



(REVIEW ARTICLE)



## Anticariogenic action and safety profile of a cacao bean husk extract: A systematic review and meta-analysis

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### Abstract

Nowadays, there is not a meta-analytic synthesis of the clinical reports that used a cacao bean husk extract (CBHE) solution as an anticariogenic mouth rinse. Thus, the aim of this study was to evaluate that information through a systematic review and meta-analysis methodology, conducted in accordance with PRISMA guidelines. Scientific databases were searched for studies published up to June 2021. Inclusion and exclusion criteria were applied to studies found and then, their data was analyzed. The five selected studies were categorized with a 36.6, 58.5, and 4.9 % of a low, unclear, and high risk of bias, respectively. Under appropriate heterogeneities ( $I^2$  values from 0 to 65 %,  $p$  values > 0.09) and absent reporting bias (symmetrical funnels), the meta-analyses show that the use of a CBHE mouth rinse reduced the salivary count of *Streptococcus mutans* ( $Z$  values from 2.45 to 10.61,  $p$  values < 0.01), similar to the chlorhexidine rinse performance ( $Z$  value= 0.55,  $p$  value= 0.58), and produced an insignificant presence of adverse events ( $Z$  value= 0.92,  $p$  value= 0.36) in children and adults, all these effects compared with those volunteers under an ethanol rinse or their pretest conditions. In conclusion, the CBHE mouth rinse reduced a cariogenic bacterium under an acceptable safety profile, but more clinical studies with high quality and more parameters are needed.

**Keywords:** *Streptococcus mutans*; Cacao bean husk; Adverse event; Meta-analysis

### 1. Introduction

Dental caries is a worldwide public health problem, which affects a large number of people [1, 2]. This disease condition starts with a homeostatic disbalance of the plaque biofilm, which in turn is caused by a selective ecological advantage of acidogenic and aciduric members of the resident oral microbiome over other commensal microorganisms [1]. Despite of the dental caries has a polymicrobial nature, *Streptococcus mutans* has a critical function for the caries pathogenesis [1]. Consequently, one of the practical interventions is the reduction of the oral *Streptococcus mutans* (MS) load by chemical agents to arrest or reverse the development of caries lesions [2, 3]. In this way, chlorhexidine gluconate, a cationic biguanide, is the most potent non-fluoride agent and the gold standard for the reduction of MS and plaque [2-4]. Nevertheless, there is an urgent need for alternative therapies, such as the use of natural by-product extracts, given the resistance to antibacterial agents by the excessive use of antibiotics in dentistry [5-7].

Cacao bean husk is the main by-product generated by the cocoa industry. About 3240 tons per year of the cacao bean husk residue are generated solely in Mexico [8]. Some clinical studies have claimed that a mouth rinse enriched with a cacao bean husk extract (CBHE) is a promising alternative to the anticariogenic mouth rinses [3, 9-12]. Nevertheless, one of the main limitations of those studies was the small sample size to reach a proper clinical decision. For this type of conflicts, the use of a meta-analysis should be used to combine the results of independent studies. Since a meta-

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analysis provides a more precise estimate of the health effects, compared with those derived from the individual studies [13].

To our knowledge, there is not a review or meta-analysis of the beneficial or harmful effects by the use of a CBHE mouth rinse against the dental caries in children and adults. The aim of this study was to examine the usefulness as an anticaries agent and clinical safety of the CBHE mouth rinse, reflected by an evaluation of the caries incidence, count of salivary cariogenic bacteria, plaque index score and number of adverse events in the participants, under a systematic review and meta-analysis methodology.

