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Evaluation of antibacterial efficacy of various herbal products in reduction of *Streptococcus mutans* in children- A systematic review

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ABSTRACT

The objective of the present systematic review is to evaluate and assess the antibacterial efficacy of various herbal products in reducing the levels of *Streptococcus mutans* in pediatric population. The inclusion criteria of the present study consist of all randomized clinical trials, in vivo study designs in which herbal (natural) and conventional oral health care products were compared and published in English language. A search was done in electronic databases using MeSH terms like herbal products, Pediatric dentistry, anticariogenic properties of herbal products, etc. in different data bases which included PubMed central, Google scholar, Science direct, and Cochrane library from January 2001 to 2023. The articles were analyzed by three reviewers independently for assessment of the quality of the studies, and data was derived. Results of the present systematic review showed that many of the herbal products studied had antibacterial properties against *Streptococcus mutans*. Within the limitations of this study, it can be concluded that phyto therapeutic compounds, when compared to chemotherapeutic agents, have demonstrated equal anti-microbial and anti-inflammatory characteristics with lack of adverse effects, making them a superior choice.

Keywords: Clinical efficacy, Herbal products, *Streptococcus mutans*, Chlorhexidine.

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INTRODUCTION

Dental caries is a biofilm-mediated, diet modulated, multifactorial, non-communicable, dynamic disease resulting in net mineral loss of dental hard tissues [1] .

Streptococcus mutans (S. mutans) is the primary causative agent of dental caries in humans. The virulence of *S. mutans* is attributed to its ability to form biofilm known as dental plaque on tooth surfaces[2]. With the help of the enzymes, glucosyl transferase and fructosyltransferase, *S. mutans* produces the extracellular polysaccharides glucans and fructans from sucrose. These glucans, are the causative in the aetiology of dental caries because they are insoluble in water and have the property to induce adhesion when synthesised on diverse solid surfaces. An acquired pellicle of salivary origin typically forms before bacterial adhesion to the tooth surface which is necessary for the early phases of plaque formation on tooth surfaces. Later a complex of glycoprotein and polysaccharide is formed on the bacterial surface followed by multiplication of various gram positive and gram negative micro organisms, resulting in maturation of the plaque. Dietary carbohydrates especially sucrose, and colonisation of increased levels of *S. mutans* are responsible in the development of dental caries [3].

Many therapeutic agents have been developed to reduce the microbial load, thereby contributing to reduction in plaque accumulation and dental caries. Many chemo therapeutic agents are known to have antimicrobial, bactericidal and bacteriostatic properties against *S. mutans*. Chlorhexidine (CHX), a quaternary ammonium compound, is one of the most commonly used chemotherapeutic agent to reduce the plaque accumulation.

Among the conventional chemotherapeutic agents, 0.2% CHX mouth rinse was found to reduce the population of *S. mutans* by around 30 to 50 % [4]. However CHX has certain side effects like brown discolouration of teeth, oral mucosal erosion and brittle teeth. This has lead to find an alternative to the conventional antimicrobials and switch to the use of herbal products especially in paediatric dentistry.

India is a country where traditional medicine is practiced since ancient times. The information about the healing properties of herbs was found in many ancient texts. Botanically speaking, herbs are any plants that don't have the woody tissue that shrubs and trees have. Herbs are more specifically plants that are used medicinally, for flavour or smell, or both [5].

Up to 80% of people worldwide, according to the World Health Organisation (WHO), rely on traditional medicine (herbal) for their basic medical requirements. The utilisation of medicinal plants and the production of homegrown medications both have significant financial advantages in the treatment of many diseases. 25% of medical medications in affluent nations are based on herbs and their by-products [6].

Herbal extracts are drugs themselves by interaction with specific chemical receptors within the body and are therefore effective [7]. Currently, herbal remedies are used in dentistry to treat a variety of conditions, including mouth sores, gum inflammation, and tooth discomfort [8].

