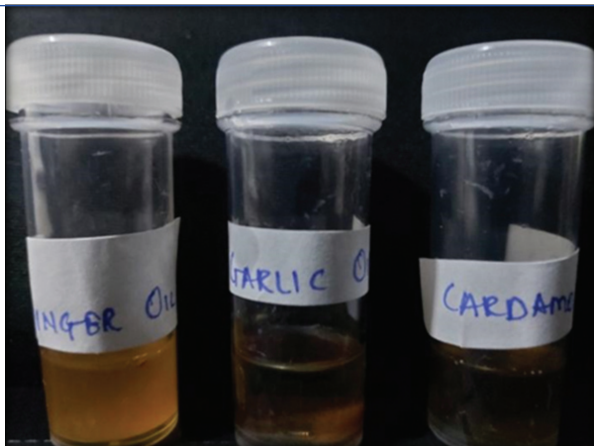


[Table/Fig-1]: a) Dried form of ginger; b) Cardamom.



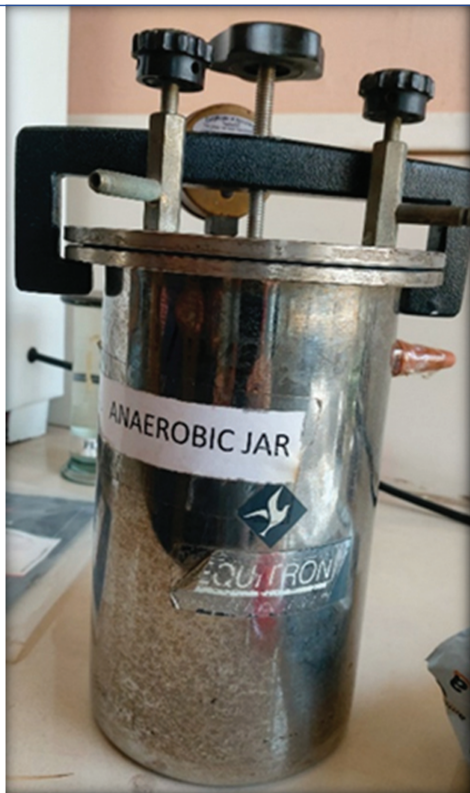
[Table/Fig-2]: Coarse particle form of a) ginger; b) Cardamom; c) Garlic.

[Table/Fig-3]: Steam distillation.



[Table/Fig-4]: Oil extracts of ginger, garlic, and cardamom. [From left to right].

In the second part of the study, the *S.mutans* strain (ATCC no. 25175) was obtained from the Microbiology lab of MGM Medical College and Hospital, cultured, and incubated in an anaerobic gas jar at 37°C for 48 hours, as shown in [Table/Fig-5] [1,9].



[Table/Fig-5]: Anaerobic gas jar.

Screening of Antibacterial Activity (Disc Diffusion Technique)

A suspension of *S. mutans* was prepared in peptone water using 3 to 4 colonies from the mentioned culture plates. This suspension was then anaerobically incubated at 37°C for 3 to 4 hours, and its turbidity was matched with the 0.5 McFarland standard. The aerobic condition has been shown to create an unfavourable environment for the growth of *S. mutans*, thus affecting the virulence of this pathogen [9]. A lawn culture was created from this suspension on Muller-Hilton agar (MHA-Hi media M1084) plates using a sterile cotton swab, and the plates were dried for 15 minutes in an inverted position [1,10]. Filter paper discs loaded with the aforementioned oils at different concentrations (50 μL, 100 μL, and 200 μL) and 2% Chlorhexidine (Hexidine) were placed on the plates [1], which were then anaerobically incubated at 37°C for 48 hours [1]. The zone of inhibition observed on the culture plates was measured in millimeters [1].

RESULTS

Ginger oil exhibited the highest zone of inhibition, indicating its superior effectiveness compared to the other two essential oils. The zone of inhibition measured for oils extracted from cardamom and garlic was less, other zone of inhibition measured for oils from garlic, ginger, and cardamom shown in [Table/Fig-6,7]. Hence, ginger oil demonstrated the highest antibacterial efficacy in terms of the zone of inhibition, with the 200 μL concentration proving to be more effective compared to other concentrations of these essential oils.

Variables	Zone of inhibition as per 3 concentrations (in mm)		
	T1	T2	T3
Garlic oil	50 μL (0 mm)	100 μL (7 mm)	200 μL (12 mm)
Ginger oil	50 μL (13 mm)	100 μL (16 mm)	200 μL (25 mm)
Cardamom oil	50 μL (9 mm)	100 μL (13 mm)	200 μL (18 mm)
Chlorhexidine (2%)	50 μL (22 mm)	100 μL (22 mm)	200 μL (22 mm)

[Table/Fig-6]: Zone of inhibition (in mm).

[Table/Fig-7]: Zone of inhibition measured on Muller- Hinton agar plates for 200 μL, 100 μL and 50 μL concentration, respectively (From left to right).