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## 2. Material and methods

### 2.1. Data sources

Our study was conducted in accordance with the guidelines for the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [14, 15]. The protocol for this work was registered with the International Prospective Register of Systematic Reviews (PROSPERO) of the United Kingdom's National Institute for Health Research. A literature search was performed in PubMed, Cochrane, LILACS, MedLine, and Imbiomed databases taking account from the earlier records to 01 June 2021, in order to identify clinical studies, where a CBHE was employed for the reduction of the caries incidence, cariogenic microorganisms and/or plaque formation. The following terms were used for the PubMed, Cochrane, LILACS and MedLine databases: cacao AND dental caries; cacao AND saliva; cacao AND clinical trial; cacao AND cariogenic bacteria; cacao AND *Streptococcus mutans*; cocoa AND dental caries; cocoa AND saliva; cocoa AND clinical trial; cocoa AND cariogenic bacteria; cocoa AND *Streptococcus mutans*; bean husk AND dental caries; bean husk AND saliva; bean husk AND clinical trial; bean husk AND cariogenic bacteria; and, bean husk AND *Streptococcus mutans*. For the Imbiomed database, the following single search term was used: cacao; cocoa; bean husk; dental caries; saliva; clinical trial; cariogenic bacteria; or, *Streptococcus mutans*. Also, the list of references of the articles found was reviewed to prevent the omission of studies.

### 2.2. Eligibility criteria

Papers were selected based on the following criteria: randomized or nonrandomized controlled clinical trial under a parallel or crossover design applied to volunteers with or without a diagnosis of dental caries; at least one of the following evaluations: 1) comparison of subjects under a CBHE therapy with a control, placebo, or chlorhexidine therapy, or 2) comparison of the data obtained from participants before and after a CBHE rinse; at least one of the following measurements: 1) dental caries incidence, 2) count of cariogenic bacteria, or 3) plaque index; and, report published in English or Spanish. Studies were excluded based on the following criterium: participants who taken an antibiotic during the study.

### 2.3. Data collection and risk of bias

Two researchers independently screened the titles and abstracts of the articles found and then, they reviewed the complete papers. Subsequently, both researchers compared their evaluations, and any disagreement was resolved by consensus with the assistance of a third researcher. The following characteristics were extracted from each study: first author; year of publication; the patient's age; study design; number of participants; intervention characteristics (active principle, concentration, dose interval, and route of administration); diagnostic criteria for the dental caries; outcome data (caries incidence, colony count of bacteria, and/or score of the plaque index); adverse effects; and, author's conclusion. For crossover studies with a comparison of three or more intervention groups, the data from the first phase was only extracted.

For the parallel design studies, their quality was evaluated using the Cochrane Collaboration's risk of bias tool from the Review Manager (RevMan) version 5.3 statistical analysis software (The Cochrane Collaboration, Copenhagen, DK, NE). In this sense, the following criteria were selected to assess the risk of bias: random sequence generation; allocation concealment; blinding of participants and personnel; blinding outcome assessment; incomplete outcome data; selective reporting; and, other biases. For the crossover design studies, their quality was evaluated using the nine-item checklist derived from the Cochrane Collaboration's risk of bias tool [16]. Then, the following criteria was used to assess the risk of bias: appropriate cross-over design; randomized order of receiving treatment; carry-over effects; unbiased data; allocation concealment; blinding; incomplete outcome data; selective outcome reporting; and, other bias. For both evaluations, each criterion was classified with a low, unclear, or high risk of bias, in according to the procedure described by Higgins *et al.* [17] or Ding *et al.* [16], and then, a risk of bias graph was used to show the proportion of studies into each category. Both quality assessments were performed individually by two researchers and a third researcher resolved the discrepancies between them to obtain a consensual evaluation.