A variety of natural herbal/plant products are known to exhibit antimicrobial properties and therapeutic benefits. Considering the side effects of the conventional chemical agents that are used to reduce the *S. mutans* counts in the oral cavity it is time to harness the beneficial effects of herbal products.

Due to an increased awareness of indigenous medical practices in various parts of the world, the use of herbal medicine has engendered interest and facilitated the growth of complementary and alternative therapies in health care promotion.

Many of the herbal or plant extracts have been promoted as possessing anti-inflammatory, antipyretic, analgesic, antibacterial, antiviral, anticarcinogenic and antioxidant activities by means of in vitro, in vivo, and animal studies [9] .Several herbal extracts like triphala, terminalia chebula and neem are known for their therapeutic benefits in oral cavity [10].

Many commonly available herbs which are used in day to day life such as tulsi, neem, aloe vera, turmeric, green tea, honey and many traditional spices used in Indian cooking are known to have anti-inflammatory, antimicrobial and antioxidant properties have been studied by many researchers [11]. These products are used directly or indirectly and delivered in various forms either as mouth rinse, tooth paste or dentrifice. There is no concrete evidence that supports their role in reducing the incidence of dental caries thereby improving the oral health [12]. The present systematic review aimed to evaluate the available literature regarding the anti bacterial efficacy of the herbal products against *S. mutans* [13].

Data Sources

The methodology followed in the present systematic review was based on PRISMA guidelines (Preferred reporting items for systematic reviews and meta analysis). The protocol was registered in the prospero data base (International prospective register of systematic reviews) with ID - CRD42022382765.

A search was done in electronic databases using MeSH terms like herbal products, dentistry, anticariogenic properties of herbal products, herbal products and *S. mutans* etc. in different data bases like PubMed central, Google scholar, Science direct, and Cochrane. The various MeSH words used to search articles were as follows

1. “herbal products” OR “herbs” OR “herbal materials”

“tooth, deciduous” OR “tooth” AND “deciduous” OR “deciduous tooth” OR “primary “tooth” OR “anticariogenic properties

2. “chlorhexidine” OR “aloe vera” OR “aloe” OR “turmeric” OR “garlic” OR “green tea” OR “Camellia sinensis” OR “ neem” OR “triphala” OR “Terminalia Chebula “ OR “Terminalia Bellirica” OR “Embilica Officinalis” OR “neem”OR “nutmeg” OR “ chamomile” OR “Pomegranate “ OR “grape seed extract” OR “guava extract” OR “neem stick” OR “Ocimum sanctum” OR “ Psidium Gujava”

3. “miswake” OR “Tulsi” OR “thyme” OR “honey” OR “Rosmarinus Officinalis” OR “ guava” OR “Tea tree oil” OR “Amla fruits” OR “Garlic bulbs” OR “Ginger” OR “lime” OR “ curry leaves” OR “moringa “ OR “lemon “ OR “cranberry” OR “cardamom” OR “ cinnamon” OR “clove oil” OR “Tulasi”

4. “streptococcus mutans” OR “s.mutans” OR “dental caries” OR “caries” OR “early childhood caries” OR “pediatric dentistry” OR “mouth wash” OR “mouthrinse” OR “tooth paste” OR “dentrifices” OR “oragel” OR “ tooth brush” OR “plaque control”

Titles and abstracts were utilised in the initial screening stage to locate complete papers about the use of herbal products as oral hygiene aids in paediatric patients.

One article was chosen in the second stage of the screening procedure after the duplicates from the corresponding searches were eliminated. These articles were selected based on inclusion and exclusion criteria in the third step.

Inclusion Criteria

1. Articles published in English language only
2. Studies published between January 1, 2001, and September 30, 2023
3. Randomized clinical trials,
4. Invivo study designs in which herbal(natural) and conventional tooth-pastes and mouthwashes have been compared.

Exclusion Criteria

The following studies were excluded,

1. In vitro studies
2. Animal studies
3. Adult studies
4. Review articles, abstracts, case reports, expert opinions.
5. Interventions done on children with special health care needs.

Data extraction forms that were particularly created were used to extract the data. The qualitative and quantitative data for all included studies was extracted, along with the publication year.

