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A COMPARATIVE STUDY OF THE ANTIMICROBIAL ACTIVITY OF CACAO ETHANOL EXTRACT AND CHLORHEXIDINE DIGLUCONATE ON SALIVARY *STREPTOCOCCUS MUTANS*

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ABSTRACT

Background: Dental caries is caused by interaction between three factors; microorganisms (dental plaque), the host (teeth and saliva) and the substrate (fermentable carbohydrates). Tooth decay is highly associated with *Streptococcus mutans*. Chlorhexidine (CHX) is an effective chemotherapeutic agent in reducing *S. mutans*. Suggestion of possible natural substitutes to chlorohexidine is cacao bean ethanol extract (CBEE). There are insufficient studies on evaluating the antibacterial activities of cacao bean ethanol extract versus chlorhexidine on oral salivary *S. mutans*.

Aim of the study: The aim of the current study is to compare the antimicrobial capability of cacao bean ethanol extract versus chlorhexidine on oral *S. mutans*.

Materials and methods: Stimulated saliva was segregated from pre-school children and was evaluated microbiologically by the agar diffusion method. Saliva was cultured to isolate *S. mutans*. After incubation, the diameters of zones of inhibition were measured.

Results: The statistical analysis using paired t-test declared a significant difference ($p < 0.05$) for CBEE and CHX groups with reference to their antimicrobial power.

Conclusion: The antimicrobial effect of CHX on *S. mutans* was greater than that of CBEE.

KEYWORDS: *Streptococcus mutans*; Cacao bean ethanol extract; Chlorhexidine; Antimicrobials.

INTRODUCTION

Dental caries is considered the most popular dental disease in children [(1)]. It is an unchangeable bacterial decay of calcified tissues of the teeth, identified by demineralization of inorganic portion and disintegration of organic substance of tooth, which often induces cavitation[(2)]. *Streptococcus mutans* is a Gram positive, facultative anaerobic,

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coccus bacteria that inhabits the oral cavity[3]. It is one of the foremost dominant disease-causing organisms that plays a role in dental caries. It spreads from the mother to the child's mouth[4]. Unfortunately, a considerable share of newborns is heavily populated by *S. mutans* throughout the "window of infectivity". *S. mutans* activity in tooth decay is that, it mainly transform sucrose by using glucan sucrase enzyme to lactic acid [5]. Moreover, large amount of acid and extracellular polysaccharides are produced due to the bacterial colonization that benefit plaque formation[6].

Chlorhexidine (CHX) mouth rinse aids to minimize dental plaque and oral bacteria in the oral cavity[7]. Nevertheless, the presence of side effects such as unacceptable tooth stains, displeasing taste, dry mouth and burning sensation in the oral cavity divert some patients from utilizing it[8]. Natural products which can be easily prepared such as cacao bean ethanol extract (CBEE) has polyphenols which are occurring molecules originated in tea, chocolate and plants[9]. Polyphenols function similarly to chlorhexidine and has special therapeutic qualities with few or no negative side effects[10]. Cacao extract has antibacterial activity, antioxidant properties and anti-glucosyltransferase activity[11]. Some studies found out that when cacao extract was used as a mouthwash by children, it was very effective in decreasing *S. mutans* and contained cariostatic chemicals that can suppress dental caries and plaque buildup [12].

To our knowledge, few studies [13-15] have been escorted to differentiate the antimicrobial efficacy of chlorhexidine and cacao bean ethanol extract. Therefore, the aim of the current work is to compare the antimicrobial effects of cacao bean ethanol extract versus chlorhexidine on *S. mutans* isolated from saliva of pre-school children.

MATERIALS AND METHODS

A double-blinded, randomized controlled study to compare the effectiveness of CBEE versus CHX on salivary *S. mutans* isolated from children saliva. Ethical approval was obtained from the Unit of Research Ethics Approval Committee (UREAC), Pharos University in Alexandria (approval No. 175/27-1-2024).