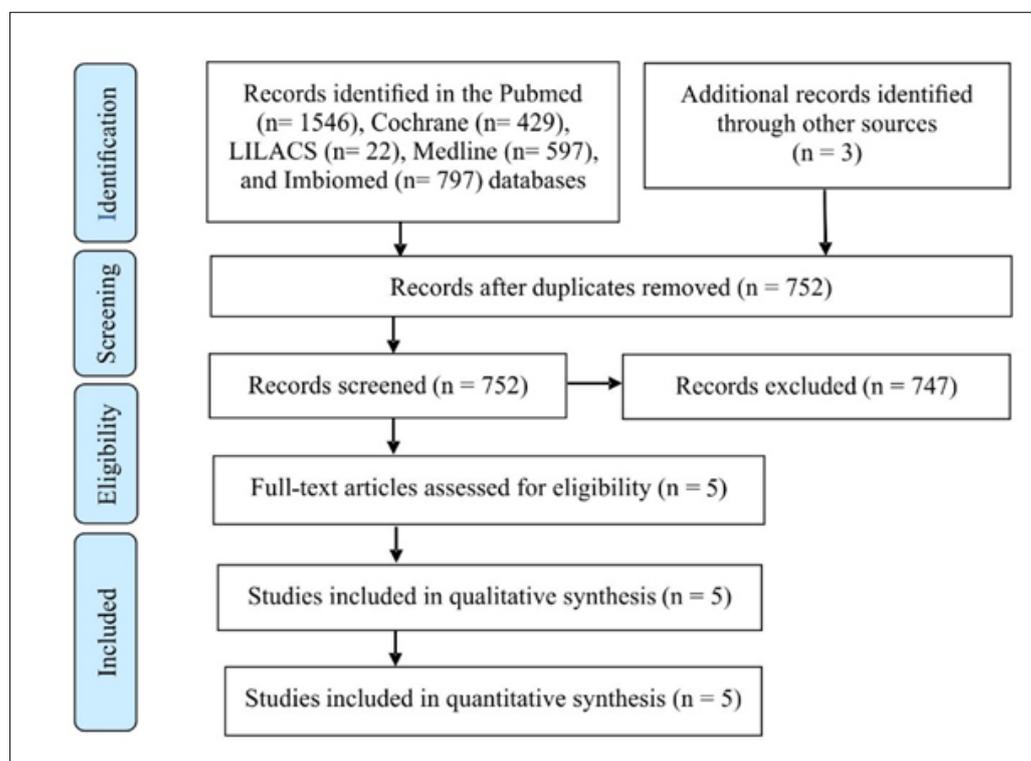
## 2.4. Data analysis

For dichotomous or continuous data, each meta-analysis was performed with a Mantel-Haenszel or inverse variance method. The risk difference, mean difference, and 95% confidence interval (CI) were analyzed with a fixed effects model. For each meta-analysis, Z, static  $I^2$ , and p values were used to evaluate the overall effect, heterogeneity, and probability, respectively. A static  $I^2$  ranged from 0 to 40, 40 to 70, or 70 to 100% determined an absent, acceptable, or considerable heterogeneity, respectively. For the overall effect and heterogeneity, a probability of less than 0.05 was accepted as significant. Finally, a funnel plot was used to detect reporting biases in each meta-analysis, where the presence of any reporting bias produced an asymmetrical funnel [18]. All the above-mentioned meta-analyses and parameters were obtained and calculated using the RevMan version 5.3 statistical analysis software (The Cochrane Collaboration, Copenhagen, and DK, NE).

## 3. Results

### 3.1. Characteristics and evaluation of the studies

Five relevant studies were identified, in which the use of a CBHE mouth rinse modified the number of MS or plaque index score in participants. It is important to mention that one of the 747 records excluded was the study of Fajriani and colleagues [9], since that study is not a clinical trial under a parallel or crossover design and consequently, it do not have one of the inclusion criteria (Figure 1).



**Figure 1** Search strategy

**Table 1** Relevant characteristics of the included studies

First author, year	Study design, health status, and age of participants	Groups and sample size	Intervention characteristics	Author's conclusion
Shrimathi, 2019 [3]	Randomized, double blinded, controlled crossover study.	Experimental group: 0.5% CBHE mouth rinse flavoured and sweetened with 0.1%	During the first phase of the study, each participant used 10 mL of the CBHE or	In a similar fashion, both therapies reduced the salivary MS count.