The author's conclusion, the number and age of patients, the experimental and control therapies, the length of the treatments and follow-ups, and all the data required for methodological quality assessment was assessed.

Do the herbal products delivered in different forms to children reduce *S. mutans* count in the oral cavity?

PICO analysis

- Population - children with dental caries
- Intervention - Herbal products
- Comparison – chlorhexidine
- Outcome - Reduction in *Streptococcus mutans*

Table 1: Resource selection - articles included in the systematic review

Eligibility criteria

Types of studies – randomised clinical trials

Types of participants **-** Child patients between 4 to 12 years of age with dental caries.

Types of Interventions **-** Herbal products and chlorhexidine

Types of Outcome measures- reduction in *Streptococcus mutans*

Resource Selection

A title search of different electronic data bases including PubMed central, Google scholar, Science direct, and Cochrane revealed 197 publications, of which 32 duplicates were eliminated. 165 articles were screened by their abstract 121 articles were excluded after title search for comparative studies. Finally, 44 full text articles were acquired and assessed. After evaluation, 33 articles were excluded as they did not meet the included age criteria. Finally, 10 articles were chosen based on the afore mentioned criteria. The selected articles in the present systematic review are included in Table 1.

Study Selection

Selection of studies was done initially by reading the title and abstract of the articles obtained from each database. Articles reporting information regarding use of herbal products and conventional products as oral hygiene measures in children were assessed further for the review. Full-text articles of the selected abstracts were then evaluated independently. The analysis was done by two independent authors and in case of disagreement, decision of third reviewer is considered as final. Missed data if any was also searched based on reference articles. The study selection was done using the PRISMA 2020 guidelines as mentioned in Figure 1.

S. No.	Study	Year of Publication
1.	Avisek Mukherjee et al	2021
2.	Swara Shah et al	2018
3.	Shamika Kamath et al	2021
4.	Fatemeh Sadat Sajadi et al	2021
5.	Fatemeh Sadat Sajadi et al	2021
6.	Fatemehsadat Sajadi et al	2022
7.	Jyothsna Pinni et al	2018
8.	Ann Thomas et al	2016
9.	N.P. Kamath et al	2019
10.	Amro M. Moness Ali et al	2019