Sample size estimation

A total sample size of 30 preschoolers(16) (3-6 years) divided into 2 groups (15 in each group) according to the following equation(16);

$$N = \frac{2\,(Z_{\alpha} + Z_{1-\beta} - \beta)\,2\,\sigma^{2}}{\Delta^{2}}$$

Where n is the desired sample size. For Z_{α}, Z is a constant set by convention according to the accepted α error. For $Z_{1-\beta}$, Z is a constant set by convention according to power of the study. σ is the standard deviation and Δ is the difference in effect of two interventions.

The candidates were healthy and their caregivers provided informed consent for inclusion. They had several carious teeth. The participants ceased any drug for two weeks. Furthermore, they were instructed to avoid any food intake or drinks for at least two hours prior to saliva contribution. Children who had been subjected to topical fluoride application or an antibiotic medication two weeks before the beginning of the study were prohibited from the study. Additionally, children who had been given a mouthwash 48 hours before the onset of the study were also ruled out. Moreover, any candidate with systemic diseases, mental disorders, or physical difficulties was also excluded[14].

Preparation of the cacao bean ethanol extract was attained by adding 100 g of cacao powder to

96% ethanol at a ratio 1:4[17].

Participants samples of stimulated saliva were assembled in sterilized beakers then transported to the laboratory for microbiological investigation. For each sample, an aliquot of 1 ml saliva was cultured on mitis salivarius bacitracin agar to isolate *S. mutans*. Measurement of the antimicrobial activities of CBEE and CHX (0.2%) was performed by the agar diffusion method [18, 19]. Briefly, a loopful of the pure culture of *S. mutans* was grown overnight on trypton soya broth. The culture was calibrated at 10^5 cfu/ml then a dipped swab was used to disseminate the bacterial suspension on the surface of Muller Hinton agar medium. An aliquot of 10 µl of CBEE or CHX was placed at the center of well previously punched at equidistant points in agar by means of sterile cork borer. After incubation for 24h, the diameters of zones of inhibition were measured[16, 20].

Statistical analysis

The data were subjected to paired t-test by using Microsoft Excel 2016 to estimate the p-value.

RESULTS

The diameters of the inhibition zones (Figure 1) are tabulated in Table 1. The inhibition zones of CHX ranged from 18 to 30 mm whereas, the inhibition zones of CBEE ranged from 6 to 15 mm. As shown in Table 2, the statistical analysis using paired t-test for CHX and CBEE groups with reference to their antimicrobial effectiveness against salivary *S. mutans* has revealed highly significant reduction ($p < 0.05$) in the inhibition zones of CHX followed by CBEE group.

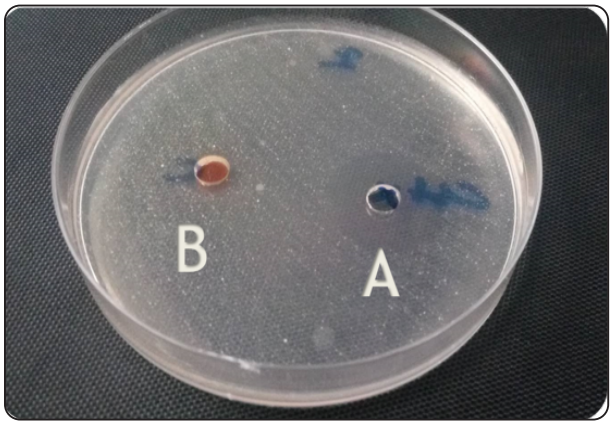

Fig. (1) The inhibition zone around a well previously filled with Chlorhexidine (A) or cacao bean ethanol extract (B) on culture medium inoculated with *S. mutans*.

TABLE (1) The diameter of inhibition zones (mm) of cacao bean ethanol extract (CBEE) and chlorhexidine (CHX) groups.

Sample number	*Inhibition zone diameter (mm)*	
	CBEE	*CHX*
1	*0*	*20*
2	*7*	*23*
3	*8*	*25*
4	*15*	*20*
5	*0*	*20*
6	*6*	*27*
7	*12*	*27*
8	*8*	*18*
9	*9*	*30*
10	*0*	*27*
11	*13*	*25*
12	*9*	*25*
13	*0*	*23*
14	*9*	*23*
15	*0*	*27*

TABLE (2) Statistical analysis using paired t-test between cacao bean ethanol extract (CBEE) and chlorhexidine (CHX) with respect to their antibacterial activity against salivary *S. mutans*.