	Healthy subjects aged 18 to 25 years.	peppermint oil and saccharine (n= 25). Comparator group: 0.2% Chlorhexidine mouth rinse (n= 25).	chlorhexidine mouth rinse once a day. An examination was performed at baseline and after 7 days of the treatment.	
Dua, 2017 [19]	Randomized, nonblinded, parallel arm clinical study. Children aged 10 to 14 years.*	Experimental group: 0.1% CBHE mouth rinse (n= 25). Comparator group: 0.12% Chlorhexidine mouth rinse (n= 25).	Each child used 10 mL of the solution to rinse once a day for 30 seconds. An evaluation was performed at day 1 and 15.	The plaque index was reduced by both therapies with a similar action between them.
Venkatesh, 2011 [10]	Randomized, nonblinded, parallel arm clinical study. Children aged 6 to 10 years.*	Experimental group: 0.1% CBHE mouth rinse (n= 25). Comparator group: 0.2% Chlorhexidine gluconate mouth rinse (n= 25).	Each child used 10 mL of the mouth rinse to rinse twice daily for 30 seconds. An examination was performed at the pre-rinse condition and after 7 days, 1 and 2 months of the therapy.	A reduction in the salivary MS count was obtained by the CBHE rinse at all follow-up intervals, compared with the pre-rinse value. Both rinses produced a similar action against the MS. The CBHE rinse caused a nauseating effect in volunteers, whereas chlorhexidine rinse did not produce anything.
Srikanth, 2008 [11]	Randomized, single blind, crossover clinical study. Children aged 10 to 14 years.*	Experimental group: 0.1% CBHE mouth rinse underwent scaling of teeth (n= 26). Placebo group: 0.1% ethanol mouth rinse underwent scaling of teeth (n= 26).	The placebo rinse was administered to volunteers. After a one-week washout period, the CBHE rinse was given to the same subjects. Each mouth rinsing session consisted of five consecutive rinses with 20 ml, for a total volume of 100 ml. Each session lasted 50 seconds and nine sessions were performed per day. An evaluation was performed at the morning of day 4.	The CBHE rinse produced a lower salivary MS count and plaque deposition than the placebo rinse. Both therapies did not produce side effects during the time of the study.
Matsumoto, 2004 [12]	Nonrandomized, single blind, crossover clinical study.** Adult volunteers aged 19 to 29 years.*	Experimental group: 0.1% CBHE mouth rinse (n= 28). Control group: 1% ethanol mouth rinse (n= 28).	The CBHE rinse was administered to the volunteers. After a one-week washout period, the control rinse was given to the same subjects. The mouth rinsing session was equal to the intervention described in Srikanth <i>et al</i> 2008. An evaluation was performed before and after four days of starting the procedure.	The plaque deposition and salivary MS count were reduced by the CBHE rinse, compared with the control values. During the study, side effects were not reported.

\*The health status of the participants was not described. \*\*The second phase of that work was a clinical study. CBHE, cacao bean husk extract; MS, *Streptococcus mutans*.

Table 1 summarizes the characteristics and outcomes of the included studies. None of the selected studies evaluated the incidence of dental caries in their participants. Figure 2 shows the quality assessment, where a 36.6, 58.5, and 4.9 % of the selected studies were categorized with a low, unclear, and high risk of bias, respectively.

