



Review Article

Effect of Garlic (*Allium sativum*) Extract on Salivary *Streptococcus mutans*: A Systematic Review and Meta-Analysis

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Abstract

Background: Oral and dental diseases are among the common health conditions which can have different effects on the individuals' health and quality of life. The objective of this study was to examine the effect of garlic (*Allium sativum*) extract on saliva *Streptococcus mutans* (primary outcome) and its side effects (secondary outcome).

Methods: In the present systematic review and meta-analysis, English and Persian databases (PubMed, Cochrane Library, Google Scholar, Scopus, SID, and Magiran) were systematically searched until February 25, 2021. The quality of the included studies was examined using Cochrane handbook, and the meta-analysis was carried out using RevMan 5.3. Heterogeneity of the studies was analyzed by index I². Moreover, the quality of the evidence was assessed through GRADE approach.

Results: A total of 93 studies were found on the databases; 89 articles were screened by title and abstract review and were removed as 19 studies were repetitive and 70 studies were unrelated to the subject under study. Ultimately, 4 articles with a sample size of 171 participants were included in this study, and two of the four studies were meta-analyzed. In all four studies, garlic extract was effective in reducing the number of saliva *Streptococcus mutans* colonies compared to the control group. The results of the meta-analysis showed that using garlic extract mouthwash significantly decreased the average number of saliva *Streptococcus mutans* colonies (Mean Difference: -3.32; 95%CI: -4.39 to -2.26; P<0.00001).

Conclusion: Although this review study indicated the significant effect of garlic extract in reducing saliva *Streptococcus mutans*, the evidence is not sufficient enough to recommend garlic extract to fight saliva *Streptococcus mutans*, and more clinical trials with stronger designs and larger sample sizes are needed.

Introduction

Dental and oral diseases are among the common health conditions which have different effects on the individuals' quality of life and health. Dental diseases cause pain and discomfort and affect physical functions such as chewing, talking, smiling, and personal-social roles.¹ Caries-causing microorganisms can be controlled with different chemicals and natural agents.²

Chlorhexidine and fluorides are among the chemical agents. Chlorhexidine is an anti-bacterial agent which helps control plaques, eliminate bacteria, and reduce the clinical symptoms of gum inflammation. However, it can also cause undesirable effects such as tooth discoloration,

resistance to disinfecting agents, and rare allergic reactions.³ Regular use of mouthwashes containing with fluoride considerably decreases caries in permanent teeth in children and adolescents.⁴ Nonetheless, it can also cause adverse effects.⁵ Natural agents such as chemicals extracted from plants are used as effective and economic therapies to prevent dental caries with fewer side effects.² The tendency to use the anti-caries properties of natural products that can control bacterial virulence without disrupting the beneficial activity of oral microbiome has been increased in recent years.⁶

Garlic, known as *Allium sativum L* in botany, is one of

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the natural agents used in dentistry because of its anti-microbial effects.⁷ The word garlic in English comes from “garleac”, literally meaning “spear-leek” (gar means spear, referring to the shape of a clove resembling spear, and leac is the Old English form of leek).⁸ Garlic has sulfurous components such as allicin, alliin, vinyldithiols, sulfides, and ajoenes. Moreover, it has biological properties as it is anti-bacterial, anti-carcinogen, antioxidant, anti-diabetic, anti-atherosclerosis, anti-fungal, and anti-inflammation. Furthermore, it helps kidneys and reduces blood proteins and blood pressure.⁹ The anti-bacterial property of garlic is mostly related to allicin, which can be effective against gram-positive and gram-negative bacteria.¹⁰ Several laboratory studies have shown the effect of garlic extract in the inhibition of *Streptococcus mutans*.¹¹⁻¹³

Streptococcus mutans is a gram-positive bacterium and the main cause of dental caries. It can synthesize large numbers of glucan extra-cellular polymers from sucrose, immediately resulting in the permanent colonization of hard surfaces and creation of extra-cellular polymer matrix. Moreover, it can transform and metabolize a vast spectrum of carbohydrates into organic acids and grow in environmental stress, particularly at low pH (acidity).¹⁴

Streptococcus mutans contributes to severe dental caries, plaques, and endocarditis, which can have serious effects on health.¹⁵ On the other hand, herbal agents have positive effects with fewer side effects, and dental treatments and general health interventions, so they can improve life quality.¹ Therefore, the aim of this study was to examine the effect of garlic (*Allium sativum*) extract on salivary *Streptococcus mutans* (primary outcome) and its side effects (secondary outcome).