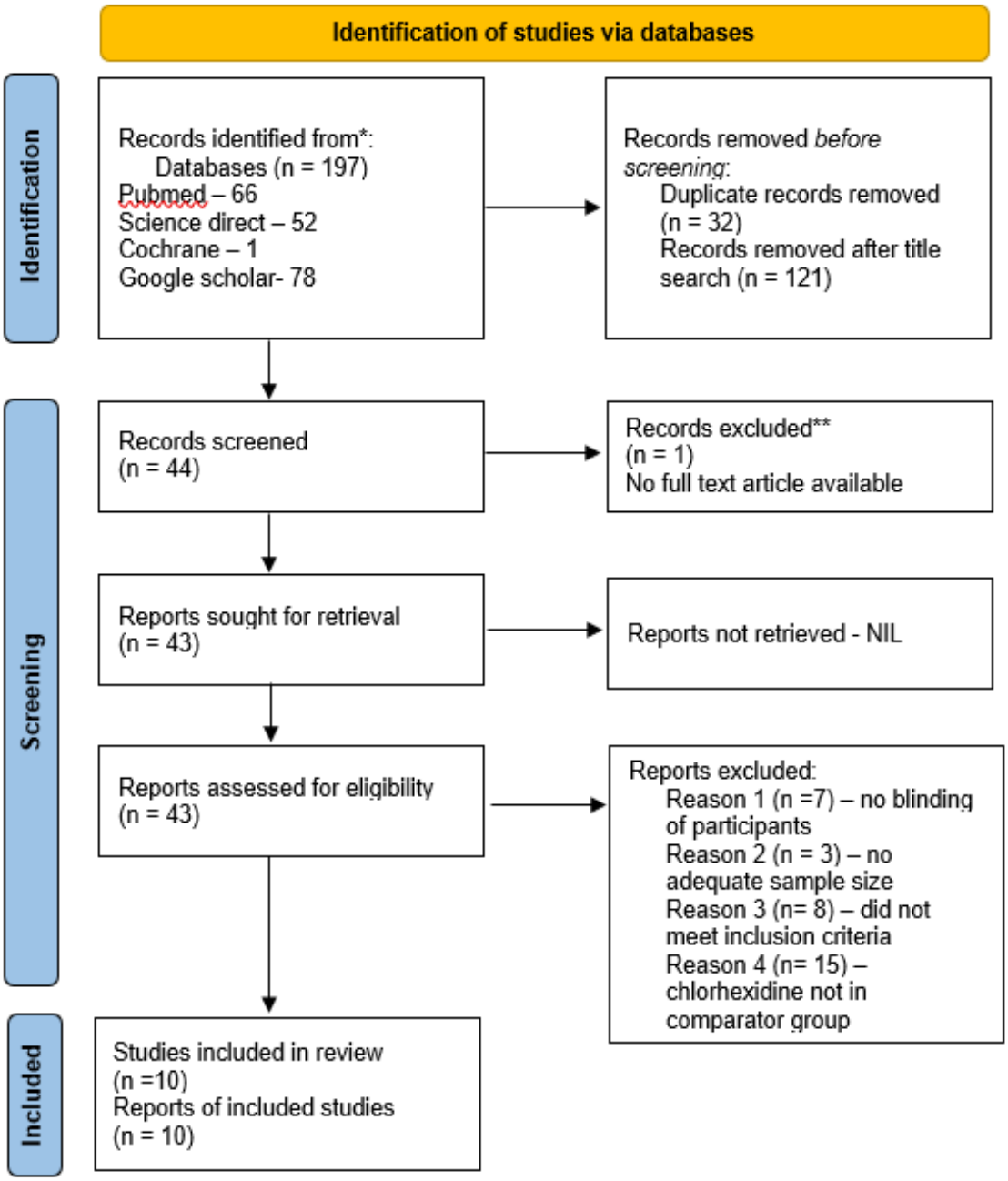

Figure 1: PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

Study Characteristics

A standard pilot data extraction form was produced in consultation with the evaluator, an expert, and the data were gathered and analysed by the same investigators who picked each article on discussion. Searches turned up 197 studies. 121 full-text publications were chosen following the elimination of duplicates and the screening of the abstracts. Only ten of the 121 articles met the inclusion criteria. A summary of all the included trials is shown in Table 2.

DISCUSSION

Dental caries is considered to occur due to interaction between genetic and environmental factors [14]. Colonisation of tooth surfaces by bacteria is one of the important etiological factor in dental caries [15] . *S. mutans* play a major role in the initiation and progression of dental caries due to the inherent ability to adhere to the tooth surface leading to the formation of dental plaque, hence by reducing the colony counts of *S. mutans* in the oral cavity can lead to decreased incidence of dental caries. Plethora of antimicrobial, antibacterial chemical agents are manufactured and marketed to be used against *S. mutans*. Among these, 0.2% CHX has gained attention in dentistry due to its properties such as substantivity, antiplaque and antimicrobial efficacy. CHX has a bacteriostatic effect at low doses (0.02%–0.06%), where it causes displacement of Ca2+ and Mg2+ and loss of K+ from the cell wall. CHX has a bactericidal (cell lysis and death) action at high doses (>0.1%) by causing leaking of all the major intracellular components out of the cell [36] .

