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Evaluation of Silybum marianum Extract and Natural Antimicrobial Dental Disinfectant Solutions for Toothbrush Decontamination and Oral Hygiene Infection Control

Keywords

Silybum Marianum; Herbal Antimicrobial; Dental Pharmaceutical; Toothbrush Decontamination; Oral Hygiene; Natural Antimicrobial Agents

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Abstract

Background: Toothbrushes may act as reservoirs for oral and extraoral microorganisms and contribute to cross-contamination and oral microbial reinfection. This is clinically relevant for patients requiring restorative, periodontal, and prosthodontic maintenance. Regular toothbrush disinfection may help reduce bacterial contamination.

Aim: To evaluate toothbrush microbiological contamination and the antimicrobial effectiveness of Silybum marianum extract and other natural dental disinfectant solutions.

Materials and Methods: Sixty volunteers aged 15–45 years used standardized toothbrushes for seven days. Toothbrushes were disinfected using baking soda, 50% vinegar, 3% garlic solution, aqueous Silybum marianum extract, 0.2% chlorhexidine gluconate, and tap water control. Bacterial counts before and after disinfection were analyzed statistically.

Results: All disinfectant solutions except tap water showed significant bacterial reduction ($P<0.05$). The highest reduction was observed with 0.2% chlorhexidine, followed by Silybum marianum, garlic, vinegar, and baking soda. No significant difference was found between Silybum marianum, garlic, and chlorhexidine groups ($P>0.05$). Bacillus subtilis was the most prevalent bacterial isolate.

Conclusion: Silybum marianum demonstrated promising antibacterial activity and may serve as a natural adjunctive dental disinfectant for toothbrush decontamination; however, further clinical validation is required before routine dental recommendation.

Introduction

In order to prevent periodontal diseases and dental caries, brushing is essential for removing dental plaque and maintaining oral hygiene[1]. Toothbrushes are colonized by oral and environmental microorganisms, which can survive between the bristles for one to seven days. Many bacteria, viruses, and fungi can spread or reinfect through contaminated toothbrushes [2,3]. Effective microbial control is important not only for maintaining general oral hygiene but also for supporting restorative and prosthodontic dental maintenance, where plaque accumulation and oral microbial reinfection may compromise long-term oral health outcomes. Toothbrush decontamination may therefore represent a simple supportive measure for reducing bacterial transmission and improving oral hygiene maintenance in susceptible dental patients.

Regular usage of infected toothbrushes can spread bacteria between people or within the same person's oral cavity.

To stop spreading of diseases, toothbrush disinfection is essential [4,5]. Therefore, there is a need for disinfection techniques that are quick, economical, safe, and simple to use. Finding a substitute for current medications is crucial since the prevalence of drug resistance in common pathogens is rising over time, and there is a danger connected with chemotherapeutic treatments. The best source of these alternative medications may be the plants, which are recognized to have medicinal qualities. The antibacterial qualities of therapeutic natural substances have been reported more frequently in recent years from all over the world [6].

Chlorhexidine gluconate is a widely used dental antimicrobial agent with broad-spectrum antibacterial activity and is commonly regarded as the gold standard chemical disinfectant in oral healthcare. Although it is regarded as the "gold standard," it has a few adverse effects, including tooth discolouration, mucosal irritation, changed taste perception, parotid edema, and increased supragingival calculus formation [7,8]. In various regions of the nation, garlic (Allium sativum) is used in cooking. Garlic's antibacterial properties stem from the presence of allicin, which has been utilized in traditional medicine since ancient times to cure a variety of illnesses. Mouthwash with garlic may help prevent tooth decay [9]. White vinegar is favored as a practical substitute disinfectant in many places because of it was found have a potent antibacterial properties and affordable pricing. A few research have examined with the using of white vinegar in dental practice. White vinegar was often used to disinfect toothbrushes despite the fact that it kills a lot of microorganisms. Acetic acid produces up 4% to 18% of white vinegar [10]. Baking soda, often known as sodium bicarbonate, is a cheap and highly efficient method of lowering cariogenic S. mutans in saliva and plaque. By acting as a mild abrasive to remove plaque and kill specific bacteria, it has been used to treat oral infections and prevent dental diseases, particularly Streptococcus mutans. It was found to be worked by raising pH levels, which can damage bacterial membranes and boost immunity. Bacterial growth is inhibited by the bicarbonate ion itself, which is not just an osmotic impact. Bacterial cells suffer structural damage (cytoplasm shrinkage) when exposed to high concentrations (e.g., 1% or greater) [11,12]. Milk thistle, or Silybum marianum, has significant antibacterial qualities against both gram-positive and gram-negative bacteria, including drug-resistant strains like MRSA. This is especially true of silybin, which is the primary component of silymarin. It performs by preventing the formation of biofilms, damaging cell membranes, and restricting bacterial growth [13,14].