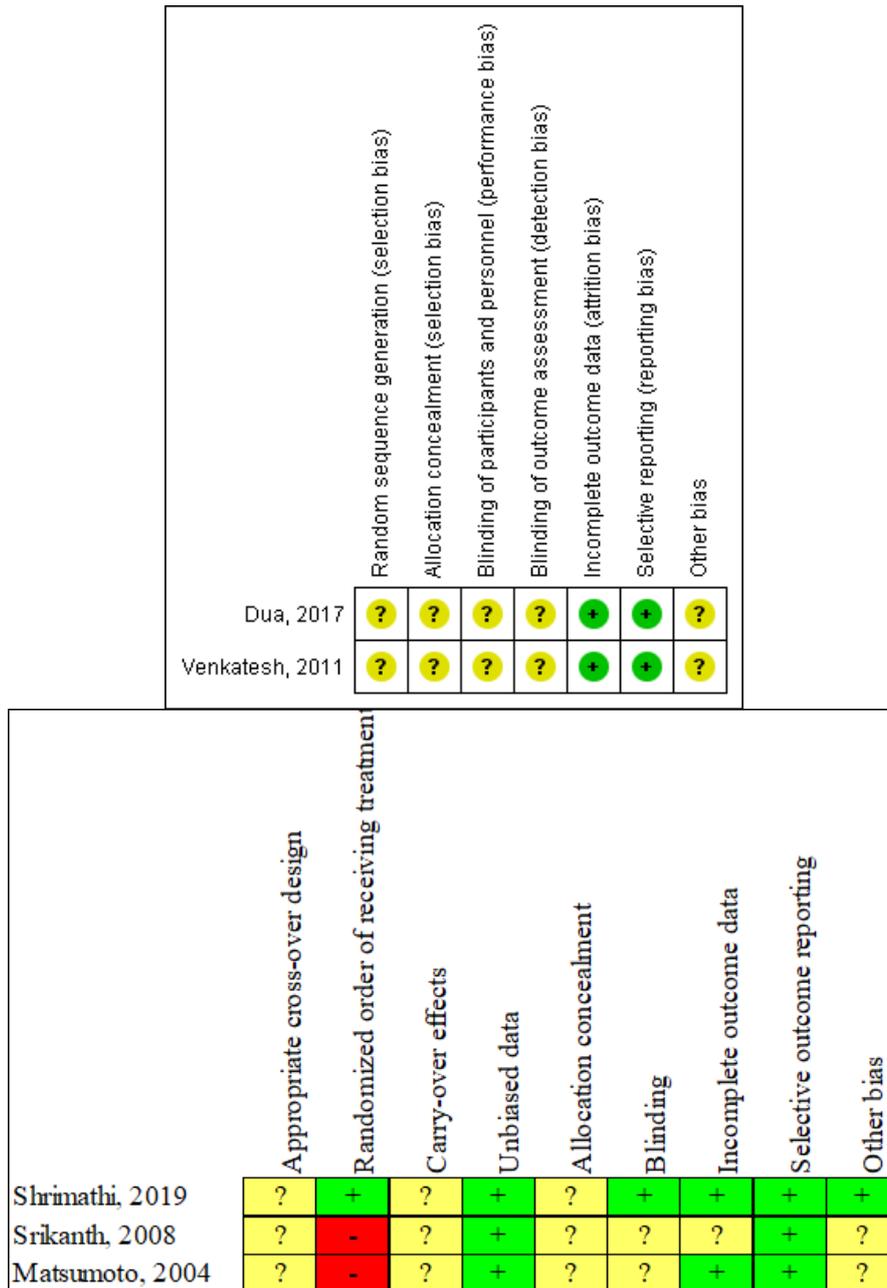


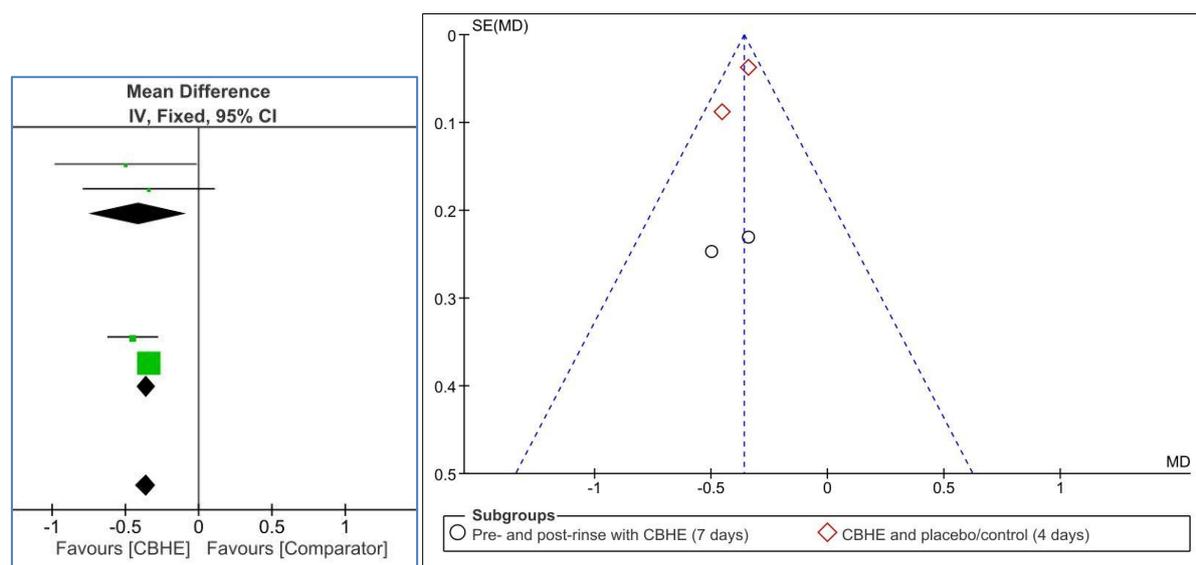
Figure 2 Risk of bias graph of the included studies

