SYSTEMATIC REVIEW

Herbal Mouthrinses for Prevention of Dental Caries in Children and Adolescents: A Systematic Review

Ankita M Saikia[1], Abirami Sivasubramanian[2], Muthu MS[3], Akila Ganesh[4], Krithika Chandrasekaran[5], Richard Kirubakaran[6]

Abstract

The primary aim of this study was to evaluate the effectiveness of herbal mouthrinses (HMR) on caries prevention in children and adolescents. In addition, this systematic review assessed its effectiveness in remineralization of white spot lesions, reduction of halitosis, and improving gingival and periodontal health in orthodontic patients and patients with special healthcare needs (SHCN). A comprehensive bibliographic search was conducted in PubMed, Cochrane Central, EMBASE, AMED, ProQuest, CINAHL, AYUSH, Digital Helpline for Ayurveda Research Articles (DHARA), and Clinical Trial Gov databases. A total of 3,918 titles were identified during the initial search. Of these, 32 studies were selected for quality assessment. A total of 5,038 participants from 10 countries were thus included in this review, with 22 (66.7%) studies conducted in India. All included studies were published between 2004 and 2021. Included studies investigated the effect of HMR on caries increments, which record decayed, missing, filled (DMF) [International Caries Detection and Assessment System (ICDAS)], decayed, missing, filled teeth/surfaces (DMFT/S), and incipient caries. Changes in bacterial count (*Streptococcus mutans* and *Lactobacillus*) and alterations in levels of *Candida albicans* from saliva or plaque samples were also reported. The effect of HMR on gingival and plaque indices among adolescents undergoing orthodontic treatment and children with SHCN was reported in two studies. The variance in the HMR formula across studies, short follow-up period, and limiting grade of evidence do not allow for conclusive evidence of the efficacy of HMR. This warrants high-quality randomized controlled trials (RCTs) with longer intervention periods involving children under 6 years to yield more conclusive results.

Keywords: Caries prevention, Herbs, Herbal mouthrinse, *Streptococcus mutans*, Systematic review.

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Introduction

According to the World Health Organization (WHO) Global Centre for Traditional Medicine (GCTM), nearly 88% of all countries rely on traditional medicines, including herbal medicines (WHO 2020). Herbal products continue to gain popularity among patients and healthcare professionals worldwide (WHO). Evidence of natural products being used for the prevention and treatment of oral diseases backdates thousands of years ago in Western and Eastern societies.[1] Numerous recipes for mouthrinses and toothpaste composed of natural substances can be found in the Ebers Papyrus.[2] In 5000 BC, the Babylonians introduced "chewing stick" which continues to be popular to date among communities in Asia and Africa.[3–5] Herbal mouthrinses (HMR) are generally formulated with extracts of sanguinarine, propolis, neem, green tea, charcoal, clove, and miswak.[6] Epidemiological studies revealed that miswak had strong anticaries effects[7–9] while *Galla chinensis* was reported to be more effective in inhibiting demineralization and promoting remineralization.[10,11] Though the mechanisms for these actions remain inconclusive, polyphenols in *G. chinensis* may potentially aid in delaying demineralization by stabilizing remnants in the organic matrix and blocking ion diffusion pathways.[12,13] The remineralization properties of *G. chinensis* are attributed to polyphenol compounds that act as Ca^{2+} ion carriers.[14–16]

Dental caries remains the most common chronic disease of childhood across the globe. Its prevalence is significant, afflicting 60–90% of school-age children.[17,18] An overall prevalence of 49.6% has been reported.[19] A study among the South Indian population reported every fourth child to be affected by early childhood caries (ECC).[20] In 2015, Hambire et al. conducted a randomized, blinded, controlled trial to assess 0.5% *Camellia sinensis* (green tea) extract, 0.05% sodium fluoride (NaF), and 0.2% chlorhexidine (CHX) mouthwashes and their effectiveness among aged 9–14 years. The results of the trial confirmed *C. sinensis* to have superior effects over NaF and CHX.[21] Later, Thomas et al. compared green tea and CHX mouthwash against cariogenic microbes, such as *Streptococcus mutans*, *Lactobacilli*, and *Candida albicans*,

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Conflict of interest: None

in 4–6 years children reporting ECC. A significant decrease in *S. mutans* counts was reported with green tea, while CHX fared better in the reduction of lactobacilli.[22] A meta-analysis conducted by Kommuri et al. in patients undergoing fixed orthodontic treatment reported CHX to be more effective against *S. mutans*; however, the results remain debatable as most included studies were assessed as low to moderate quality.[23]

Despite the reports of a few reviews[11,24–27] on HMR, their benefits still remain inconclusive. There are no reports comparing the caries preventive effects of herbal vs conventional mouthrinses in children and adolescents to the best of our search. Hence, this systematic review aims to evaluate existing evidence on HMR and caries prevention in children and adolescents. Additionally, this review also intends to report the findings on efficacy in remineralizing white spot lesions, reducing halitosis, and improving gingival and periodontal health among orthodontic patients and those patients with special healthcare needs (SHCN).

Methods

Protocol and Registration

This review was conducted in alignment with the recommendation of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.[28] The protocol was registered priori in the International Prospective Register of Systematic Reviews (PROSPERO) (Registration No: CRD42021273019).

Eligibility Criteria

All studies were selected based on the following population, intervention, comparison, outcome, study design (PICOS) criteria:

- Participants: All trials involving children and adolescents under 19 years of age, irrespective of SHCN, were included.
- Intervention group: All trials assessing mouthrinses containing any herbal extract.

Comparison groups can include fluoride, CHX, or placebo-containing mouthrinses. Studies—all trials measuring at least one of the outcomes mentioned above (caries prevention, remineralization of white spots, change in bacterial counts, halitosis status, and gingival and periodontal health).