Because long-term chlorhexidine use may be associated with adverse effects, interest has increased in natural antimicrobial agents and herbal dental pharmaceutical preparations as alternative or adjunctive oral disinfectants. Comparative evaluation of herbal agents with chlorhexidine may therefore provide clinically relevant information regarding their potential role in oral hygiene and toothbrush decontamination. This study aimed to evaluate toothbrush microbiological contamination and compare the antimicrobial effectiveness of aqueous Silybum marianum extract and other natural disinfectant solutions with 0.2% chlorhexidine gluconate as a standard dental antimicrobial agent.

Materials and Methods

Sixty volunteers aged between 15 and 45 years from the College of Dentistry, University of Babylon, participated in this study. Medical history, oral hygiene habits, and oral examinations were recorded for all participants. Individuals with systemic disorders, recent antibiotic or antiseptic mouthwash use, orthodontic appliances, intraoral prostheses, smoking habits, or narcotic use were excluded from the study. All participants received standardized toothbrushes and toothpaste to minimize variations in mechanical plaque control. Participants were instructed to brush twice daily for 2–4 minutes over a seven-day period. After brushing, toothbrushes were rinsed under running tap water for 30 seconds and stored upright in open air conditions outside the bathroom environment. After seven days of use, toothbrushes were collected individually in sterile plastic bags, labeled, and immediately transported to the microbiology laboratory for analysis.

To recover microbial species, toothbrush samples were immersed in sterile nutrient broth and incubated at 37°C for 48 hours. Following incubation, samples were vortexed for one minute, and 0.1 mL aliquots were inoculated onto nutrient agar and blood agar plates. The plates were incubated at 37°C for an additional 48 hours before bacterial colony-forming units (CFU) were recorded.

Disinfection Groups:

The toothbrush samples were randomly allocated into six disinfection groups:

1. Tap water control group
2. 0.2% chlorhexidine gluconate
3. Baking soda solution
4. 50% white vinegar solution
5. 3% garlic solution
6. Aqueous Silybum marianum extract

To make the baking soda solution, use one or two tablespoons of baking soda with one cup of water[15]. Half To prepare the white vinegar solution, equal portions vinegar was combined with warm water [16]. A 3% garlic solution was made by cleaning 100 grams of fresh bulbs, blending them with 100 milliliters of distilled water, filtering the mixture through membrane filter paper measuring 0.2 micrometers to create a clear liquid, and then adding 12 milliliters to the distilled water that had just been made using [17]. Ten grams of crushed plant material (from the Hilla City local market) were put in a Soxhlet extractor with 300 milliliters of distilled water for twelve hours to create the Silybum marianum solution, Whatman's No. 1 filter paper was subsequently utilized for filtering it[18].

Statistical analysis:
Statistical analysis was performed using Jamovi software (Version 2.6). The Shapiro–Wilk test was used to evaluate data distribution. Kruskal–Wallis and Dwass–Steel–Critchlow–Fligner post-hoc tests were applied for intergroup comparisons. Statistical significance was set at $P < 0.05$.