DISCUSSION

S. mutans is the most cariogenic among all oral Streptococci and a primary initiator of dental caries [1]. Various antimicrobial agents, such as triclosan, chlorhexidine digluconate, and xylitol, are employed in preventing dental caries. Although chlorhexidine is a gold standard antimicrobial agent, it has its drawbacks, such as brown pigmentation, an unpleasant taste, allergies, and lack of specificity [4]. Essential oil extracts from cinnamon, turmeric, cloves, ginger, and garlic are recognised for their antimicrobial activity against oral disease-causing pathogens [5]. These extracts are readily available, cost-effective, easy to use, and devoid of adverse effects.

The objective of this study was to assess the antimicrobial efficacy of garlic oil, ginger oil, and cardamom oil against *S. mutans* in comparison to chlorhexidine. Diallylthiosulphonate (allicin), an active ingredient in garlic *(Allium sativum)*, disrupts the structure and metabolic processes of bacterial cells [11]. In a systematic review and meta-analysis by Torbati M et al., a significant reduction in the average number of *S. mutans colonies* was observed using garlic extract mouthwash (Mean difference: -3.32; 95% CI: -4.39 to -2.26; p-value<0.00001) [12]; however, more clinical trials with larger sample sizes were recommended [12]. Numerous studies have demonstrated the effectiveness of green cardamom *(Elettaria cardamomum)* against *S. mutans* due to the presence of its active ingredients 1,8-cineole, α-terpinyl acetate, and linalool [13]. In a study by Karimi N et al., the Minimum Inhibitory Concentration (MIC) of green cardamom was effective in nanoemulsion form compared to free cardamom essential oil [14]. Karimi N et al., mentioned that candies with cardamom oil exhibited antimicrobial activity against *S. mutans*, with the oil sourced from Zardbond Co. (Iran), although the methodology for oil extraction remains undisclosed [14]. The primary components of ginger *(Zingiber officinale)* are gingerol and related compounds, paradol, and zingerone, which have displayed antibacterial activity against *S. mutans* [15]. In a study by Jain I et al., it was noted that the alcoholic form of ginger has the potential to exhibit antibacterial effects against *S. mutans* [16].

Extraction of oils from these essential herbs can be performed using various methods such as maceration, percolation, soxhlet extraction, steam distillation, and supercritical fluid extraction [9]. Soxhlet extraction is a commonly used method for oil extraction, followed by steam distillation. However, steam distillation was selected as the preferred method for oil extraction due to its ability to yield pure oil with active ingredients [9,17]. A major drawback of this method is that it requires higher expenditure and is time-consuming.

The results of present study demonstrated that ginger oil proved to be an effective antimicrobial agent against *S. mutans* compared to chlorhexidine, with a maximum zone of inhibition of 25 mm at a 200 μL concentration. Zones of inhibition were observed for all three essential oils at a concentration of 200 μL compared to chlorhexidine. However, the findings of a study conducted by Prabhakar AR et al., indicated that garlic oil was effective against *S. mutans* [18]. This difference in results may be attributed to the authors using white bulbs of garlic and obtaining extracts through grinding, followed by mixing in distilled water and direct filtration using membrane filter paper [18,19].

As the study suggests the effectiveness of ginger, cardamom, and garlic oil in decreasing order, their potential role in formulations for topical use such as creams, ointments, gels, and mouthwashes can be considered. Since this project only involves a preliminary evaluation of the antimicrobial effects against *S. mutans* of these herbal agents, further clinical research may be necessary to correlate their actual impact on the pathogen and potential alteration or elimination of the disease process. The zone of inhibition serves as a surrogate outcome for the actual disease process, and the efficacy of these agents could be further assessed through a well-planned randomised controlled trial as the next logical step in the evidence hierarchy for evaluating the effectiveness of these agents. Future research opportunities include developing a balanced formulation to enhance the effectiveness of these agents with good oral biocompatibility for clinical application.

Limitation(s)

The oil extraction process was very time-consuming, which was one of the limitations of the study as it impacted the study's duration. Additionally, the shelf-life of the extracted oils could not be determined. As it was a pilot study, no consideration was given to sample size. Due to its in-vitro design, only the antibacterial effects against *S. mutans* could be determined; the actual effects on children and adults will only be understood through conducting in-vivo studies.

CONCLUSION(S)

As per the results of the present study, the effectiveness of ginger oil at a concentration of 200 µL was higher than that of 2% Chlorhexidine (the highest concentration used clinically). The effectiveness of cardamom oil was also substantial at 200 µL, but the effect of garlic oil is moderate at the same concentration. The antimicrobial action of these agents was directly proportional to their concentration, and their effect was comparable to 2% chlorhexidine. It is important to assess clinical correlation with respect to these findings. Thus, essential oils such as ginger oil, cardamom oil, and garlic oil have shown antimicrobial activity against *S. mutans*. Furthermore, research in in-vivo settings is needed to test the oil extracts as an alternative to chlorhexidine, especially for topical use in the oral environment.

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