Materials and Methods

PICOS (Participants, Intervention, Comparison group, Outcomes, Study Type)

All age groups from both genders were included in this systematic review. All clinical trials in which garlic extract was used as an intervention on *Streptococcus mutans* were included regardless of their preparation and production methods, duration of treatments, and dosage. The trials in which the intervention group received garlic extract combined with other herbs were excluded. Also, the trials with control groups taking placebo and other treatments or without intervention were included in this systematic review. The primary and secondary outcomes showed the effect of garlic extract on saliva *Streptococcus mutans* and its side effects, respectively.

All randomized controlled trials and quasi-experimental trials published in English and Persian were included in this systematic review. Qualitative studies, systematic reviews, observational researches and letters to the editor were excluded from the present systematic review.

Search strategy

English databases including PubMed, Cochrane Library, Embase, Google scholar, Scopus, and Web of Science

as well as Persian databases including SID and Magiran were systematically searched until February 25, 2021 using the keywords *Streptococcus mutans* [MeSH], garlic [MeSH], saliva [MeSH], and *allium sativum*. Moreover, free keywords were used alongside MeSH keywords. Also, the references of all the included articles were assessed for additional related articles. To search for grey literature (unpublished sources), European Association for Grey Literature Exploitation (EAGLE) and Health Care Management Information Consortium (HMIC) were investigated. For example, the search strategy for PubMed database was as follows:

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(((((("Garlic"[Mesh]) OR (Garlic)) OR (Allium)) OR (allicin)) OR (allium sativum)) AND (((("Saliva"[Mesh]) OR (saliva)) OR (salivas))) AND ("Streptococcus mutans"[Mesh])).
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Selection of studies and Data extraction

The eligibility of the studies was analyzed by two authors (MT, MM) independently. The disagreement between the authors on the eligibility of the studies was resolved by discussion and, in cases of disagreement, by consulting a third party (MM). Furthermore, the references of the included studies were examined.

The data extraction form was designed based on the Cochrane handbook for systematic reviews of interventions¹⁶ including the author's name, publication year, country, clinical trial type, sample size, participants' age, intervention, control group, measurement instrument, results and side effects. Two authors (MT, MM) extracted the information of the studies independently.

Evaluation of the risk of bias in the included studies

Two authors (MT, MM) independently examined the risk of bias in terms of randomization, concealment of allocation, blinding, incomplete outcome data, and selective outcome reporting for all the studies based on the criteria in Cochrane Handbook.¹⁶ According to the Handbook, the risk of bias was assessed as low risk, high risk and unclear risk. Next, any disagreement between the authors was resolved by discussion, and a third author (MM) was consulted if required.

The evidence quality was assessed using the GRADE approach in the four categories of high, moderate, low and very low.¹⁷ Based on this method, the random controlled experiments that do not have serious limitations are evaluated as high quality. However, the general quality of the evidence can be reduced with the presence of each one of the following factors: indirectness of the evidence, imprecision of the results, high likelihood of bias, inconsistency of the results or unexplained heterogeneity, and publication bias.

Data analysis

RevMan was used for meta-analysis.¹⁸ The mean difference (95% confidence interval) was considered for continuous outcomes. Heterogeneity was identified by I squared (I²)

statistic. Heterogeneity >50% with p-value <0.1 were considered as the criteria for judgement.

