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ORIGINAL ARTICLE

Comparative evaluation of antimicrobial properties of chicory extract and chlorhexidine mouthwash against *Streptococcus mutans* and *Lactobacillus acidophilus*

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Abstract

Background: Dental caries is the most widespread disease affecting human race where *Streptococcus mutans* and *Lactobacillus acidophilus* are prime microorganisms involved. Chlorhexidine is used as antiplaque agent, but it has some side effects such as unpleasant taste and staining on long-term use, leading to search of a suitable alternative. Chicory, an ayurvedic plant, has antiplaque, anti-inflammatory, and healing properties along with aromatic cooling property.

Aim: This study aims to evaluate the antimicrobial efficacy of chicory extract mouthwash and chlorhexidine mouthwash (0.12%) against *S. mutans* and *L. acidophilus*.

Methods: Strains of *S. mutans* and *Lactobacillus* were grown and subjected to test solutions (chicory and chlorhexidine) and zone of inhibition measured by agar well diffusion method. Results obtained were subjected to statistical analysis.

Results: Zone of inhibition of chicory extract and chlorhexidine against *S. mutans* and *Lactobacillus* shows highly significant $P < 0.001$ with +value 19.982 and 21.731, respectively.

Conclusion: Chicory extract showed statistically significant inhibition against *S. mutans* as compared to chlorhexidine rinse mouthwash. While chlorhexidine mouthwash has shown statistically significant inhibition against *S. mutans* as compared to chicory extract.

Clinical Significance: Chicory has a promising antibacterial activity. Thus, it can be incorporated as one of the ingredients in mouthwash.

Introduction

Bacterial plaque or biofilm is composed by bacterial deposits and food debris strongly attached to tooth surfaces, which is colonized by multiple microorganisms. According to Miller (Chemicoparasitic theory) and the modern concepts of Cariogram show microorganisms as one of the etiological factors for dental caries.[1] Frequent biofilm exposure to saccharose diminishes its pH, favoring aciduric and acidogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus.* In developing countries like India, the major cause for dental caries is microbial infections.[2]

S. mutans is the main cause of dental caries,[3] which ferments the carbohydrates to acid, resulting in demineralization of enamel surface.[2] *Streptococcus* bacteria are mainly responsible for the initial phase of the caries lesion, especially in the enamel (initiation), whereas *Lactobacillus* is more involved with the progression of caries.[4]

Mouthwashes mainly help in the removal of plaque and gingivitis and in the removal of infections during extraction of tooth, intraoral surgical procedures, or immunosuppression during cancer therapy.[5]

Chlorhexidine is the antimicrobial agent used for the prevention of dental caries by decreasing the number of microorganisms or inhibiting the formation of dental plaque. However, because of the regular use of chlorhexidine mouthwash resulted in several side effects such as unpleasant taste and staining, which stimulated the search for an alternative for young children.

Chicory (*Cichorium intybus*) is also called as Kasni which is a perennial herb with a long taproot. It is dried, roasted, and grounded to prepare a natural healthy beverage. It is used topically for the treatment of acne, ophthalmia, and throat inflammation. It has antiplaque, anti-inflammatory, and healing properties along with aromatic cooling property. It purifies the blood, reduces the soft tissue inflammation and joint pain.[6]

It also regulates the sugar level in blood, helps in cleansing of colon, digestion, stimulates appetite, removes extra water and toxins reducing strain on liver, nourishes optic nerves, decreases the level of low-density lipoprotein cholesterol, and is mild diuretic.

The null hypothesis is that there is no difference in antimicrobial efficacy of chicory extract mouthwash and chlorhexidine mouthwash (0.12%) against *S. mutans* and *Lactobacillus acidophilus*.

Thus, the aim of the study is to evaluate antimicrobial efficacy of chicory extract mouthwash and chlorhexidine mouthwash (0.12%) against *S. mutans* and *L. acidophilus*.

Materials and Methods

Strains of *S. mutans* and *L. acidophilus* were obtained commercially (Microbial Type Culture Collection Center, Chandigarh).

The chlorhexidine mouthwash and the chicory powder were obtained from the local market. The aqueous form of chicory extract was obtained by mixing 25 m of chicory powder with 250 ml of distilled water. The solution was boiled with the water for 2 min and filtered and stored in a sterilized container [Figure 1].

Of the 24 sample size, two groups were made:

Group 1 - 12 (chicory extract)

Group 2 - 12 (0.12% chlorhexidine).

Agar well diffusion method

Each group was subdivided into six for *S. mutans* and six for *L. acidophilus*. Indicator strains are grown in blood agar plates. For agar well diffusion method, 6 mm diameter and 4 mm in depth, wells were made in the medium with the help of a sterile steel borer in a Petri dish containing blood agar [Figures 2 and 3]. The test solutions were added in the wells of the plate and under aerobic conditions, it is incubated at 37°C, for 48 h. The experiment was performed in strict aseptic conditions. The antimicrobial activity was measured as size of zone of inhibition (in millimeter) using a digital Vernier caliper.