It should be noted that only randomized controlled trials (RCTs), quasi-randomized trials, and cluster randomized trials were included to synthesize high-quality evidence. Studies where participants were aged beyond 19 years were excluded. Included studies were limited to English language. Narrative reviews, laboratory-based studies (*in vitro* studies, animal studies), letters to the editor, case reports, observational studies, crossover trials, and conference abstracts with no subsequent full-text publications were excluded.

Information Sources and Literature Search

The search was conducted in the following databases for relevant studies from inception to January 2022: Cochrane Oral Health Group's Trials Register, CENTRAL, MEDLINE *via* OVID, PubMed, CINAHL *via* EBSCOhost, EMBASE *via* OVID, Natural Medicines Comprehensive Database, ProQuest (Dissertations and Theses), ProQuest (Conference Proceedings), AMED, AYUSH Portal, Digital Helpline for Ayurveda Research Articles (DHARA), US National Library of Medicine (clinicaltrials.gov), and Clinical Trials Registry of India (CTRI—www.ctri.nic.in). Hand searching was performed in the following key journals: Community Dental Health, International Journal of Paediatric Dentistry, Pediatrics, Pediatric Dentistry, European Archives of Paediatric Dentistry, European Journal of Paediatric Dentistry, Pediatric Dental Journal, Community Dentistry and Oral Epidemiology, and International Journal of Clinical Pediatric Dentistry. The SHODHGANGA, the national dissertation abstracts database, was also screened for gray literature. Both Medical Subject Headings (MeSH) and free text using keywords—herbal, plant extracts, mouthrinses, mouthwash, dental caries, and oral health—were used for the search. The following filters were

Table 1: Search strategies formulated and used in PubMed/MEDLINE/EMBASE databases

40	#13 and #37
39	#13 and #37
38	#13 and #37
37	#34 and #35 and #36
36	(((((((((dental caries) OR (dental plaque)) OR (gingival disease)) OR (periodontal disease)) OR (remineralization)) OR (bad breath)) OR (halitosis)) OR (medically compromised patients)) OR (orthodontic patients)
35	(((mouth washes) OR (mouthwash)) OR (mouthrinse)) OR (mouthrinses)
34	((((((herbal) OR (herb)) OR (herbs)) OR (natural)) OR (organic)) OR (plant extracts)) OR (herbal medicine)
33	((herbal) OR (herb)) OR (herbs)
32	(herb) OR (herbal)
31	#13 and #25 and #28
30	#13 and #25 and #28
29	#13 and #25 and #28
28	#26 or #27
27	Mouthwash
26	Mouthrinse
25	#16 or #17 or #18 or #19 or #20 or #21 or #21 or #22 or #23 or #24
24	Oral health
23	Tooth decay
22	Halitosis
21	Orthodontic patients
20	Medically compromised
19	Remineralization
18	Gingival disease OR periodontal disease
17	Bad breath
16	Dental caries
15	#9 not #10
14	(humans) NOT (animals)
13	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9
9	Groups
12	Medicinal plant extracts
11	Plant extracts
10	Plant
8	Trial
7	Randomly
6	Herbal
5	Herbal medicine
4	Placebo
3	Randomized
2	Controlled clinical trial
1	RCT

applied to these terms: clinical trial, English. The detailed search strategy is provided in Table 1. The following set of terminologies were used in AYUSH research portal and DHARA along with the general keywords: Ayurveda (Dantagata/Dantamulagata Roga, Mukha/Jiva/Oshtha Roga, Mukha/Jhiva/Osthharoga); Siddha (Vai/Naakku/Udhadu Noigal and Pal Eru Noi); Unani [Dental Carries (Taakkul E Asnan) and Stomatitis (Qula)]. The final search results from each database were imported and converted into research information systems (RIS) format in Zotero software before uploading into the Covidence (www.covidence.org) software.

Study Selection

Initial screening of titles and abstracts was performed by two reviewers (AS and SA) independently against the eligibility criteria using Covidence software. Duplicates were removed. Full text of potentially included studies was retrieved and checked for eligibility and independently screened by the same two authors and grouped as "included" "may be" and "excluded." The reasons for exclusion were recorded in the software. In cases of any disagreement regarding inclusion or exclusion of the study, arbitration was done through discussion with the experienced third review author (AG).

Data Extraction

Data extraction was performed independently by two reviewers (AS and SA) independently using a predetermined data extraction template. In instances of any missing or unclear reporting of data, the corresponding authors were contacted through e-mail.

Quality Assessment

The Cochrane Risk of Bias (RoB) tool[29] was used to assess the quality of included studies. This tool consists of seven domains such as allocation concealment, sequence generation, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. The reporting assessment were "low risk," "high risk" and "unclear risk." The RoB for all included studies were performed in Covidence software by independently by both authors (AS and SA).

Summary Measures

The clinical outcomes evaluated in this review were caries increments in primary tooth surfaces [decayed, extracted due to caries, filled primary teeth/surfaces (deft/s)] permanent tooth surfaces [decayed, missing, filled teeth/surfaces (DMFT/S)] reported as change from baseline; change in bacterial count from saliva or plaque sample (*S. mutans*, *Lactobacillus*, or other cariogenic pathogens); remineralization of white spots in orthodontic patients as measured by the devices using laser fluorescence detection systems, change in halitosis status measured by any organoleptic devices, and change in gingival and periodontal health status measured by indices as a change from baseline.

Data-analysis

The heterogeneity across included studies were assessed based on study characteristics, methodological heterogeneity, and type of analysis.

Results

The initial search identified 3,918 papers. After elimination of 256 duplicates, 3,662 papers were screened. This screening initially resulted in 356 full-text articles. After full-text screening, 182 articles were excluded for reasons such as wrong patient population—71; wrong study design—37; wrong intervention—25; only protocols—16; not in English language—9; ongoing studies—8; wrong outcomes—15; and study published twice—1. Among the remaining 174 full-text papers, 142 studies which reported any one of the desired outcomes were with overlapping age-group (children/adolescents and adults in the same study). These authors were contacted through e-mail to share the raw data for children and adolescents <19 years from their studies. Two authors replied. One author (Pratibha Taneja) shared the data from which we could not retrieve data <19 years. Another author (Vinayak Joshi) reported exclusion of children. Hence, no relevant data was obtained for further analysis from these 142 studies. Finally, 32 studies[22,30–60] with 5038 total participants were included. Figure 1 illustrates the screening process using the PRISMA flow diagram.