Results

Results of the search

In total, 93 articles were obtained from the databases; 89 articles were screened by title and abstract review and were removed as 19 studies were repetitive and 70 studies were unrelated to the subject under study. Four articles were included in this systematic review, and two trials, in which the effects of garlic extract on saliva *Streptococcus mutans* were reported as mean (standard deviation) compared with the control group were entered into the meta-analysis (Figure 1).

Information on the included studies

Table 1 indicates the information on the included trials. The participants were divided into 4 groups in two of the trials^{19,20} and into 3 groups in the other two trials.^{21,22} In Chavan *et al.*²¹ study, the effect of garlic extract mouthwash was compared to that of chlorhexidine 0.2% as well as Sorbitol 5% and Spearmint oil 5% (control group); in Padiyar *et al.*¹⁹ study, it was compared to Triphala mouthwash and chlorhexidine 0.2%; in Prabhakar *et al.*²⁰ study, it was compared to tea tree 0.2% mouthwash, curry

leave mouthwash 2.5% and placebo group; and in Groppo *et al.*²² study, it was compared to chlorhexidine 1.2% and tea tree 0.2%.

The mouthwashes were used as follows: 10 mL before sleep for a week,²¹ 10 mL once a day for 15 days,¹⁴ 10 mL twice a day for seven days,²⁰ and 10 mL once a day for 7 days²² in the studies by Chavan *et al.*²¹, Padiyar *et al.*¹⁹, Prabhakar *et al.*²⁰, and Groppo *et al.*²² respectively. The sample size was 171 in the included studies and ranged from 30 participants in the study by Groppo *et al.*²² to 60 participants in the study by Padiyar *et al.*¹⁹ All the included studies were carried out in India. The participants in the included review studies were school children (9-12 age group)^{20,21} and adults (18-35 age group).^{21,22} Saliva samples were taken from the participants for microbiological evaluation in order to examine the effect of garlic extract mouthwash, and *Streptococcus mutans* colony forming unit was measured in the laboratory after preparation.

In the study by Chavan *et al.*²¹ saliva *Streptococcus mutans* count was significantly lower with 3.82 (1.33) in 2.5% garlic extract mouthwash compared with that in the chlorhexidine group with 5.62 (2.17) and the control group with 6.4 (2.16) ($p < 0.05$). The side effect of garlic extract mouthwash was a burning sensation after use as all of the participants complained about it.

