ORIGINAL RESEARCH

Evaluation of Antiplaque and Antimicrobial Activity of Cocoa Bean Extract: An *In Vivo* Study

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Abstract

Aim and objective: To develop experimental mouthwash, cocoa extract added with honey and assessed antiplaque and antimicrobial activities.

Materials and methods: A mouthwash was formulated from aqueous extracts of cocoa and honey. Sixty children aged 9–13 years participated in the study and were equally divided into two groups. Group I children were asked to rinse with 10 mL of cocoa with honey mouthwash and group II with 0.2% of chlorhexidine mouthwash for 21 days. Gingival index (GI), plaque index (PI), *Streptococcus mutans* (SM), and *Lactobacilli* (LB) counts were assessed of both groups at baseline, 14th day, and 21st day. Data were subjected to statistical analysis.

Results: Significant decreases in both gingival and plaque indices from baseline to 14th day and further up to 21st day ($p < 0.001$) were seen in both groups. The microbiological analysis revealed a significant reduction of SM and LB counts in both groups from baseline up to 21st day. However, no statistically significant differences were seen in percentage reductions of SM and LB counts between the two groups. When subjective and objective criteria were assessed, the majority of the children found the experimental mouthwash acceptable in taste and free of side effects.

Conclusion: Cocoa mouthwash with honey demonstrated effective antiplaque, anti-inflammatory, and antimicrobial properties comparable with 0.2% chlorhexidine digluconate.

Clinical significance: Cost-effective and easily available herbs as an adjuvant to oral hygiene maintenance may have a far-reaching effect on the prevention as well as the prevalence of oral diseases. Our study indicated that cocoa with honey mouthwash can be used as a suitable alternative to chlorhexidine mouthwash in children, as an adjunct in their regular oral hygiene maintenance.

Keywords: Antimicrobial properties, Antiplaque, Cocoa extracts.

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Introduction

The most common illnesses among the oral diseases of mankind are dental caries and disease of the periodontium.[1] Cavities and gingivitis are caused when teeth and their supporting structures are exposed to infection by *Streptococcus* bacteria.[2] Paramount factor in the initiation and progression of gingival and periodontal diseases is dental plaque and it has been proved by extensive research.[3] A direct relationship has been established between the severity of gingivitis and plaque levels. Regular, effective removal of plaque by the personal oral hygiene protocol is the highest rationale methodology toward the prevention of periodontal diseases.[4]

The most fundamental type of dental care initiates at home. In maintaining healthy teeth and gums, daily oral hygiene plays a vital role. A combination of toothbrushing, flossing, and use of a suitable mouthwash is the ideal personal oral hygiene regime. Yet, regardless of socioeconomic status, the degree of motivation and dexterity required for an optimal oral hygiene level may be beyond the capability of the majority of the patient.[5] This is especially true in children, the majority of whom lack sufficient manual dexterity for effective toothbrushing till the early teenage. From this point of view, the use of antimicrobial mouth rinses has been considered a useful adjunct to oral hygiene.[6]

Several chemical agents have been assessed over the years with respect to their antimicrobial effects in the oral cavity; among the chemical agents, the bis-biguanides constitute an important group. However, all are connected with consequences that prohibit routine long-standing usage.[2] The quest for a substitute product remains and natural phytochemicals isolated from plant products used in traditional medicine are now contemplated as a good substitute to synthetic chemicals,[7] also which are safer, biodegradable, and have fewer side effects. Hence, newer agents that are effective, safe, and economical need to be developed.

Cocoa production generates substantial quantities of waste. Cocoa bean husk is a processing by-product generated in the chocolate industry. It has been shown to possess two types of cariostatic substances, one exhibiting antibacterial activities by its unsaturated fatty acids and the other its anti-glucosyl transferases activities by its epicatechin polymers.[8] In our study, since cocoa is very bitter, honey was added as a sweetener to increase patient compliance.

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Since ancient times, honey has been used as a source of nutrients as well as a medicine.[9] Honey is an effective broad-spectrum antibacterial agent.[10] Its antibacterial action is attributed to the presence of inhibitory factors such as flavonoids,[11] hydrogen peroxide,[12,13] low pH, and high osmolarity[14] due to its sugar concentration. These features play a major role in controlling inflammation and stimulating microbial control and healing processes.[9]

In our study, we sought to estimate the antiplaque and antimicrobial effect of the cocoa extract in the form of mouthwash in a group of school-going children. We also sought to assess the subjective and objective criteria regarding acceptability and unwanted side effects of the mouthwash.

Materials and Methods

Sixty children between the age groups of 9 years and 13 years of both genders were selected from Sri Swami Sadananda Saraswati Vidyalaya, Mangaluru. Children with poor to fair oral hygiene, mild to moderate gingival inflammation, and DMFT/dft >3 were chosen for the study. This study is a single-blinded study with the participants being unaware of their grouping.