Results
Following toothbrush disinfection with different dental disinfectant solutions, a significant reduction in bacterial count was observed in all groups except the tap water control group ($P<0.05$). These findings demonstrated the antimicrobial effectiveness of the tested disinfectant solutions in reducing toothbrush microbial contamination (Table 1 and 2).

Table1: Comparison of bacterial counts (colony-forming units, CFU) before and after toothbrush disinfection using different disinfectant solutions

Groups	P value	W
Before baking soda use vs After baking soda use	0.008	-5.357
Before white vinegar use vs After white vinegar use	0.008	5.382
Before garlic use vs After garlic use	0.008	5.370
Before Silybum marianum use vs After Silybum marianum use	0.008	5.392
Before CHX use vs After 0.2% chlorhexidine use	0.007	5.421
Before tap water use vs After tap water control use	1.000	-0.660

Table 2: Dwass–Steel–Critchlow–Fligner post-hoc pairwise comparisons of bacterial counts (CFU) before and after toothbrush disinfection

Groups	Mean	Median	SD
CFU before baking soda use	85.400	84.500	3.098
CFU after baking soda use	18.300	18.500	3.057
CFU before white vinegar use	72.600	72.000	2.319
CFU after white vinegar use	10.400	10.000	1.430
CFU before garlic use	81.200	81.500	1.476
CFU after garlic use	3.100	3.000	1.449
CFU before Silybum marianum use	89.200	90.000	3.584
CFU after Silybum marianum use	1.300	1.000	1.418
CFU before CHX use	83.900	83.000	1.969
CFU after CHX use	0.700	0.500	0.823
CFU before tap water use	74.700	73.000	2.869
CFU after tap water use	74.300	73.000	2.791

According to Table 3 and Figure 1, the greatest percentage reduction in bacterial count after disinfection was observed in the 0.2% chlorhexidine group, followed by Silybum marianum, 3% garlic solution, 50% white vinegar, and baking soda groups. The lowest reduction was observed in the tap water control group.

Table 3: Mean differences and percentage reduction in bacterial counts after toothbrush disinfection

Groups	Mean difference	Percentage reduction
baking soda	67.100	78.57%
50% white vinegar	62.200	85.67%
3% garlic	78.100	96.18%
sillybum marianum	87.900	98.54%
0.2% CHX	83.200	99.16%
Tap water	0.400	0.05%

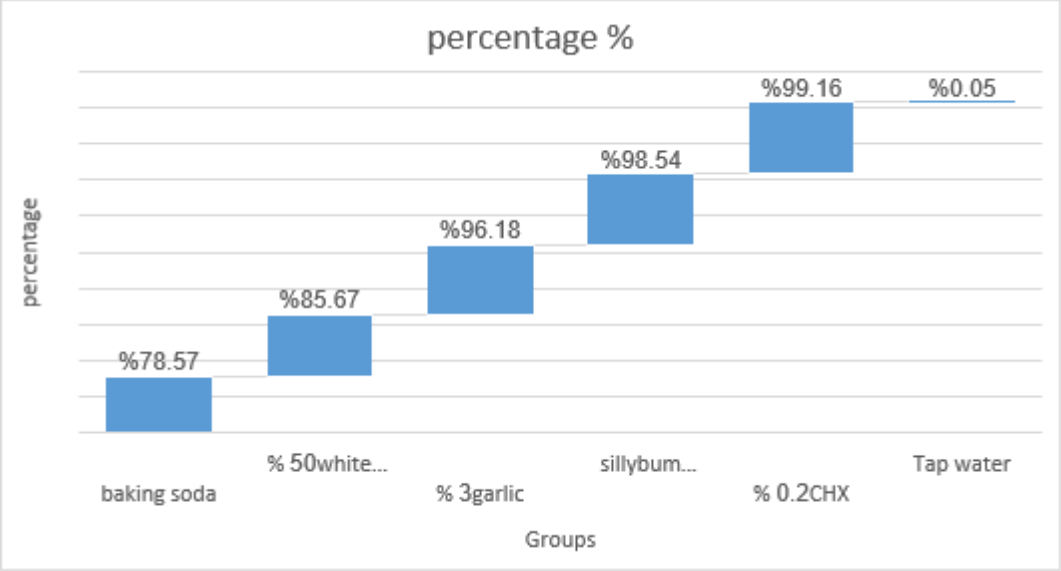

Figure 1: Percentage reduction in bacterial counts following toothbrush disinfection

It was reported highly significant differences between baking soda, 50% vinegar and tap water groups in comparison to 0.2% chlorhexidine group ($p<0.05$), while no significant differences between 3% garlic, silybum marianum groups as showed in Table 4 in contrasts to chlorhexidine group with P value >0.05.