The results obtained were then statistically analyzed using unpaired *t*-test.

Figure 1: Chicory powder and chicory extract

Results

S. mutans

Chicory extract showed greater diameter of inhibition zone when compared to chlorhexidine mouthwash for *S. mutans* [Table 1].

L. acidophilus

Chlorhexidine mouthwash showed greater diameter of inhibition zone when compared to chicory extract for *S. mutans* [Table 2].

Table 1: *S. mutans* count

Chicory extract (mm)	Chlorhexidine (0.12%) (mm)
20.1	15
20	15.2
19.5	16.2
20.5	15.6
20.2	16.3
19.5	15.7

S. mutans: Streptococcus mutans

Figure 2: Group A- chicory extract

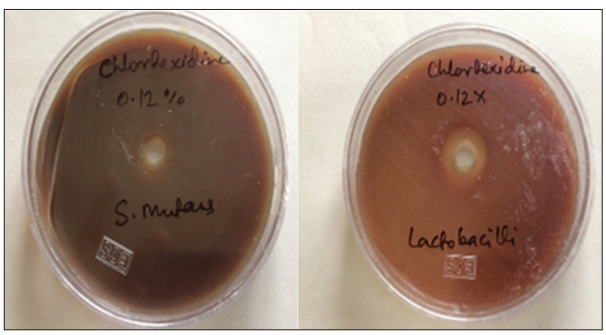


Figure 3: Group B- chlorhexidine mouthwash

Statistical analysis

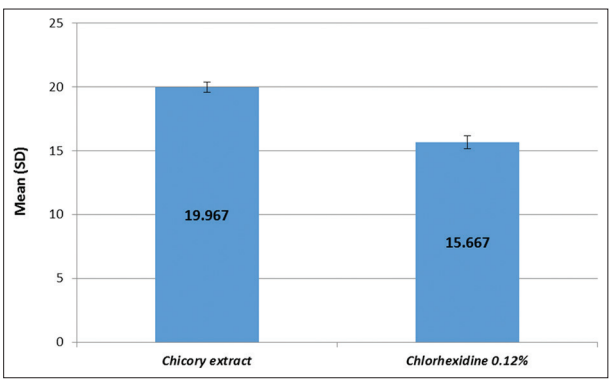
Data collected were entered into a computer and analyzed using the SPSS software. Since the level of significance was fixed at $P = 0.05$ and $P \leq 0.05$ was considered as statistically significant.

According to unpaired *t*-test, chicory extract showed statistically significant inhibition against *S. mutans* as compared to chlorhexidine rinse mouthwash [Graph 1].

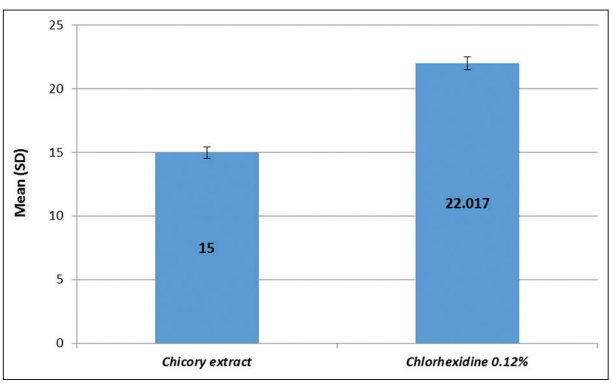
While chlorhexidine mouthwash has shown statistically significant inhibition against *S. mutans* compared to chicory extract [Graph 2].

Table 2: *Lactobacillus* count

Chicory extract (mm)	Chlorhexidine (0.12%) (mm)
14.4	22
15.8	22.1
14.9	21.8
15	22.4
15.1	22.6
14.8	21.2



Graph 1: Comparison of the zone of inhibition in terms of mean (SD) against *Streptococcus mutans* when treated with chicory extract and chlorhexidine (0.12%) using unpaired *t*-test. (Both the solutions against *S. mutans*)

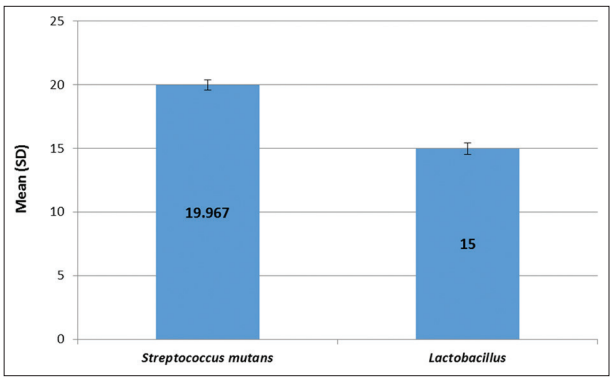


Graph 2: Comparison of the zone of inhibition in terms of mean (SD) against Lactobacillus when treated with chicory extract and chlorhexidine (0.12%) using unpaired t-test