Study Characteristics

The characteristics of 32 qualified RCTs are presented in Table 2. The mean age ranged from 4 to 19 years were included in the present review. The studies included participants from 10 countries with majority of the studies 22 (66.7%) conducted in India and the publication years of included studies ranged from 2004 to 2021. Of 32 included studies, 30 reported on HMR and its effect on dental caries [using International Caries Detection and Assessment System (ICDAS), DMFT/S assessments]. Two studies report on HMR and its impact on bacterial count (*S. mutans*, and *Lactobacillus*, *C. albicans*). Changes in gingival and plaque scores of adolescents undergoing

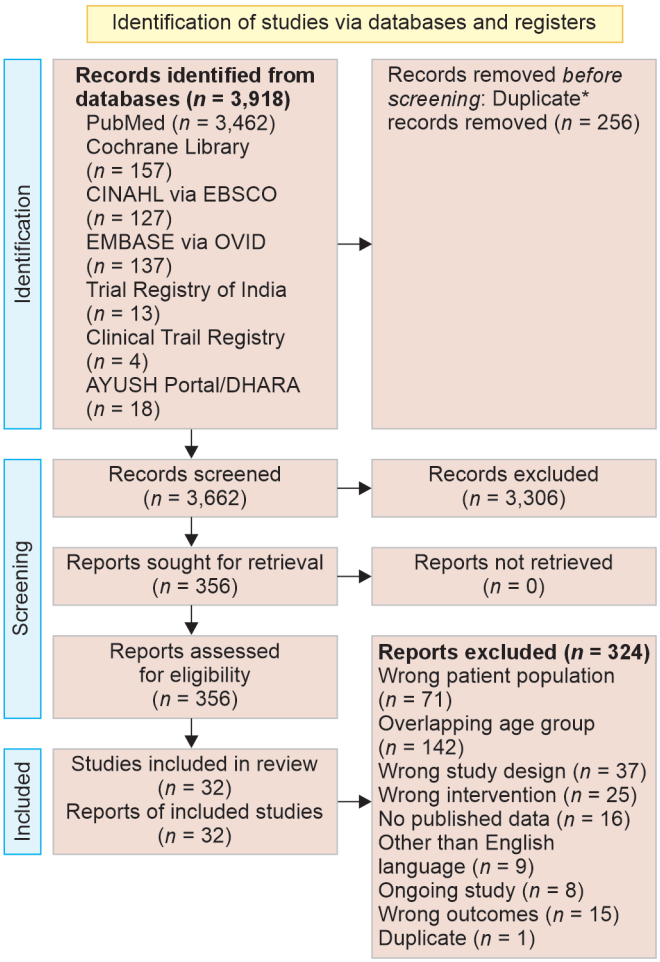

Fig. 1: Preferred Reporting Items for Systematic Reviews and Meta-Analysis flow to illustrate the screening process

orthodontic treatment and among children with SHCNs were reported in two studies. None of the studies reported on the effect of HMR on remineralization and halitosis. The outcome measures reported in the included studies were debris index, calculus index, Oral Hygiene Index (OHI), ICDAS, bacterial counts, salivary pH, buffering capacity of saliva, glucan concentration, density of bacterial growth, and zone of bacterial inhibition from the saliva and plaque samples.

Herbal extracts analyzed in the included studies[22,30–60] were mainly derived from *Azadirachta indica* (neem), *Terminalia chebula* (black-myrobalan or kadukai), *Salvadora persica* (miswak), *Plantago lanceolata* (ribwort plantain—a species of flowering plant), *Punica granatum* (pomegranate), *Vitis vinifera* (wine grape), *Glycyrrhiza glabra* (licorice), *Aloe barbadensis miller* (Family: Liliaceae—*Aloe vera*), *Phyllanthus emblica* (amla), *Allium sativum* (garlic), *Zingiber officinale* (ginger), *Mangifera indica* (mango), *Psidium guajava* (guava), *Salvia officinalis* (Family: Lamiaceae—sage), *Stevia rebaudiana* (stevia) and other polyherbal formula that includes Triphala (polyherbal medicine including plant species *Emblica officinalis*, *Terminalia bellerica*, and *T. chebula*). The control groups were gold standard CHX or normal saline or placebo or the comparison among the other herbal preparations. The duration of intervention ranged from immediate prerinse vs postrinse or baseline comparison with various follow-up periods which varied among each study with minutes, weeks, days, and months.