Table 4: Pairwise comparison of bacterial counts (CFU) between disinfectant groups and the 0.2% chlorhexidine group

Groups	W	P value
Baking soda	-5.398	0.008
50% vinegar	-5.419	0.007
3% garlic	-4.577	0.055
Silybum marianum	-1.248	0.999
Tap water	5.407	0.007

Several bacterial species were identified in the used toothbrush samples, including Bacillus subtilis, Streptococcus pneumoniae, Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Pseudomonas spp., Klebsiella spp., Bacillus cereus, Streptococcus salivarius, Actinobacillus suis, lactobacillus firmicutes, and sarcina. Among these isolates, Bacillus subtilis demonstrated the highest prevalence (>20%) compared with the other bacterial species (Table 5 and Figure 2).

Table 5: Prevalence of bacterial species identified on used toothbrush samples

Bacterial species	Percentage %
Bacillus subtilis	21
Streptococcus pneumoniae	5
S. mutans	10
Staphylococcus aureus	9
Lactobacillus acidophilus	9
Pseudomonas spp	6
Klebsiella spp	8
Bacillus cereus	7
S. salivarius	8
Actinobacillus suis	5
lactobacillus firmicutes	6
sarcina	6

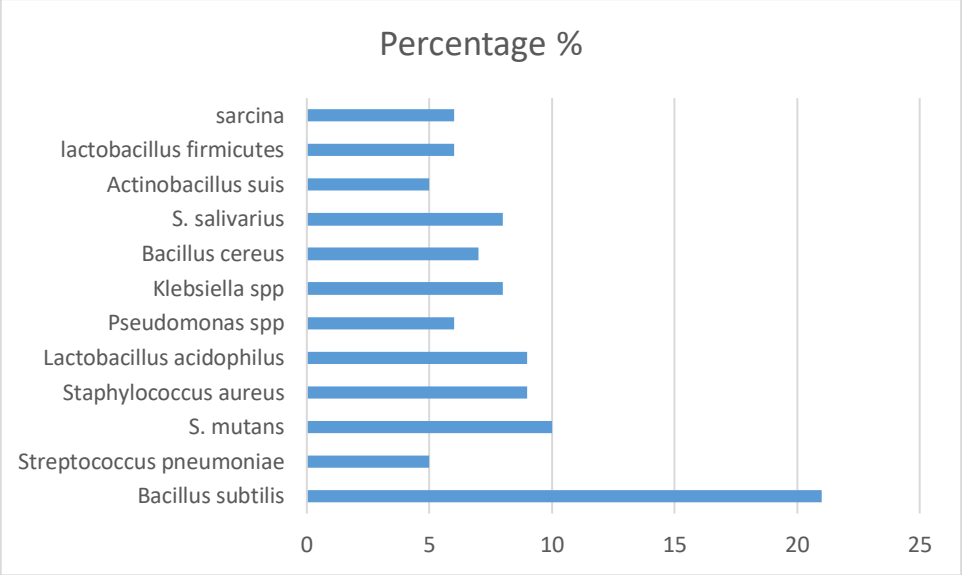

Figure 2: Distribution of bacterial species isolated from used toothbrush samples