**3.2. Comparison of the CBHE rinse with the pre-rinse condition or placebo/control rinse**

The 95% CI, Z and p values of the meta-analysis showed a reduction in the salivary MS count by use of the CBHE mouth rinse, compared with the pretest data or ethanol rinse group, under an absence of heterogeneity and reporting biases (Fig. 3). Meanwhile, the meta-analysis of the plaque index scores showed a reduction of the plaque biofilm by use of the CBHE, compared with the ethanol rinse outcomes (108 participants from two studies: Z value= 41.47 with a p value <0.001), but a considerable heterogeneity and reporting bias were obtained (I<sup>2</sup>= 97% with a p value <0.001 and asymmetrical funnel).

**Table 2** Meta-analysis and funnel plot of the data obtained from the cacao bean husk extract (pre- and post-rinse) and placebo/control mouth rinse on the MS count

Study or subgroup	CBHE			Comparator			Weight	Mean difference IV, Fixed, 95% CI
	Mean	SD	Total	Mean	SD	Total		
<b>Pre- and post-rinse with CBHE (7 days)</b>								
Shrimathi, 2019	1.61	0.792	25	2.108	0.952	25	1.9%	-0.50 [-0.98, -0.01]
Venkatesh, 2011	1.83	0.78	23	2.17	0.78	23	2.2%	-0.34 [-0.79, 0.11]
Subtotal (95% CI)			48			48	4.0%	-0.41 [-0.74, -0.08]
Heterogeneity: Chi <sup>2</sup> = 0.22, df = 1 (p = 0.64); I <sup>2</sup> = 0%								
Test for overall effect: Z = 2.45 (p = 0.01)								
<b>CBHE and placebo/control (4 days)</b>								
Srikanth, 2008	1.7	0.04	26	2.15	0.45	26	14.6%	-0.45 [-0.62, -0.28]
Matsumoto, 2004	4.16	0.15	28	4.5	0.13	28	81.4%	-0.34 [-0.41, -0.27]
Subtotal (95% CI)			54			54	96.0%	-0.36 [-0.42, -0.29]
Heterogeneity: Chi <sup>2</sup> = 1.31, df = 1 (p = 0.25); I <sup>2</sup> = 23%								
Test for overall effect: Z = 10.33 (p < 0.00001)								
Total (95% CI)			102			102	100.0%	-0.36 [-0.43, -0.29]
Heterogeneity: Chi <sup>2</sup> = 1.63, df = 3 (p = 0.65); I <sup>2</sup> = 0%								
Test for overall effect: Z = 10.61 (p < 0.00001)								
Test for subgroup differences: Chi <sup>2</sup> = 0.11, df = 1 (p = 0.74); I <sup>2</sup> = 0%								