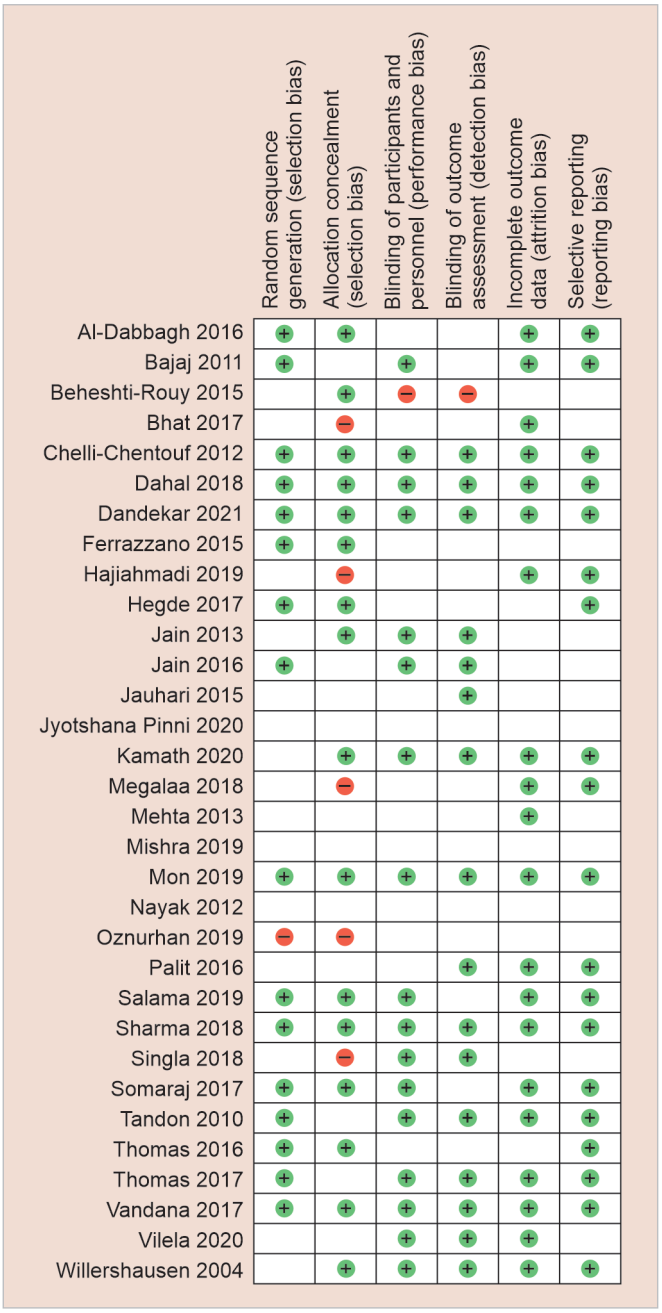

Fig. 2: Risk of bias graph

Quality Assessments of Studies

Among the 32 included studies, about 16 (50%) were of "low risk" and 1 (3.1%) study assessed as "high risk" based on their randomization process and incorrect random sequence generation. The assessment of allocation concealment showed that about 15 (46.9%) and 6 (15.6%) were categorized under "low risk" and "high risk" category, respectively. Nearly, 16 (50%) studies were rated as "low risk" while 1 (3.1%) study rated as "high risk" for the domain—"Blinding of participants and personnel for all outcomes." Figures 2 and 3 represent the risks of bias assessment for the included studies.

Descriptive Analysis

The detailed included studies characteristics are provided in Table 2. Table 3 represents descriptive summary including statistical analysis between the intervention and comparison group.

Discussion

The primary aim of this study was to assess the effects of HMR in caries prevention. The results of the 32 included trials involving 5,038 children and adolescents affirms a positive effect of HMR in controlling caries and altering bacterial levels. Of the 32 included studies,[22,29–59] 22 trials were conducted across India, 2 in Iran, 1 each in Germany, France, Italy, Turkey, Brazil, Iraq, Saudi Arabia, and Nepal. Our analysis reported 27 trials evaluating bacterial counts (*S. mutans*, *Lactobacillus*, and *C. albicans*) as outcome assessment while the effects of HMR on caries increment using indices such as DMFT and OHI were analyzed in five trials. Two RCTs evaluated plaque and gingival health using Plaque Index (PI) and Gingival Index (GI).

Secondary aims of the review were to determine the efficacy in remineralizing white spot lesions, reducing halitosis, and improving