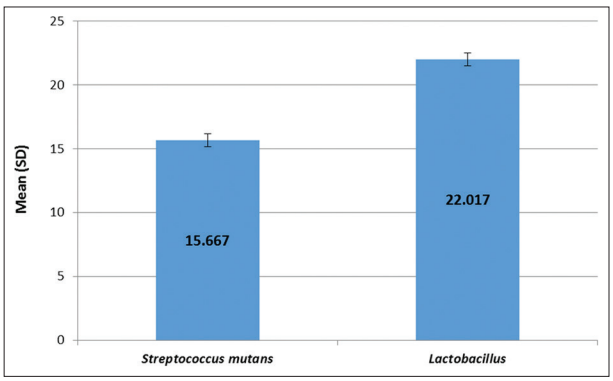
Discussion

Streptococcus mutans are the initiators of dental caries. The study shows that chicory has a potent antimicrobial activity against it [Graph 3]. Polyphenols found in chicory leaf extract have antibacterial properties.[7] Also it has anti-inflammatory because of its alkalizing nature and rich in sesquiterpene lactones.[8]

Chlorhexidine is the gold standard against which the efficacy of newer antiplaque agents is compared because of its superior antiplaque effect and its substantivity for 10–12 h.[9] It has shown the significant zone od inhibition against lactobacillus followed by *S. mutans* [Graph 4]. However, the side effects of chlorhexidine such as tooth and tongue staining, taste alterations, and mucosal erosions limit patient compliance. The mechanism of action of a chlorhexidine mouthwash seems to be an immediate and probably short-lived bactericidal effect, followed by a prolonged bacteriostatic action that is dependent on antiseptic absorbed by the pellicle coating tooth surface.[10] The zone of inhibition (Dalinsali *et al.*, 2011, Fereshten *et al.*, 2012, and Hasanali *et al.*, 2012) of chlorhexidine mouthwash has a greater zone of inhibition than any herbal mouthwash, and also



Graph 3: Comparison of the zone of inhibition in terms of mean (SD) against Streptococcus mutans and Lactobacillus when treated with chicory extract using unpaired t-test. (Chicory extract against both organisms)



Graph 4: Comparison of the zone of inhibition in terms of mean (SD) against Streptococcus mutans and Lactobacillus when treated with chlorhexidine (0.12%) using unpaired t-test. (Chlorhexidine (0.12%) against both organisms)

other two studies (Agarwal *et al.*, 2010, and Hegde *et al.*, 2011) mention greater zone of inhibition of chlorhexidine mouth when compared with other herbal mouthwash.[11]

While in this study, chicory extract showed statistically significant inhibition against *S. mutans* as compared to chlorhexidine rinse mouthwash.

Chicory extract is one of the common ingredients used in beverages, especially coffee. Chicory is grown in India since 1918 at Coimbatore and Nilgiris in Tamil Nadu and at Broach, Amalsad and Jamnagar in Gujarat.[12]

Sharma *et al*. conducted a study to compare the antimicrobial effect of different concentrations of chicory, where he concluded that 100% concentration of chicory is more effective against *S. mutans*.[13] Patel *et al.* conducted a study where 50 patients with gingivitis and bleeding gums were advised to massage the gums with alcoholic extract of chicory roots, twice a day for 3 weeks. He concluded that chicory helped in the relief of inflammation and bleeding gums.[14] Haffajee *et al.* concluded that herbal mouthwash was less essential than the chlorhexidine mouthwash while the herbal mouthwash was more effective than the essential oil rinse in reduction in the oral bacteria *in vitro*.[15]

Advantages of chicory are ease of its availability, cost effective, can be used by masses, non- staining, and biodegradable. The shelf life of chicory is for 3 years and has no side effects. It is available currently in the form of toothpastes, used as additive in coffee, powder supplements and in the form of capsules.

Chicory has shown marked antimicrobial property so it can be incorporated in the formulation of mouthwash. It will be easily accepted by pediatric patient. Moreover, the drug resistance associated with the misuse of chemotherapeutic agents can be considerably reduced with the use of plant extracts.

Conclusion

Chicory has a promising future because of its superior antimicrobial property. Chicory, a natural bioactive extract with aromatic and cooling property, no side effects, better shelf life, and less cost-effective can be incorporated in the oral hygiene products and has been proved as a boon in the countries like India where because of its poor economic conditions discourages the use of regular oral hygiene products which are commercially available.

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