Ethical clearance was obtained from the ethical committee of the institution. Informed consent was acquired from the parents before the study. The study was conducted in accordance with the Declaration of Helsinki.

Preparation of the Mouthwash

A byproduct of cocoa manufacture, the ground husk of cocoa beans (1 kg), was obtained from CAMPCO factory, Puttur, Dakshina Karnataka. The mouthwash was prepared in the Nitte Gulabi Shetty Memorial Institute of Pharmaceutical Sciences. Cocoa bean husks were then treated with 5 g of cellulose in 4.75 L of distilled water at 50°C for 4 hours. This mixture was refluxed for 1 hour after adding ethanol up to 50% (v/v final concentration). Ethanol was removed by evaporation after filtration and the aqueous solution lyophilized to generate a powder. The yield of processed extract was analyzed. The powder was then liquefied in distilled water to obtain a mouth rinse with a definitive concentration of 1 mg/mL in 0.1%[15] which was carried out at NGSM Institute of Pharmaceutical Sciences, Mangaluru. Formulation of the mouthwash included cocoa extract, natural honey as sweetener, and compound sodium chloride mouthwash.

Characterization of the Mouthwashes

Once the mouth rinse was developed, the prepared formulations were placed in the clear glass vials and checked for the presence of any particulate matter or fiber by placing against the black and white background.[16]

The pH was noted using a pen pH meter (Model pH Tester 10) by making the electrode contact the surface of the formulation and permitting it to equilibrate for 1 minute and reading was noted down.[17]

The viscosity of the mouthwash was recorded by using Brookfield Viscometer (Model DV-II plus Pro) with spindle no 61. The viscosity was measured using 25 mL of mouthwash filled in 50 mL of the clean glass beaker. The spindle was dropped perpendicular in the center and care was taken that it does not touch the base of the beaker. The features such as sample size, temperature, and pressure, which disturb the viscosity, were maintained. The viscosity measurement was done at room temperature and rotating the spindle at 100 rpm.[18]

According to modified ICH guidelines,[19–21] the stability findings were executed for all the preparations. Preparations were stored at 25 ± 2°C and 60 ± 5% RH using a stability chamber (Lab top instruments) and at 4 ± 2°C in a refrigerator (Whirlpool, India), and they were estimated periodically for 12 weeks. Their physical stability and appearance were examined for a period of 3 months at 1-month interval. As per ICH guidelines, the parameters like clarity, pH, and viscosity were evaluated at the end of every month for a period of 3 months.

Commercially obtainable 0.2% chlorhexidine gluconate mouthwash (chlorhexidine mouthwash) was used in our study.

All children were subjected to thorough oral prophylaxis. After a period of 2 weeks, a single trained and calibrated investigator recorded the caries experience of the children using the DMFT/dft indices. Turesky–Gilmore–Glickman modification of the Quigley–Hein plaque index (PI)[22] and Loe and Silness gingival index (GI)[23] were used to measure and record the plaque and gingival scores. Examination of children was done seated on an ordinary chair, under good illumination, either natural light or hand torch, using a sterile mouth mirror and CPI probe while taking protective cross infection control measures using disposable gloves and masks. Collection of salivary samples[24] and estimation of *Streptococcus mutans* (SM),[25,26] and *Lactobacilli* (LB)[27] colony count were done using unstimulated saliva, by means of sterile collection bottles, sealed and transported immediately to the laboratory. All investigations were accomplished by the same examiner.

The children were now randomly divided into two groups:

- Group I: 30 children given cocoa mouthwash with honey.
- Group II: 30 children given chlorhexidine mouthwash (0.2%).

Children were asked to rinse their mouths with 10 mL of the mouthwash dispensed into a disposable cup for 30 seconds, once daily, 1 hour after brushing, in the morning, for 3 weeks. Ten milliliters of mouthwash were dispensed by the investigators daily to each of the participants and mouth rinsing was done under supervision during school hours.

Plaque and gingival indices were recorded at baseline and again at the end of 14 and 21 days, SM and LB colony count were also similarly assessed. During the period of study, children followed their everyday oral hygiene habits and were asked to abstain from using commercial mouth rinses and notify if they initiated any antibiotic or anti-inflammatory drug therapy.[4] Subjective and objective criteria regarding the acceptability and unwanted side effects of the cocoa mouthwash were assessed on the 14th and 21st day.[4]

Statistical Analysis

To calculate mean scores of gingival and plaque indices of all groups at different time periods, mean and standard deviations were used. To assess the significance of changes in both indices and LB counts within each group between the different time periods (intragroup comparison), paired *t*-tests were used. Critical *p* values of significance were set at 0.05 and a confidence of 95%. Changes in salivary SM counts from baseline to the different time periods (intragroup comparison) were analyzed by the Wilcoxon signed-rank test. ANOVA tests were used to ascertain significant

differences among the percentage reduction of the indices and microbial counts of the study groups (intergroup comparison). Statistical analysis was done using SPSS version 20.0.