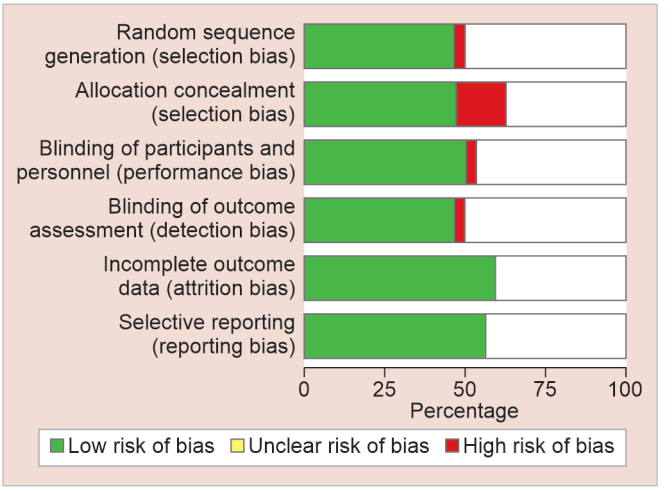

Fig. 3: Risk of bias summary

Table 2: Characteristics of included studies

No.	*Study (author/ year)*	*Country*	*Designduration*	*Participation/ inclusion criteria*	*Sample size*	*Study duration*	*Source of funding*	*Author's conclusion*
1	Al-Dabbagh et al./2016	Iraq	Randomized control trial	40 students (20 males and 20 females) aged 16–18 years, presence of at least 2 carious teeth	40 (10 each group)	Salivary samples were collected at 3-time intervals: Before, immediately after use, and after 2 weeks of use	Nil	Miswak mouthrinse was found to be more effective in reducing the growth of cariogenic bacteria
2	Bajaj and Tandon/2011	Karnataka, India	*In vivo* study: Double-blind randomized control trial	1,431 students in the age-group 8–12 years	1,431	Baseline, 3 months, 6 months, and 9 months	Indian Council of Medical Research	The reports of the study stated no significant difference in the *S. mutans* count except for lactobacillus where Triphala had shown better results than CHX
3	Beheshti-Rouy et al./2015	Iran	Double-blind randomized clinical trial	Female 11–14- year-old school children of Hamadan, Iran. Mean age not mentioned	40 (35 each group)	Baseline: Plaque sample, after 21 days of intervention	Nil	The sage mouthwash was found to be effective in reduction of the *S. mutans*
4	Bhat et al./2017	Karnataka, India	Randomized control trial	Children aged 8–14 years in Mangalore Residential School, Karnataka	20 (10 each group)	Basline, 0.5 hours after rinsing and after 5 days	Nil	Daily use of herbal alternatives can be as effective and safe alternative to conventional mouthrinse
5	Chelli-Chentouf et al./2012	France	Single blind, randomized, and placebo-controlled clinical trial	School children aged from 6 to 12 with and without caries	20 (10 children with caries/10: children without caries	Saliva sample (1 mL) collected in sterile tube. The PH was measured immediately after collection using a calibrated PH meter	Nil	Hoggar miswak extract displayed a stronger antimicrobial effect
6	Dandekar and Winnier/2021	Mumbai, India	A three-arm double-blind RCT	300 children between the age-group of 8 and 13 years. With dmft/DMFT scores between 3 and 6 were selected for the study	90 (30 each group)	Salivary *S. mutans* was evaluated at baseline, 7th day, and 21st day	Nil	Neem and mango mouthrinses were proven to be an effective alternative to CHX in children
7	Ferrazzano et al./2015	Italy	*In vitro* and *in vivo*: Trial	44 adolescents (24 males and 20 females) ranging from 12 to 18 years old	44 (22 each group)	Baseline and on the 4th and 7th days: Saliva/plaque sample	Nil	*P. lanceolata* extract could represent a natural anticariogenic agent via an antimicrobial effect useful to control the proliferation of cariogenic flora
8	Hajiahmadi et al./2019	Isfahan, Iran	Double-blind randomized controlled clinical trial	64 children aged 6–12 years (35 girls and 29 boys) mean age of participants was 10.73 ± 2.82 years	64 (32 each group)	Salivary counts of bacteria were determined at the baseline and after 2 weeks of intervention	Isfahan University of Medical Sciences, Isfahan, Iran	The effect of "green tea with xylitol" mouthwash on reducing the number of salivary colonies of *S. mutans* and *Lactobacillus* was found to be significantly higher than that of the "green tea" mouthwash
9	Hegde and Kamath/2017	Maharashtra, India	Randomized control Trail	75 school children aged 8–12 years. Mean age of the participants was 11.2 (SD: 0.93) years	71 (24, 23, and 24 in groups 1, 2, and 3, respectively)	Nonstimulated whole salivary sample (2 mL) was collected at baseline and postrinsing	Nil	Green tea mouthrinse can be a promising preventive therapy worldwide for the prevention of dental caries. However, no significant difference was found between groups
10	Jain et al./2013	Uttar Pradesh, India	Double-blind pilot study: Randomized control Trial	Pediatric patients aged 7–14 years, mean age not mentioned	60 (20 each group)	Baseline prerinse and three postrinse saliva samples were evaluated for the changes in ph and mutans streptococci colony counts	Nil	The study affirms that both aqueous and ethanolic licorice extracts are potent cariostatic agents and are found to be palatable by child patients
11	Jain and Jain/2016	Uttar Pradesh, India	Triple-blinded randomized controlled field trial	15–17 years old school children in Ghaziabad, mean age not mentioned	120 (30 each group)	Salivary and plaque sample were estimated prior to the use of mouthrinses and repeated again after a period of 24, 48 hours, 1 and 2 weeks Pre- and postrinse salivary samples are collected at each time interval	Nil	Both CHX and multi-HMR showed statistically significant reduction in the *S. mutans* CFU count, in terms of efficacy and substantivity both

Contd…

Table 2: *Contd…*

No.	Study (author/ year)	Country	Design duration	Participation/ inclusion criteria	Sample size	Study duration	Source of funding	Author's conclusion
12	Jauhari et al./2015	Rajasthan, India	Randomized double-blinded controlled trial	52 subjects in the age of 6–12 years	52 (13 each group)	Caries activity and *S. mutans* counts were estimated using or a test and Dentocult SM Strip mutans kit prior to the use of mouthrinses and oil pulling technique and repeated again after a period of 2 weeks	Nil	HMR were found to be effective antimicrobial agent in reducing the bacterial colonization of an individual
13	Kamath et al./2020	Karnataka, India	Double-blinded, placebo-controlled prospective interventional study	School children in the age range of 8–14 years with plaque scores and gingivalscores >1 and similar oral hygiene practices (89 boys, 63 girls)	152 (38 groups each)	Baseline, 4 weeks after supervised mouthrinse and after 2 weeks of stopping the mouthrinse	Nil	The use of *Aloe vera* and tea tree oil mouthwashes can decrease plaque, gingivitis and *S. mutans* in the oral cavity in children
14	Megalaa et al./2018	India	Double-blinded, placebo-controlled prospective interventional study	School children 8–12 years, 152 (38 each group) children were equally divided by simple randomization into four groups. The mean age in groups 1, 2, 3 and 4 was 11.92 ± 1.84 years, 12.15 ± 1.94 years, 12.16 ± 1.97 years, and 11.29 ± 2.16 years	152 (38 each group)	Saliva sample baseline, 2 weeks washout period and at the end of 2 weeks wash out period	Nil	The results of the study suggest that HMR could be tried as an adjunctive anticaries agent against dental caries causing microorganisms
15	Mehta et al./2013	Andhra Pradesh, India	Double-blind randomized clinical trial	55 healthy children between 8 and 14 years	55 (35 group A, 20 group B)	Phase 1 and phase 3 were the clinical trial periods of 10 days. Phase 2 was the washout period of 2 weeks. Baseline data were collected at the onset of phase 1 and the final value collected at the end of phase 3	Nil	Freshol was found to be better than CHX in reducing the salivary mutans streptococci count and equieffective to CHX in altering plaque and gingival scores
16	Mishra et al./2019	Rajasthan, India	Randomized clinical double-blinded study	80 children of 8–15 years of age with decayed missing filled teeth (DMFT)/decayed, missing and filled teeth (dmft) >4 and no history of an orthodontic appliance and no medical history	80 (20 each group)	Baseline, days 16, and 31	Nil	This study concluded that *V. vinifera* had shown the lowest plaque reduction owing to its antioxidant and phytochemical properties. And *P. granatum* showed the maximum substantivity
17	Mon et al./2019	Tamil Nadu, India	Parallel multiarm RCT	Schoolchildren aged 10–12 years, DMFT/deft score ≤3	100 (25 each group)	Baseline (T1), after 15 days (T2), and after 30 days (T3). Duration of the study was 1 month	Nil	Herbal water can be used in children instead of chemical mouthrinses to avoid any adverse effects
18	Nayak et al./2012	Karnataka, India	Triple blind randomized field trial	12–15-year-old school children of Belgaum city, 36 were male and 24 were female with the mean age 13.4	60 (36 and 24 groups 1, 2, respectively)	Baseline, 6 and 12 hours postrinsing	Nil	*S. mutans* counts significantly reduced 6 hours postrinsing for 80% of the children
19	Salama and Alsughier /2019	Saudi Arabia	Randomized controlled clinical trial	Healthy children 4–5 years of age have primary dentition	40 (20 each group)	Baseline, 2 and 4 weeks	Nil	Green tea extract showed promising effect in decreasing the count of salivary *S. mutans* and in the prevention of dental caries
20	Sharma et al./2018	Ghaziabad, India	Randomized control trail	Healthy children between ages 6 and 12 years having one or more interproximal carious lesions, well into the dentin, visualized radiographically	60 (15 each group)	Saliva samples baseline and again after 15 days of using the mouthrinse	Nil	CHX and fluoride showed statistically significant reduction in *S. mutans* count compared to herbal rinse