**Figure 3** Meta-analysis and funnel plot of the data obtained from the cacao bean husk extract (pre- and post-rinse) and placebo/control mouth rinse on the MS count. Comparator includes the pre-rinse or alcohol rinse group. CBHE, cacao bean husk extract; MD, mean difference; MS, *Streptococcus mutans*; IV, inverse variance; SD, standard deviation; SE, standard error

### 3.3. Comparison between the CBHE and chlorhexidine mouth rinse

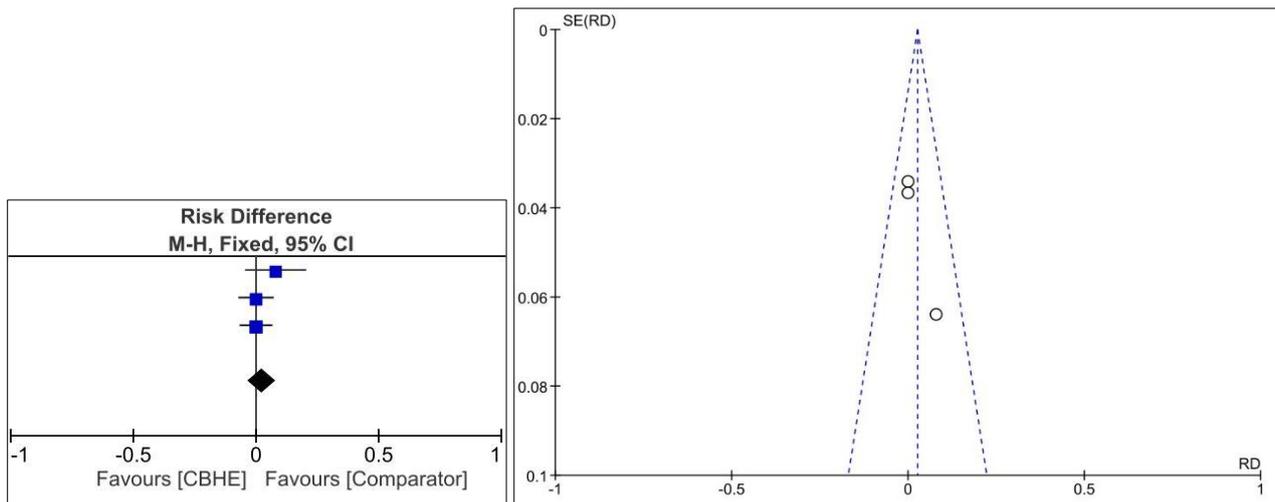
The analysis of the information of 98 participants from two studies [3,10] showed a similar action between the CBHE and chlorhexidine therapy against the MS found in saliva, under an acceptable heterogeneity and reporting bias (95% CI of the mean difference: -0.36 to 0.20, Z value= 0.58 with a p value= 0.58, I<sup>2</sup>= 65% with a p value= 0.09, and symmetrical funnel). For the plaque index evaluation, only one study compared these two therapies [19].

### 3.4. Clinical safety of the CBHE rinse

The 95% CI, Z and p values of the meta-analysis did not show a difference in the presence of adverse events by use of the CBHE mouth rinse, compared with the chlorhexidine and ethanol mouth rinse (Figure 4). An absence of heterogeneity and reporting biases was obtained for this analysis (Figure 4).

**Table 3** Meta-analysis and funnel plot of adverse events by use of the cacao bean husk extract (CBHE) and comparator mouth rinse

Study or subgroup	CBHE		Comparator		Weight	Risk difference
	Events	Total	Events	Total		M-H, Fixed, 95% CI
Venkatesh, 2011	2	25	0	25	31.6%	0.08 [-0.05, 0.21]
Srikanth, 2008	0	26	0	26	32.9%	0.00 [-0.07, 0.07]
Matsumoto, 2004	0	28	0	28	35.4%	0.00 [-0.07, 0.07]
Total (95% CI)		79		79	100.0%	0.03 [-0.03, 0.08]
Heterogeneity: Chi <sup>2</sup> = 1.76, df = 2 (p = 0.41); I <sup>2</sup> = 0%						
Test for overall effect: Z = 0.92 (p = 0.36)						