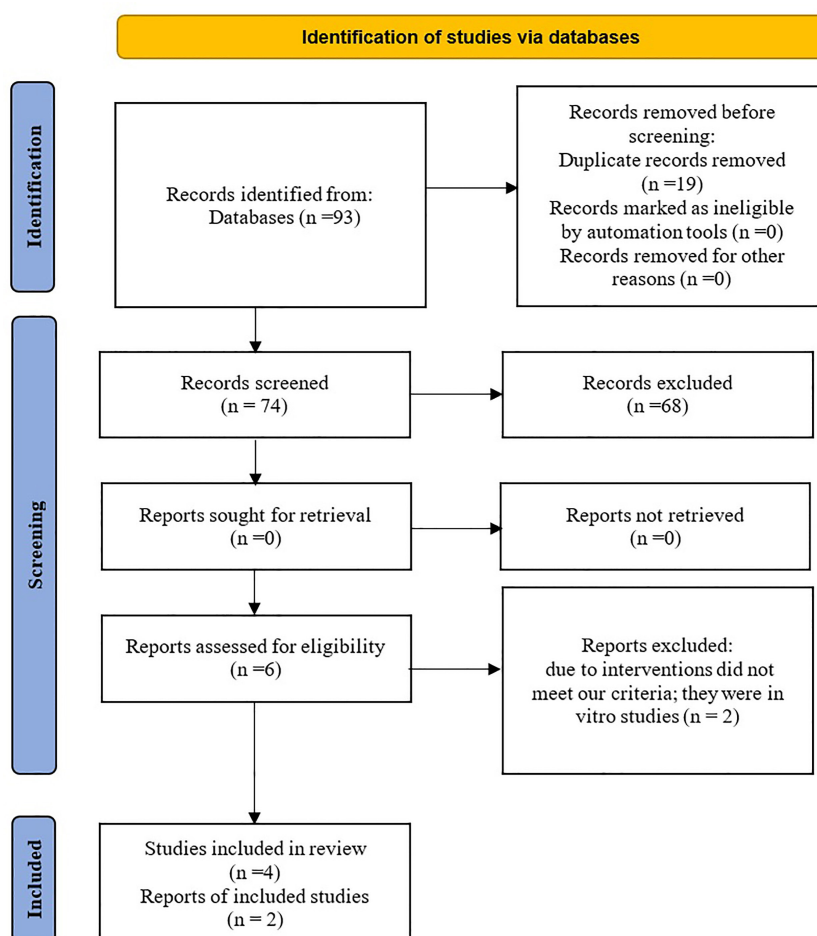


Figure 1. Flow diagram of the study

Table 1. Information of included studies.

| Authors | Study type | Sample Size | Age of participants (years) | Intervention (dosage + months of treatment) | Comparison (dosage + months of treatment) | Duration of follow up | Outcomes | Outcome | Results | Adverse effects |
|---------------------------------------|--------------------------------------|--|-----------------------------|---|---|-----------------------|---|---|---|---|
| Padiyar <i>et al.</i> ¹⁹ | Randomized clinical blind study | 2.5% garlic extracts mouthwash group: 15 6%triphala mouth-wash group: 15 0.2% chlorhexidine mouthwash group: 15 Control group: 15 | 9 -12 | Garlic extract mouthwash (2.5%): 10 ml was used at night once a day and swish it in all quadrants of the mouth for a period of 2 minutes for 15 days. For the next 15 days, the participants in all the groups discontinued the mouthwashes and continued regular tooth brushing twice a day. Streptococcus mutans count on days 1, 15, and 30. | Control group: "mouth rinsing with distilled water once a day at night for 15 days." | 30 days | Salivary streptococcus mutans count | The colony counts were obtained by a clinical microbiologist | Garlic extract mouthwash was efficient in decreasing the salivary Streptococcus mutans count | It has not been mentioned. |
| Prabhakar <i>et al.</i> ²⁰ | A clinical and microbiological study | 2.5% garlic mouth-wash group: 9 2.5% curry leaves mouthwash group: 9 0.2% tea tree oil mouthwash group: 9 Placebo group: 9 | 9-11 | 2.5% garlic mouthwash group: 10 ml of mouthwash daily twice rinse for one minute. It was carried out after last tooth brushing for 7 days. Mouthwash regimen was discontinued after 7th day, and salivary samples were collected on the 14th day to observe the substantivity. Salivary samples were collected on baseline (0 th day), ½ hour, 3rd and 7 th and 14 th day morning. | Placebo group: dosage of placebo was not mentioned. This group received the placebo in the same way of garlic mouthwash group | 14 days | Salivary streptococcus mutans count and adverse effects | 1- Using digital colony counter. 2- An analytical scale (10 cm) was used to assess the above mentioned parameters in which a line was drawn from 0 cm to 10 cm and subjects marked the scale with an 'x' and the length was measured | 2.5% garlic was able to decrease streptococcus mutans count to a significant level and also indicated more adverse effects than mouth-washes with 0.2% tea tree oil and 2.5% curry leaves | Unpleasant taste ($\chi^2 = 20.2$, $p = 0.003$). Burning sensation ($\chi^2 = 17.9$, $p = 0.006$) and nausea ($\chi^2 = 29.3$, $p < 0.05$), the bad breath ($\chi^2 = 27.9$, $p < 0.05$) was more intense in garlic compared to the tea tree oil and curry leaves mouthwash |

Table 1 Continued.