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Table 2: *Contd…*

No.	Study (author/ year)	Country	Design duration	Participation/ inclusion criteria	Sample size	Study duration	Source of funding	Author's conclusion
21	Singla et al./2018	Bhopal, India	*In vivo* study: randomized control trial	40 subjects were recruited from a local boarding school, age-group of 8–10 years with mean age of 9.7± 1.3, out of which 26 were males and 14 werefemales	40 (10 each group)	Salivary samples on blood agar media at the end of 48 hours, and 7 days	Not mentioned	The aqueous extracts of the chosen herbal plants showed an acceptable antibacterial efficacy against oral streptococci
22	Somaraj et al./2017	Karnataka, India	RCT (parallel group, placebo controlled	240 schoolchildren (12–15 years old) e mean age of study participants was 13.86 ± 0.85	240 (80 each group)	Baseline, 6, and 12 months	Nil	Both herbal and fluoride were found to be equally effective and could be recommended for use in school-based health education program to control dental caries
23	Tandon et al./2010	Manipal, India	Randomized control trail	1,501 students in the age-group of 8–12 years, belonging to classes 4–6th were the subjects for this study	1,501 (514, 495, and 492 in groups 1, 2, and 3, respectively	The Dsi (incipient caries) were done at 3, 6, and 9 months intervals from baseline to the caries assessment was done at 9 months interval from baseline	Funded by the Indian Council of Medical-Research	No significant difference was found between the Triphala and the CHX mouthwashes
24	Thomas et al./2016	India	A randomized, double-blind, active, and controlled clinical trial	30 children aged 4–6 years. Mean age of the participants was 5 [standarddeviation (SD): 0.69] years	30 (15 each group)	2 mL saliva samples at baseline and after 2 weeks rinsing	Nil	From the results of our study, it can be concluded that green tea mouthrinse could be very good cost-effective mouthrinse
25	Vandana et al./2017	Andhra Pradesh, India	A randomized, controlled, triple blind, and parallel repeated measure study	Female children aged 12–15 years selected from secondary school	108 (27 each group)	Basline, 3 months, and 6 months	Nil	Stevia demonstrated very potent antiplaque and antigingivitis properties compared to other mouthrinses at the end of 6 months trial
26	Vilela et al./2020	Brazil	Randomized control trial	47 healthy children aged 5–12 years	47	Nonstimulated salivary sample (pre-/ postrinse)	Individual scholarship (CAPES PROEX)	Both EGCG and green tea could be used as alternatives to CHX-based mouthwashes
27	Pinni et al./2018	Andhra Pradesh, India	An *in vitro* and *in vivo* study	Children between the age-group of 6 and 12 years who were following a routine oral hygiene practice with dmft score 4 were screened without sex predilection	30 (10 each group)	Salivary samples which were collected before and after (5 minutes) mouth rinsing	Not mentioned	Pomegranate Pericarp mouthrinse may be considered a potential anticaries mouthrinse
28	Oznurhan et al./2019	Turkey	Randomized control trial	Children aged 10 –13 years (mean age: 11.33) with clinical picture of gingivitis. 90 individuals [48 girls (53.3%, 42 boys (46.7%)]	90 (30 each group)	Saliva sample: Baseline 5 minutes (T1) and 60 minutes (T2) differences were calculated within 5–60 minutes (T3). Plaque sample for bacterial counts (CFU)/mL	Supported by CUBAP	Licorice was found to be more effective than CHX
29	Palit et al./2016	Uttar Pradesh, India	Single-blinded (microbiologist) randomized control trial	Children between 8 and 12 years	60 (20 each group)	Baseline ph of both the extract groups were compared to the ph at 10, 30, and 90 minutes	Nil	Results of this study showed that both types of aqueous extract of *T. chebula* may be used as potential anticariogenic mouthwash with acceptable taste in children
30	Thomas et al./2017	Karnataka, India	Randomized double-blind active controlled clinical trial	45 children aged 4 to 6 years with severe early childhood caries [S-ECC; based on decayed extracted filled (defs) score] were selected	45 (15 each group)	A base-line and postrinsing nonstimulated whole salivary sample (2 mL) was collected and tested for the number of CFUs	Nil	The findings of this study indicate that green tea and garlic with lime mouthrinse can be an economical alternative to NaF mouthrinse both for prevention and therapeutics
31	Willershausen et al./2015	Germany	Prospective randomized, double-blind clinical study	40 patients (15 males, 25 females) mean age 16.1 ± 2.3 years: Fixed orthodontic patients with mild-to-moderate gingivitis	40 (20 each group)	Saliva/plaque baseline, 4, 8, and 12 weeks	Nil	HMR, used as an adjunct to mechanical oral hygiene measures was found beneficial for gingival health in patients wearing fixed orthodontic appliances
32	Dahal et al./2018	Nepal	Randomized controlled clinical trial with parallel groups study: Triple blinded	82 visually impaired students of age 4–20 years from Shree Purwanchal Gyanchakshu Vidhyalaya, Dharan, Nepal	60 (20 each group)	Baseline and follow-up study visit after 2 weeks	Nil	Within the limitation of this study, herbal mouthwash could be useful in management of gingival health among visually impaired children