Table 2: Interpretation of The Included Studies

S. No.	AUTHOR AND YEAR OF STUDY	STUDY TYPE	SAMPLE SIZE	AGE GROUP	METHOD OF DELIVERY OF HERBAL PRODUCT	NAME OF HERBAL PRODUCT	TIME OF ASSESSMENT	Method of evaluation	Overall Interpretation
1.	Avisek Mukherjee 2021	*In vivo*	N= 90 Group 1 (30)-triphala, green tea, neem Group 2 (30)- tulsi	6-12-Years	Mouth wash	Triphala 0.6%, green tea, neem	At baseline and after 7 days	One-way ANOVA test , post hoc Tukey test, repeated-measures ANOVA	Statistical significant difference was seen in group 1, 2 and 3 at three different time intervals
2.	Swara Shah 2018	*In vivo*	N=45 Group 1(15)- CHX Group 2(15)- Herbal mouthwash with Terminalia chebula Group 3 (15)-Distilled water	7–8 years	Mouth wash	Terminalia chebula	At baseline,2 weeks	Paired t-test, ANOVA test, and post hoc tests	statistically significant difference between group 1 and group 2
3.	Shamika Kamath 2021	*In vivo*	N=50 Group 1(25)- CHX Group 2 (25)- green tea extract	8-12 Years	Mouth wash	green tea extract	At baseline and after 2 weeks	paired t-test, Shapiro–Wilk test of normality and Levene's test	No statistically significant difference between group 1, group 2
4.	Fatemeh Sadat Sajadi 2021	*In vivo*	N=60 Group 1 (20)-green tea 5% gel, Group 2(20)- CHX 2% gel Group 3(20)- fluoride 0.2% gel	4-6 Years	Ora gel	Green Tea	Baseline and after 1 week	ANOVA and Tukey test	statistically significant difference between group 1, 2 and group 3
5.	Fatemeh Sadat Sajadi 2021	*In vivo*	N= 90 Group 1(30)-CHX Group 2(30)-chamomile Group 3(30)-thyme	4-6 years	Mouth wash	Chamomile thyme	At baseline and after 7 days	parametric and non-parametric tests, Mann-Whitney test	No statistically significant difference between group 1, group 2 and group 3
6.	Fatemehsadat Sajadi 2022	*In vivo*	N= 90 Group 1(30)-Camelia Sinenis or Group 2(30)- Teucrium polium (TP) Group 3(30)- CHX	4-6 Years	Mouth wash	Camelia Sinenis or green tea Teucrium polium (TP))	After 30 minutes and after 1 week	Shapiro-Wilk test, Friedman test, Chi-square test, paired sample t test, repeated ANOVA and Mann-Whitney U test	Statistical significant difference was seen in group in all groups at different time intervals
7.	Jyothsna Pinni 2018	*In vivo*	N= 30 Group 1(10)- pomegranate pericarp extract Group 2(10)- CHX Group 3(10)- distilled water	6-12 years	Mouth wash	pomegranate pericarp extract chlorhexidine distilled water	At base line, after 5 min and after 7 days	Wilcoxon signed rank test. Mann Whitney test	Group 1 and 2 differed significantly from control. No significant difference between group 1 and group 2
8.	Ann Thomas 2016	*In vivo*	N=30 Group 1 (15)- Green tea mouth rinse Group 2(15)-CHX	4-6 Years	Mouthwash	Green tea	At baseline and after 2 weeks	one-way ANOVA and Tukey's POSTHOC test	Statistical significant difference was seen in group 1 group and group 2
9.	N.P. Kamath 2019	*In vivo*	N=50 Group 1 (25)- aloe vera Group 2 (35)-CHX Group 3 (35)-tea tree oil Group 4 (35)-placebo	8-14 Years	Mouthwash	Aloe vera Tea tree oil	at baseline, 2 weeks after mouth rinse and 4 weeks after stopping mouth rinse	ANOVA and Tukey's HSD test	No Statistically significant difference between group 1, group 2 and group 3
10.	Amro M. Moness Ali 2019	*In vivo*	N=42 Group A(14)- green tea mouthwash Group B(14)-CHX (0.12%) mouthwash (3) Group C: colored flavored non sweetened tap water	5 to 6 years	mouth rinse	Green tea	2 weeks	Kruskal–Wallis test (H-test) and post-hoc	no statistically significant difference in group 1 and group 2

Long term and safe use in children has never been advocated till date due to its disadvantages such as staining/ discoloration of teeth, altered taste sensation and anaphylactic reactions. Recent data suggests that CHX at low doses, causes an increase in antimicrobial resistance in Gram-negative and Gram-positive species due to mutations or alteration of genetic material, resulting in alterations in cell membrane structure and functioning of ion pumps [37]. To add, the taste of CHX is unacceptable in children as reported in many clinical trials.