**Figure 4** Meta-analysis and funnel plot of adverse events by use of the cacao bean husk extract (CBHE) and comparator mouth rinse. Comparator group includes the chlorhexidine, placebo, and control group. M-H, Mantel-Haenszel; SD, standard deviation; SE, standard error

## 4. Discussion

For the present study, we searched dental caries incidence, count of cariogenic bacteria, and plaque index in the clinical studies as an evaluation of the anticariogenic ability. Our study showed that there was a reduced salivary count of MS in children and adults, when the CBHE mouth rinse was used, in comparison with the pre-rinse or placebo/control data. Besides, the CBHE mouth rinse exhibited a similar antibacterial effect to the chlorhexidine mouth rinse, as well as an absence of any serious adverse event by the husk extract in the subjects. These results were obtained under appropriate heterogeneities and reporting biases alongside a high percentage of low/unclear risk of bias. Nevertheless, an absence of the evaluation of the caries incidence was an unexpected outcome from all the studies using a CBHE rinse. It is well

known that the incidence of caries is the preferred hard endpoint for this type of studies, since there is a controversy about the correlation of the MS level and the risk of caries development [20]. Moreover, the plaque reduction by the CBHE cannot be supported with our meta-analysis due to a high presence of heterogeneity and reporting bias. Under these conditions, the meta-analysis of the plaque index reduction should not be taken into account [21].

The CBHE can produce a cariostatic effect by a reversible, complex and beneficial action on the homeostatic balance in the oral microbiome and plaque biofilm, together with a direct antibacterial activity against the MS. Since the extract contains oleic and linoleic acids, epicatechin polymers, and other polyphenols, which have a bactericidal activity towards the MS, inhibitory action against the biofilm formation and acid production by the MS through an anti-glucosyltransferase activity, and induction of the growth of species associated with the oral health, such as *Streptococcus sanguinis* [3,8,22-24]. In fact, subjects who consumed catechins suffer a reversible change in their salivary microbiota, under a recovery determined by the catechin dose and duration of the intervention [25].

For our study, we incorporated only the data from the first phase of the crossover study with three intervention groups [3] to avoid any carry-over effect [26]. A washout period of 15 days was used in that study, but the time for the oral microbiota recovery after the CBHE exposure is unknown. In one of the two-group crossover studies [12], the effect of the CBHE was probably underestimated, since the MS count of the control group was performed 11 days after the CBHE exposure. This underestimation does not apply to the two-group crossover study of Srikanth *et al.* [11], because the placebo rinsing was administered one week before the CBHE mouth rinsing. However, the placebo mouth rinse contained alcohol and their effects over the oral microbiome were not taken into account [27].

An acceptable clinical safety for the CBHE rinse was observed in our study, since only a nauseating event was reported by the use of the CBHE in two children after one week of the rinsing [10]. That study monitored the adverse events produced by the CBHE and chlorhexidine mouth rinses for up to 2 months. On the rest of studies that the adverse events were considered, the subjects were monitored for up to four days [11, 12]. Finally, all our results cannot be compared with prior information, since there are not reviews or meta-analyses of the effects of a CBHE on a similar or different oral clinical condition. On the other hand, the main limitation of the present study was the small number of studies included in the meta-analyses, where this situation has a potential impact on the interpretation of the results. Also, the prevention of dental caries by the CBHE rinse remains to be supported with clinical trials under study designs that include a hard endpoint, such as the caries incidence. Nevertheless, the use of the available data under appropriate statistical analyses is essential to support the growing demand of dental and public health decisions [28].

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## 5. Conclusion

Within the limitations of the available information, it may be concluded that the CBHE mouth rinse is a valid therapy for the reduction of MS in saliva and a safety intervention in children and adults, compared with a chlorhexidine or ethanol mouth rinse, as well as with their pretest condition. Nevertheless, more studies with best quality and more parameters are needed.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

Authors hereby state that there is no conflict of interest.

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