| | | | | | | | | | | |
|------------------------------------|-----------------------------|---|-------|---|---|---------|--|---|---|--|
| Chavan <i>et al.</i> ²¹ | Randomized controlled trial | 2.5% garlic extract mouthwash group: 15 Chlorhexidine mouthwash (0.2%) group: 15 Control group: 15 | 18-35 | Garlic extract mouthwash group: 10 ml of the allocated mouthwash for 30 s for 1 week before going to bed. | Control group: 10 ml of the allocated mouthwash (water + sorbitol + spearmint oil) for 30 s for 1 week before going to bed. | 7 days. | Salivary streptococcus mutans count and adverse effects. | 1- the saliva sample was subjected to microbiological assessment, and the determination of <i>Streptococcus mutans</i> colony-forming units (CFUs) was performed. | Garlic extract has been indicated to be effective on <i>Streptococcus mutans</i> . | All of the participants belonging to the garlic extract and negative control groups complained of a burning sensation 5 min post-rinsing. None of the participants experienced any staining of the teeth or any other adverse effects. |
| Grosso <i>et al.</i> ²² | Randomized clinical study | Garlic group 2.5% garlic solution: White garlic (n=10) Tea tree group 0.2% tea tree oil solutions (n=10) Chlorhexidine group 0.12% chlorhexidine solution (n= 10) | 18-35 | All mouthwashes were used daily thirty minutes after the last tooth brushing | In the results, the groups were not compared. Each group was compared with its arrival time. | 7 days | Salivary streptococcus mutans count and adverse effects. | Saliva samples were collected to assess oral microorganisms and mutans streptococci | Mouthwashes with 2.5% Garlic and 0.2% tea tree oil reduced oral microorganisms as 0.12% Chlorhexidine | Burning sensation was more intense in the garlic group. Garlic caused the worst breath. The taste of garlic solution was the worst in Comparison with the Chlorhexidine and tea tree oil |

In the study by Padiyar *et al.*,¹⁹ saliva *Streptococcus mutans* count was significantly different in the four study groups. However, chlorhexidine mouthwash was more effective in reducing *Streptococcus mutans* compared with garlic extract and Triphala mouthwashes and the control group ($P < 0.05$). The mean (SD: standard deviation) of *Streptococcus mutans* counts were reported 1.24 (0.33), 1.93 (0.38), 2.31 (0.43), and 6.03 (0.55) in chlorhexidine, Triphala, garlic extract, and control groups, respectively. *Streptococcus mutans* count was significantly lower in garlic extract group compared with that in the control group ($P < 0.05$). This study did not report the side effects of garlic extract mouthwash.

In the trial by Prabhakar *et al.*,²⁰ saliva *Streptococcus mutans* count in garlic extract group significantly decreased after using the mouthwash compared with pre-treatment count. The reported side effects for garlic extract mouthwash were bad taste, burning sensation, bad odor, and nausea.

In the trial by Grosso *et al.*,²² the results indicated that the average *Streptococcus mutans* count was lower than pre-treatment average count in garlic extract group after the first, second, and third weeks of intervention ($p < 0.05$). The side effects such as bad odor, burning sensation, and worse breath were significantly more pronounced in garlic extract