Table 3: Descriptive summary of statistical analysis between groups

				Caries status						
				Caries increments			*Change in bacterial count*			
No.	*Study (author/ year)*	*Intervention*	*Comparison*	*DMFT(S)/ dmft(s)*	*ICDAS*	*D(si)*	*SM (CFU/mL)*	*LB (CFU/mL)*	*CA (CFU/mL)*	*Authors estimated RoB*
1	Al-Dabbagh et al./2016	Miswak MW + OTP Miswak TP ordinary TP (OTP)	Saline + OTP	ND	ND	ND	>	>	ND	Low
2	Bajaj and Tandon/2011	Triphala MW (0.6%)	CHX (0.1%) (+ control), distilled water (–control)	ND	ND	ND	NS	>	ND	Low
3	Beheshti-Rouy et al./2015	Sage (stevia) 5%	Normal saline	ND	ND	ND	>	ND	ND	High
4	Bhat et al./2017	Mango leaves MR (2%)	CHX (0.12%)	ND	ND	ND	>	ND	ND	Low
5	Chelli-Chentouf et al./2012	Miswak	Placebo group	ND	ND	ND	>	ND	ND	Low
6	Dandekar and Winnier/2021	Neem MR Mango MR	CHX (0.12%)	ND	ND	ND	>	ND	ND	Low
7	Ferrazzano et al./2015	*P. lanceolata*	Placebo group	ND	ND	ND	>	ND	NS	High
8	Hajiahmadi et al./2019	0.5% green tea	20% xylitol + 0.5% green tea	ND	ND	ND	<	ND	ND	High
9	Hegde and Kamath/2017	Green tea extract (0.5%) MR Combination MR: Thermokind	CHX (0.12%)	ND	ND	ND	NS	ND	ND	Moderate
10	Jain et al./2013	Aqueous licorice mouthwash (15%), ethanolic licorice mouthwash (3.75%)	CHX (0.12%)	ND	ND	ND	>	ND	ND	Low
11	Jain and Jain/2016	HMR containing active ingredient as mixture of garlic powder, aqueous extract of amla (gooseberry), and organic solvent-based extract of ginger (20 mg each), essential oil containing menthol, and eucalyptol, fluoride containing 0.2% NaF	CHX mouthrinse (0.2%)	ND	ND	ND	>	ND	ND	Moderate
12	Jauhari et al./2015	HMR containing active ingredient as *S. persica* (5 mg), oil pulling, fluoride mouthrinse containing 200 ppm NaF	Distilled water	ND	ND	ND	>	ND	ND	High
13	Kamath et al./2020	Tea tree oil (0.5 gm), *Aloe vera*	0.2% CHX (positive control), distilled water (negative control)	ND	ND	ND	>	ND	ND	Low
14	Megalaa et al./2018	Sodium fluoride rinse 0.05%, herbal ethanolic extracts of tulsi (4%)	Black myrobalans 2.5%	ND	ND	ND	>	ND	ND	High
15	Mehta et al./2013	Freshol	CHX gluconate (0.156%)	ND	ND	ND	>	ND	ND	High
16	Mishra et al./2019	*P. granatum, T. chebula, V. vinifera*	0.2% of CHX	ND	ND	ND	>	ND	ND	High
17	Mon et al./2019	Herbal water, ozone water, CHX	Water	ND	ND	ND	>	ND	ND	Low
18	Nayak et al./2012	*T. chebula*	Placebo control	ND	ND	ND	>	ND	ND	High
19	Salama and Alsughier/2019	OHI + green tea extract (8 mL/day)	OHI	ND	ND	ND	>	ND	ND	Low
20	Sharma et al./2018	10 mL of herbal (HioraR), 0.2% CHX gluconate (HexidineR), NaF	Plain water	ND	ND	ND	<	ND	ND	High
21	Singla et al./2018	Pomegranate extract, grape seed extract, guava extract	Distilled water	ND	ND	ND	>	ND	ND	High
22	Somaraj et al./2017	Herbal MR (Freshol), 0.2% Fluoride	Mint flavor added in distilled water	NS	ND	ND	ND	ND	ND	Low