	CBEE	CHX
Mean	6.4 24	24
Variance	27.11428571	11.57142857
Observations	15	15
Pearson Correlation		0.056455652
Hypothesized Mean		
Difference		0
Df		14
t Stat		11.25407507
P(T<=t) one-tail		1.06105×10^{-08}

DISCUSSION

Herbal medicaments have been launched as a dental replacement remedies to arrest and cure dental diseases. Utilization of such materials all over the population will be favorable even to individuals from lower socioeconomic backgrounds. These materials are reasonably priced and have demonstrated to have little negative effects [21].

Cacao bean ethanol extract has been outlined to reveal potentially beneficial biological properties on the oral microbiome and plaque biofilm, in conjunction with a direct antibacterial activity against *S. mutans*[15]. One of the constituents of cacao is polyphenols, which have a bactericidal action against *S. mutans* biofilm production and acid formation through an anti-glucosyltransferase activity[22].

S. mutans was the examined bacteria in the present study. It is a facultative anaerobic Gram positive bacteria with a cell wall composition that incorporates glycocalyx and capsules [23]. The non-complex cell wall morphology of the bacteria creates an uncomplicated pathway for CHX potent compounds to creep into the bacterial cell wall [24]. Apparently, the chief purpose of the antibacterial mechanism of action of CHX is the disruption of the cytoplasmic membrane and the membrane forming enzymes of the bacteria [25].

The results of the existing study demonstrated a significant difference in the antibacterial activity between CBEE and 0.2% CHX which was used as a positive control. The CBEE was capable of inhibiting *S. mutans* to an average of one fourth the current gold standard CHX. This was tabulated through the dissimilarity in the inhibition zone diameter in which the CBEE inhibition zones varies from 6 to 15 mm, while the CHX inhibition zones were from 18 to 30 mm.

The present study matched the same conclusion of the laboratory study of Siswanto et al.2022[13], which reported that the cacao bean husk extract has an antimicrobial effect on the growth of *Streptococcus alpha* which enfolded many bacterial strains such as *S. mutans,* however its efficacy was lower than CHX. Despite, cacao bean husk extract contains flavonoids, its activity against *Streptococcus alpha* was subordinate than CHX. They used cacao bean husk ethanol extract as a mouthwash with different concentrations 12.5%, 25%, 37.5% and 50%. Similarly, Shrimathi et al.2019[14], in an in vivo study concluded that mouth rinses acquired from cacao bean husks were efficacious in minimizing colony forming units (CFUs)/mL of *S. mutans*. The order of bacterial reduction followed the following order: the CHX group followed by the ginger group and finally the cacao husk group. Moreover, in the current study a little concentration of chlorhexidine (0.2%) generated greater inhibition of *S. mutans* compared to CBEE. This findings align with Amanda et al.2021[20], who carried out an in-vitro study using a concentration of 2% CHX and 6% cacao peel extract.

However, the results of the present study contradict with Babu et al.2011[26], who declared that children's mouthwash from ground husk of the cacao bean was as effective as CHX mouthwash

in inhibiting *S. mutans*. This contradiction may be due to the difference in the chemical constituents of CBEE which has been used in the present study and that of the cacao bean husk extract which has been used by Babu et al. 2011 as they are different parts of the plant. Rohini Dua et al.2017[27], also showed no significant difference between both CHX and cacao bean husk extract mouth rinses in terms of antimicrobial properties.

Some limitations have been identified in the current study including encouraging a larger sample size. Further studies are also demanded to assess results on a longer scale and effectiveness in a wider scope of age groups. It is also recommended to study the efficacy of other concentrations higher than the concentration that has been explored in this investigation.

CONCLUSION

According to the previous results, both CHX and CBEE have significant outcome on *S. mutans*. However, the antimicrobial effect of CHX had a higher significant reduction in the inhibition zone than CBEE.

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