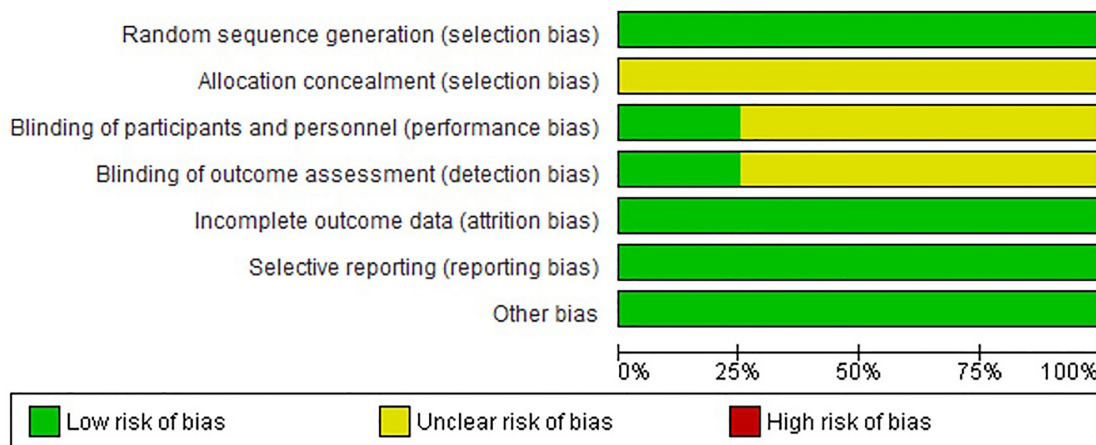


Figure 2. Risk of bias graph in included studies

group than in chlorhexidine and tea tree groups ($p < 0.05$).

Results of risk of bias evaluation

The participants were randomly assigned to the study groups in all the trials included in this systematic review; thus, random sequence generation was considered low risk. There was no explanation on allocation concealment in the included trials and, as a result, it was considered as unclear-risk. Only one study¹⁹ had blinding of participants and blinding of outcome assessor; therefore, blinding was considered as low risk in this study and as unclear risk in the other trials. Bias related to incomplete outcome data was low risk in all the four studies as all the participants completed the study. Selective reporting bias was low risk as all the outcomes were reported in all the studies. Moreover, other biases were low-risk in all the four studies (Figures 2 and 3).

Results of meta-analysis

The meta-analysis results of two trials indicated that using garlic extract mouthwash decreased the number of saliva *Streptococcus mutans* colonies compared with the control group (mean difference = -3.32 CFU/m × 10⁵; 95% CI: -4.39 to -2.26; $P < 0.00001$). The heterogeneity level was moderate ($I^2 = 64%$, $p = 0.09$). Due to high heterogeneity, random effect was reported instead of fixed effect (Figure 4). According to the GRADE approach regarding the comparison between garlic extract group and control group, the evidence was low quality. Therefore, the results were close to reality with low confidence. The results of evidence quality assessment by GRADE are shown in Table 2. Since the number of the studies included in the meta-analysis was smaller than 10, the graphical or statistical methods were not used to assess publication bias.¹⁶

Discussion

According to the results, there was a significant statistical difference between intervention and control groups regarding the average number of saliva *Streptococcus mutans*. However, due to the insufficient number of the

included RCTs and the total sample size, a final conclusion cannot be drawn for the effectiveness of garlic extract on saliva *Streptococcus mutans*.

Garlic has been known as an alternative therapy used to treat infectious diseases for years.^{23,24} Moreover, some laboratory studies have shown the positive effect of garlic in controlling *Streptococcus mutans*.^{21,25} The anti-bacterial property of garlic is mostly attributed to allicin, which acts through the chemical reactions with thiol-containing



Figure 3. Risk of bias summary in included studies.

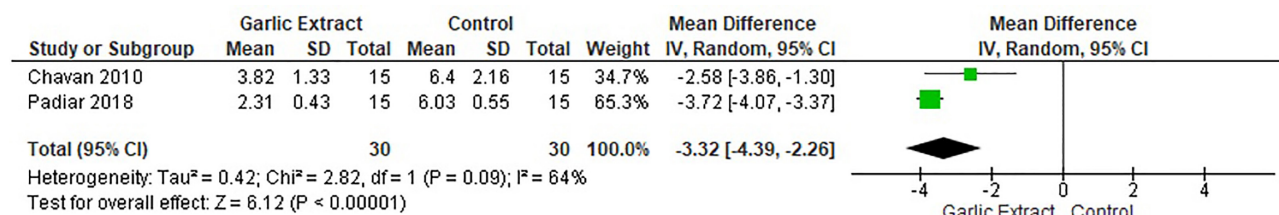


Figure 4. Forest plot of differences between garlic extract group versus the control group in terms of salivary streptococcus mutans counts