Considering the disadvantages and impact of the chemical agents on the oral and overall health of an individual, there is a surge in the use of herbal alternatives. Ayurveda, Siddha and unani system of medicine were practiced in India since decades. There is an upward trend of using the herbs to reduce the *S. mutans* level, thereby the plaque and dental caries [10] .The focus now is on providing complete herbal based alternatives in dentistry in the form of dentrifices, mouth washes, irrigating solutions as remedies for many oral lesions. The new herbal medicines are cheaper and safer making them affordable to use. Majority of the population in India belongs to lower to middle socioeconomic status and resides in rural areas which makes the availability and affordability of expensive drugs near impossible. We can hence rely on easily accessible herbal products with high safety profile, low cost and good antimicrobial efficacy.

The antibacterial effect of green tea mouthwash as reported in numerous studies claimed its use had significant anticariogenic activities, including an inhibitory effect on cariogenic bacteria by inhibition of bacterial cells adhesion to tooth surfaces. Additionally, green tea catechins keep salivary pH at a normal range, which makes it difficult for cariogenic bacteria to grow [38, 39] . Hamilton-Miller demonstrated that green tea has an indirect antimicrobial impact by activating immunoglobulins, lysosomes, lactoferrin, histatin, and mucin [40]. According to Narotzki et al, green tea also has the ability to suppress the lactate dehydrogenase enzyme, which lowers acid production after sugar consumption [41] . The results of our study are in accordance to a systematic review on Camellia sinensis mouthwashes in oral care, products made from this plant can be employed as antiseptic, antiplaque, and anti-inflammatory mouthwashes in future clinical trials and everyday practice [42]. The epigallocathechingallate in green tea, was found to lower *S. mutans* and the generation of acid in dental plaque [43].

Avisek Mukherjee et al [15] compared and assessed the efficacy of the combined herbal and non herbal mouth rinse and both herbal and non herbal mouth rinses individually on salivary pH and salivary *S. mutans* count and concluded that there was a statistically significant increase in salivary pH level and decrease in salivary *S. mutans* count in all the groups between baseline, 1 hour, and 7 days but combination mouth rinse was more effective at 1 hour and non herbal mouth rinse was more effective in increasing salivary pH level as compared to combination and herbal mouth rinse after 7 days.

Four studies, [16, 19 21, 24] concluded that green tea had more antibacterial efficacy and there was significant reduction of *S. mutans* levels than CHX. In a study conducted by Swara shah et al [16] herbal mouthwash had superior antimicrobial efficacy with no side effects when compared to 0.2% CHX mouthwash against salivary *S. mutans*. Statistically significant reduction in Colony forming units of *S. mutans* was observed between herbal mouth wash group and CHX after 15 days as compared to that of baseline. The results of this study were similar to the results of the study conducted by Mehta et al. [17] And Nayak et al in children aged 12- 15 years [18] .

Shamika Kamath et al [19] compared the efficacy of 0.12% CHX mouth rinse and 0.5% green tea extract mouth rinse on the colony-forming units (CFUs) of *S. mutans* in dental plaque in 8–12-year old children and the results indicated that 0.5% green tea extract mouthwash was just as effective as 0.12% CHX mouthwash in reducing the CFU of *S. mutans*. This is in line with an in vivo study by Goyal et al that demonstrated the superior action of green tea catechin in plaque compared to saliva as a mouthwash against *S. mutans* but long term follow up is required to identify the real benefits of green tea as a therapeutic and preventive agent for dental plaque microorganisms [20].