Discussion

Toothbrushes that are used often may be highly contaminated with bacteria, and improper storage may make the microbial load on these brushes even higher. Microbial contamination of toothbrushes can aid in the spread of numerous diseases, ranging particularly in those with gum disease and/or oral health issues. Following brushing, maintaining and storing the toothbrush has grown more crucial [19, 20]. Effective control of oral microbial contamination is clinically important not only for general oral hygiene but also for patients undergoing restorative and prosthodontic dental maintenance, where persistent plaque accumulation and bacterial reinfection may compromise oral health stability. Therefore, toothbrush decontamination may serve as a supportive preventive measure for improving oral hygiene and reducing microbial transmission in susceptible dental patients. Several chemical disinfectants and herbal remedies have been attempted in an effort to clean the toothbrushes. Nevertheless, none of these researches have offered the simplest, most affordable, and most efficient way to disinfect a toothbrush. Therefore, the current study aims to assess the 0.2% CHX gluconate therapy's efficacy in reducing the bacterial load from contaminated tooth brushes in contrast to baking soda, 50% vinegar, 3% garlic, Silybum marianum extraction aqueous solution, and tap water as a natural solution.

A week after being used orally, the toothbrushes used in this study were gathered and microbiologically examined. The current study's toothbrush collection period was seven days, which is the same as other studies [21] and [22, 23], and [24] that used different collection methods.

The present investigation indicated a significant decrease in the number of bacteria after toothbrushes had been disinfected using different disinfectant solutions. These solutions were found to be effective in decontaminating toothbrushes because of this significant effectiveness, with the exception of tap water, which showed no significant bacterial reduction[25]. Highly significant differences were observed between the baking soda, 50% white vinegar, and tap water control groups when compared with the 0.2% chlorhexidine group ($P<0.05$). However, no statistically significant differences were found between the 3% garlic, Silybum marianum, and chlorhexidine groups ($P>0.05$).

0.2% chlorhexidine gluconate demonstrated the highest antimicrobial effectiveness among the tested disinfectant solutions and remains widely regarded as a standard dental antimicrobial agent for oral disinfection and plaque control. Its broad-spectrum antibacterial activity has been reported extensively in preventive and clinical dentistry literature. According to another study, the best disinfectants were 0.2% CHX and 7.5% povidone iodine, followed by water[26] and UV toothbrush sterilizer. According to a different study, UV was the most effective method of lowering bacterial contamination on toothbrushes, followed by normal saline and 0.2% CHX [21], in conjunction with various other investigations [27, 28, 29]. Chlorhexidine salts dissociate and release the positively charged chlorhexidine cation at physiological pH. This cationic chemical binds to negative-charged bacterial cell walls to produce the bactericidal effect. This has a bacteriostatic effect at low chlorhexidine doses and causes cell death at large quantities due to membrane rupture [8].

There have been a few published research on the application of vinegar in dental care. One investigation found that 50% white vinegar proved the best disinfectant [30], whilst other studies found that the most effective combination for reducing S. mutans on dental brushes was white vinegar and NaCl, then 0.12% CHX and white vinegar for a 12-hour decontamination period at particular concentrations [31]. White vinegar was found to be effective against every type of bacteria that was examined [32], with S. mutans and S. aureus showing the greatest results [33]. Nevertheless, a different study discovered that 50% white vinegar was unsuccessful [34]. The most successful antimicrobial agent for disinfecting toothbrushes was found to be 3% garlic [35]. Several test solutions, including the garlic solution, showed a statistically significant reduction in S. mutans [36], but

other studies found limited efficacy [23]. The primary phytochemicals with antibacterial properties are oil-soluble organosulfur compounds like allicin, ajoene, and allyl sulfides. Bactericidal, antibiofilm, antitoxin, and anti-quorum sensing properties are among the many antibacterial properties of garlic's organosulfur derivatives versus a range of bacteria, including multi-drug resistant (MDR) strains. Reacting organosulfur compounds form disulfide bonds with free sulfhydryl groups of enzymes, endangering the proper functioning of the bacterial cellular membrane [37, 38].