Table 2. Quality assessment of included trials according to GRADE approach.

| Number of studies | Design | Quality assessment | | | | | Publication bias | MD (95% CI) [†] | Certainty |
|-------------------|-------------------|--------------------------------------|------------------------------------|-------------------------|----------------------------------|----------------|------------------------|--------------------------|-----------|
| | | Risk of bias | Inconsistency | Indirectness | Imprecision | Garlic Extract | | | |
| 2 | randomized trials | No Serious Risk of bias [*] | Serious Inconsistency [*] | No serious indirectness | Serious Imprecision [‡] | undetected | -3.32 (-4.39 to -2.26) | Low ⊕⊕○○ | |

* Substantial inconsistency: I²=64%; [‡]Total number of participant is less than 400; [†] Mean Difference (95% Confidence Interval)

enzymes such as RNA polymerase, thioredoxin reductase, and alcohol dehydrogenase. In addition, allicin has shown an interesting potential in general healthcare; in fact, its antioxidant and immunomodulatory effects arise from mechanisms that may define their potential in inhibiting apoptosis proliferation and induction in different human cancers.²⁶ Nevertheless, allicin, the active component of garlic, could stimulate the stomach, particularly in high doses. Additionally, it has been reported that garlic affects the pharmacokinetics of antiviral drugs and anticoagulants. Therefore, these points need to be taken into consideration when garlic is taken as a medicine to treat various diseases.⁹ *Streptococcus mutans* strains produce up to three glycosyltransferase (GtfB, GtfC, and GtfD) and use the glucose moiety of sucrose for synthesizing glucose glucan polymers.¹⁴ Increased number of saliva *Streptococcus mutans* results in more dental caries.²⁷ Live/dead staining following confocal laser scanning microscopy analysis shows that allicin has a bactericidal effect on the *Streptococcus mutans* developed in the biofilms (dental plaque).²⁸ Therefore, the mechanism of the effect of garlic on the number of *Streptococcus mutans* colonies can be explained by considering the antibacterial effects of allicin in the garlic on thiol-containing enzymes,²⁶ which are essential for bacterial nutrition.²⁹ Burning sensation, bad odor, nausea, and worse breath were reported as garlic extract side effects in the trials included in this systematic review. Garlic is an herbal medicine which has proved tolerable with the fewest side effects in the trials. No severe side effect has been reported even though most of the participants complained about its bad odor.³⁰ Smelly breath and body odor have been reported in different studies as some side effects of garlic taken orally. Other unproven side effects have been stomachache, esophagus burn, asthma, and bleeding.³¹ Although no severe side effect has been reported for garlic, bad odor in oral ingestion form could limit its use.³²

Strengths and limitations

This was the first review study on the effect of garlic

extract on saliva *Streptococcus mutans*. In all the four included studies, the participants were randomly assigned to intervention and control groups and there was no participant drop-out. Although there was no information on blinding in some studies, it could not distort the results as the endpoint of the studies was the number of *Streptococcus mutans* and a non-subjective outcome. Some weak points of this study could be the limited number of the included studies and the small sample size. Also, this study was not registered in PROSPERO or similar databases.

Conclusion

This review study indicated the significant effects of garlic extract in reducing saliva *Streptococcus mutans*. However, there is still not sufficient evidence to recommend garlic extract to fight saliva *Streptococcus mutans*, so further clinical trials with stronger designs and larger sample sizes are needed for better conclusions. Moreover, due to the language limitation, it cannot be assured that all the trials were included in this review.

Ethical Issues

The study was reviewed and approved by Ethics Committee of Tabriz University of Medical Sciences (code: IR.TBZMED.REC.1399.1113).

Acknowledgements

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Author Contributions

MT and MM performed initial search of databases and were a major contributor in writing the manuscript. PE and MT reviewed studies to investigate eligibility criteria. MM regulated methods and performed meta-analysis and supervised writing of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors report no conflicts of interest.

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