Contd…

Table 3: *Contd…*

				Caries status						
				Caries increments			*Change in bacterial count*			
No.	*Study (author/ year)*	*Intervention*	*Comparison*	*DMFT(S)/ dmft(s)*	*ICDAS*	*D(si)*	*SM (CFU/mL)*	*LB (CFU/mL)*	*CA (CFU/mL)*	*Authors estimated RoB*
23	Tandon et al./2010	Triphala (0.6%)	CHX (0.1%) (positive control)	NS	ND	ND	ND	ND	ND	Low
24	Thomas et al./2016	Green tea	CHX (0.2%)	ND	ND	ND	>	<	ND	Moderate
25	Vandana et al./2017	10% stevia	0.05% NaF, 0.2% CHX gluconate; placebo	ND	>	ND	ND	ND	ND	Low
26	Vilela et al./2020	Epigallocatechin-3-gallate (EGCG)	Green tea	ND	ND	ND	>	>	ND	Moderate
27	Pinni et al./2018	Pomegranate pericarp extract (PPE)	0.2% CHX (positive control)	ND	ND	ND	>	ND	ND	High
28	Oznurhan et al./2019	Licorice	CHX (CHX)	ND	ND	ND	>	ND	ND	High
29	Palit et al./2016	10% hot aqueous *T. chebula* extract, cold extract of *T. chebula*	Distilled water (negative control)	ND	ND	ND	>	ND	ND	Moderate
30	Thomas et al./2017	Green tea	Garlic with lime mouthrinse	ND	ND	ND	>	>	NS	Low
Gingival and periodontal health during orthodontic treatment										
No	Study (author/ year)	Intervention	Comparison	PI	GI	PD	SM (CFU/ mL)	LB (CFU/ mL)	CA (CFU/ mL)	Authors estimated RoB
31	Willershausen et al./2015	Parodontax©	Placebo	SD	>	NA	NA	NA	NA	Low
Gingival and periodontal health among children with SHCNs										
32	Dahal et al./2018	Herbal MR	CHX (+control) placebo (–control)	>	>	NA	NA	NA	NA	Low

>, significant reduction; <, lower than; MR, mouthrinse; NA, not applicable; ND, no data available; NS, no significant difference; OTP, ordinary toothpaste; OW, ozone water

gingival and periodontal health among patients undergoing orthodontic treatment and with SHCN. Our analysis revealed one RCT conducted by Willershausen et al. that assessed the "effect of HMR (Parodontax) on gingival health in patients (mean age-group of 16 ± 2 years) undergoing fixed orthodontic appliances." In this study, the subjects were examined at 4-week interval for a period of 3 months. The results of this double-blinded study established favorable beneficial outcomes in gingival therapy among children undergoing fixed orthodontic appliances when used in adjunct with regular oral hygiene measures.[58] We found one trial evaluating the gingival health using plaque and gingival scores among adolescents with SHCN.[61]

Additionally, this review also intended to report included studies involving children only below 6 years. Our analysis reported two trials conducted among 4–6 years age-group assessing the effectiveness of green tea compared to NaF and CHX on primary dentition. Both trials recommended the use of green tea and garlic-lime mouthrinse as an economic alternative to CHX and sodium flouride mouthrinse. However, the short duration of intervention period could be a subject of caution. Nevertheless, these results suggest that use of HMR such as green tea and garlic-lime mouthrinse can reduce the bacterial levels in primary dentition.

Herbal Formula and Concentration

Although, the most common herbal component used across included studies was green tea,[22,37,55,56] miswak[30,34] and Tulsi were also tested in more than one study. The wide range of herbs assessed across study is a concern to establish solid evidences. Whether a single herb is more effective than a combination of herbs, needs further investigation.

Evaluation Period

Our reports affirm most trials having short evaluation period. The period of follow-up for HMR intervention ranged from 60 minutes to 9 months. On an average, 2 weeks regime have been followed to assess the efficacy of HMR while few studies had an evaluation period beyond 9 months. Due to the wide range of herbal components used as mouthrinses across study, the estimated period required to conclude its efficacy is still unknown. We noted that most studies comparing herbal to CHX mouthrinse had an average follow-up period of 2 weeks. While this is contrary to the American Dental Association which recommends a minimum of 6 months to establish the effectiveness of any product.[62]

Level of Evidence

Overall RoB across studies was low. The RoB report for all included studies revealed less than 5% "attrition bias." All included studies reported no "reporting bias." We observed several studies with small sample size. This can potentially lead to greater variability and skewing of findings. Hence, the overall robustness of the included RCTs findings is moderate. The daily use of chemical and synthetic products has been a subject of concern among the general population, researchers, and clinicians and paving way for

herbal alternatives. Mounthrinses as an oral hygiene aid can be an effective alternative and adjunct to tooth brushing specifically in children who lack motivation, manual dexterity, and need supervision. Even though, CHX mouthwash has remained gold standard due to its substantivity, antiplaque, and antimicrobial efficacy, its shortcomings are staining, discoloration, altered taste sensation, mucosal desquamation, impaired wound healing, anaphylactic reactions, and antimicrobial resistance.[61] Its long-term effects and safety for use in children has not been examined. Moreover, the unacceptable taste of CHX experienced by children is another concern.[63]

Strengths, Limitation, and Direction for Future Research

To our knowledge, this is the first systematic review to assess the effects of HMR on caries increments in children and adolescents. The reviewers further attempted to gather evidence on the effect of HMR on halitosis, gingival and periodontal health, and its remineralization potential. The review explicitly assessed various formulations of HMR. The reviewers also used the "Covidence" software to conduct the search, screening, data extraction, and assessment of study quality. The limitation of the review could be the exclusion of possible data of RCTs with overlapping age groups beyond 19 years due to nonavailability of precise data for the included age. Another limitation is exclusion of trials published in languages other than English.

To determine and establish one or combination of effective herbal formula, long-term robust trials are recommended. Thus, the efficacy of the most superior HMR could be compared with CHX and fluoride mouthrinses to affirm its future use. Furthermore, future research is warranted to evaluate the efficacy of the herb against chemical mouthwash, pertaining to both positive and negative effects, at various levels of concentrations. The results of such research will aid us to understand and conclude if HMR could be recommended as definite alternative to chemical mouthrinses. Indices such as ICDAS can be used to assess noncavitated lesions in young children. Further research should attempt to find the effectiveness of specific formulations of HMR suitable for use in children under 6 years to reduce the burden of ECC.

Conclusion

Results of this systematic review suggest that HMR may be effective in altering microbial levels among children and adolescents. Our analysis found no RCTs that evaluated the effect of HMR on halitosis and remineralization potential. The variance in HMR formula across studies, short follow-up period and limiting grade of evidence do not allow for conclusive evidence in efficacy of HMRs. The results of this review warrant robust RCTs designed to assess the effectiveness of different HMRs in preventing ECC among children under 6 years old.

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