The present research found that utilizing an aqueous extract solution of Silybum marianum significantly reduced the number of bacteria after toothbrush disinfections. From a dental pharmaceutical perspective, the present findings support the potential role of natural antimicrobial agents as adjunctive oral hygiene disinfectants. Herbal preparations such as Silybum marianum may provide supportive antimicrobial activity for toothbrush decontamination and oral hygiene maintenance, particularly in patients requiring improved plaque control and infection prevention. However, the current study evaluated microbial reduction on toothbrush surfaces rather than direct restorative or prosthodontic clinical outcomes, and therefore clinical extrapolation should be made cautiously. Silybum marianum had been demonstrated to be a medical herb with a variety of pharmacological properties, including hepatoprotective, antimicrobial, anti-inflammatory, anticancer, and cardioprotective actions. Oral cancer and infections of periodontal are the main conditions for which silymarin is utilized. Dental caries and dental plaque can be effectively treated with this antibacterial medication. Additionally, either by itself or in conjunction with other antibiotics, it prevents the development of bacteria in the mouth. Documenting the antibacterial activity of zinc oxide and hydroxy apatite nanoparticles controlled by silymarin against oral pathogens like Streptococcus mutans, Lactobacillus acidophilus, and E. faecalis is therefore of interest [39, 40, 41]. The efficiency of herb preparations as disinfectants has been reported in a number of research. Guava leaf aqueous extracts were shown by Vignesh et al. to effectively disinfect toothbrushes, on level with CHX, making them a good natural substitute for household use [42].

Guruprasath et al. showed that CHX mouthwash were particularly successful in reducing microbial load when compared to herbal rinse (Himalaya Oro-T) [43]. Subhashini et al. [30] found that both natural and industrial disinfectants were efficient in reducing S. mutans on toothbrushes. Komiyama et al. [44] found that sodium perborate solution was less effective against the studied microorganisms than other disinfectants. According to Sato et al.[45], the most successful spray was one that contained CHX with a base composition. Shin et al showed the least effectiveness of Sodium bicarbonate-normal saline as compare to other disinfectants were used[26]. Its capacity to alter the pH gradient throughout microbial membranes, which affects both gram-positive and gram-negative bacteria, prevents the development of biofilms, has strong antibacterial as well as antifungal qualities, and can successfully stop the growth of Streptococcus mutans additionally to its antifungal effects [11,46]

Infections can enter the oral cavity through brushing. In addition to colonizing, microbes can stay on toothbrushes for extended periods of time [47, 48, 49, 50]. Abd-Ulnabi [51] discovered yeast colonies, S. aureus, S. epidermidis, and Pseudomonas on the toothbrushes of healthy individuals, which is consistent with our findings. However, this experiment did not test the materials for yeast. Bordetella spp., Salmonella, Candida, Klebsiella, Proteus, Citrobacter, Pseudomonas spp., S. aureus, Providencia, Lactobacillus, Chromobacterium, B. cereus, enterococci, and non-hemolytic streptococci were the most common species on samples with the contaminated toothbrushes, according to Bhat et al. [52]. Only half of these species were found in another study. Nonetheless, there was some agreement between the earlier study and this one [53]. The present study has certain limitations. The investigation evaluated toothbrush microbial contamination under controlled conditions and did not assess long-term clinical outcomes or direct effects on restorative, periodontal, or prosthodontic treatment success. Further in vivo clinical investigations with larger sample sizes are required to confirm the long-term effectiveness and clinical applicability of Silybum marianum as a routine dental disinfectant.

Conclusion

The present study demonstrated that used toothbrushes may harbor different bacterial species and that most tested disinfectant solutions significantly reduced bacterial counts, except the tap water control group. Among the evaluated natural antimicrobial agents, aqueous Silybum marianum extract showed marked antibacterial activity with results comparable to 0.2% chlorhexidine gluconate. These findings suggest that Silybum marianum may serve as a promising natural adjunctive dental disinfectant for toothbrush decontamination and oral hygiene maintenance. Regular toothbrush replacement and appropriate decontamination practices may help reduce microbial transmission and support preventive oral healthcare. However, further controlled clinical studies are required before recommending Silybum marianum as a routine dental disinfectant in restorative or prosthodontic maintenance protocols.

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