In a study conducted by Fatemeh Sadat Sajadi et al 2021 [21] to evaluate the effects of CHX, fluoride and green tea gel on salivary *S. mutans* in children, they found that green tea in gel form, fluoride, and CHX decreased the number of *S. mutans* in the saliva however, green tea was more durable and had acceptable flavour than gel made of chlorhexidine and fluoride. According to Neturi et al., green tea mouthwash is just as efficient in lowering *S. mutans* levels as chlorhexidine, which was typically regarded as the gold standard [22] . According to Fatemeh Sadat Sajadi et al in comparison study, thyme and chamomile extracts, like chlorhexidine, significantly reduced the salivary *S. mutans* count, in children with Early Childhood Caries [23].

In 2022 Fatemeh sadat Sajadi et al in [24] also compared the effects of CHX with Camelia Sinensis or green tea (GT) and Teucrium polium (TP) extracts on the levels of *S. mutans* in saliva, the bactericidal effects of GT, TP, and CHX were perfect in a short amount of time, but over a period of one week, TP maintained its antimicrobial activity, GT's activity significantly increased and CHG's antibacterial efficacy reduced after one week. Thus concluded that both TP and GT demonstrated antibacterial properties against *S. mutans*, making them both potent and cost effective herbal compounds when used as topical gels. The results of this study were supported by Khoramian Tusi et al [25] .

Comparing the antibacterial effectiveness of commercially available Chlorhexidine mouthwash and aqueous pomegranate pericarp extract (PPE)against caries-causing microorganisms in both in-vitro and in-vivo, Jyothsna Pinni et al [26] concluded that there was no statistically significant difference between PPE mouthwash and CHX mouthwash in terms of effectiveness at lowering the salivary *S.mutans* count but PPE mouthwash may be used as anti-caries mouthwash because of its antimicrobial, antioxidant, anti-inflammatory, and inhibitory effect on invasion of microbes into cell [27] .

Ann Thomas et al evaluated the anti-microbial efficacy of 0.5% green tea and 0.2% CHX mouth rinses against *S. mutans, Lactobacilli* species and *Candida Albicans* and assessed that green tea showed a statistically significant fall in the colony counts of *S. mutans* similar to

chlorhexidine, so it could be considered safe, economical alternative to CHX [28] .

N. P. Kamath et al compared two herbal mouthwashes containing aloe vera and tea tree oil, with CHX mouth wash and found that Aloe vera and tea tree oil mouthwashes can reduce *S. mutans*, gingivitis, and plaque accumulation in children and these two substances have similar effects to chlorhexidine in terms of activity [29]. Amro M et al also compared the antimicrobial efficacy of green tea (0.5%) mouth rinse to that of CHX (0.125%) against *S. mutans*, and found that there was no statistically significant difference between CHX and green tea's antibacterial effectiveness against *S. mutans* [30] .

Out of all included studies, the only research article that assessed the efficacy of herbal products in the form of gel, on *S. mutans* reduction was the study conducted by Fatemeh Sadat Sajadi et al in 2021 [21], rest of the included studies evaluated the effects of green tea over CHX as mouthwash. Although A.R. Pradeep et al, [31] Kuldeep Singh et al 2016, [32] Sílvia Morgana Araújo de OLIVEIRA et al 2008, [33] evaluated the antimicrobial efficacy of herbal products as dentrifices, these studies were done in adult population, so they were excluded. Neha Bhati et al, [34] Rashmi N Shetty et al [35] evaluated herbal dentrifices with flouridated dentrifices, as flouride seems to have high antimicrobial activity, and there may be bias in the results, these articles were excluded from the study

CONCLUSION

The various herbal products reviewed in this study have shown to be equally effective in reducing *S. mutans* level in the oral cavity. Although a lot of routinely available herbs such as ginger, aloe vera, neem, turmeric etc. have been studied for their efficacy , however there is a lacunae in providing substantial evidence in reducing the levels of *S. mutans*. There is lack of literature with respect to studies in regards to sample size, pre and post operative follow up and proper method of randomisation.

Limitations

There is a need for further research in this area so that many routinely available herbs can be used as a replacement for chemotherapeutic agents.

Conflict of interest

There is no conflict of interest.

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