Results

The clarity of the mouthwash and the values obtained for pH and viscosity at the end of the 1st month were seen to be maintained at the end of 3 months and there were no significant differences seen in the clarity, pH, and viscosity in the cocoa with honey mouthwash even after the addition of honey (Table 1).

Table 2 shows the mean scores of gingival and plaque indices at different time intervals in both groups. In group I (cocoa with honey mouthwash), the GI decreased from 2.23 ± 0.629 (baseline) to 1.09 ± 0.41 (14th day) and further to 1.1 ± 0.510 on the 21st day (Table 2). The PI decreased from 3.55 ± 1.35 (baseline) to 1.40 ± 0.54 (14th day) and showed no further decrease till the 21st day. In group II (0.2% chlorhexidine), the GI decreased from 2.42 ± 064 (baseline) to 1.123 ± 0.35 (14th day) and further to 1.1 ± 0.51 (21st day). The PI decreased from 4.2 ± 1.76 (baseline) to 1.70 ± 0.94 (14th day) and remained the same till the 21st day (Table 2). These changes in the GI and PI scores in both groups were found to be statistically significant ($p < 0.001$). However, when the changes in gingival and plaque indices from baseline up to 14th and 21st day were compared between group I and II, we observed no statistically significant differences.

At the baseline, up to 73.3% of the children in group I mouthwash exhibited moderate counts of SM while 26.6% exhibited low counts (Table 3). On the 14th day, 86.6% of children showed low counts of SM while 13.3% showed moderate counts. Further, at the end of 21 days up to 93.3% of children showed low counts of SM while 6.6% showed moderate counts. These changes were observed to be statistically significant. In group II, 80% of the children exhibited moderate counts of SM while 16.6% exhibited low counts and only 3.3% exhibited high counts. On the 14th day, 80% of children showed low counts of SM while 20% showed moderate counts and none showed high counts. However, at the end of 21 days, 93.3% of children showed low counts of SM while only 6.6% showed moderate counts and none showed high counts. These changes were observed to be statistically significant.

When LB counts were assessed in group I, at the baseline, 80% of the children exhibited levels of <100,000 CFU/mL (moderate caries activity) while 16.7% showed levels of <10,000 CFU/mL (slight caries activity) (Table 4). At the end of the 14th day, up to 63% showed levels of >10,000 CFU/mL (slight caries activity) while 36.7% showed levels of 0 to 1,000 CFU/mL (light or no activity). At the end of 21 days, all children in group I showed levels of 0 to 1,000 CFU/mL. Meanwhile in group II 80% of the children exhibited levels of <100,000 CFU/mL (moderate caries activity) while 20% showed levels of <10,000 CFU/mL (slight caries activity) at baseline. At the end of the 14th day, 66.7% showed levels of 0 to 1,000 CFU/mL (light/no caries activity) while 33% showed levels of >10,000 CFU/mL. At the end of 21 days, all children showed levels of 0 to 1,000 CFU/mL.

No statistically significant differences in the percentage reductions of SM and LB counts were seen between group I and group II at any of the time intervals ($p > 0.5$) (Table 5).

In our study, we found that cocoa mouthwash with honey was acceptable in taste and biocompatible in the majority of the children. The subjective criteria scored by the children revealed that bitter taste was experienced by 5 out of 30 children using cocoa with honey mouthwash (Table 6). Objective criteria revealed staining of teeth in five children in the chlorhexidine group (Table 7). Hence, the results of our study show that cocoa with honey mouthwash was acceptable in taste in most children and free of side effects such as staining of teeth, burning sensation, and allergy.

Discussion

To control oral microorganisms and to affect plaque formation, a wide range of chemotherapeutic agents have been studied for their capability.[28] After a 40-year history of chlorhexidine (CHX) digluconate in dental medicine,[22] it has been considered as a "gold" standard in dentistry for the inhibition of plaque and gingivitis, and against which other antiplaque agents are measured. Huge declines were found in plaque formation using chlorhexidine gluconate, applied topically, or as a mouth rinse.[16,29–33] Unfortunately, studies

Table 1: Changes in the clarity, pH, and viscosity of cocoa with honey mouthwash at different time intervals

	1st month		*2nd month*		*3rd month*	
	Cocoa mouthwash	*Cocoa with honey mouthwash*	*Cocoa mouthwash*	*Cocoa with honey mouthwash*	*Cocoa mouthwash*	*Cocoa with honey mouthwash*
Clarity	Clear	Clear	Clear	Clear	Clear	Clear
pH	9.44 ± 0.005	9.45 ± 0.002	9.45 ± 0.009	9.46 ± 0.003	9.44 ± 0.005	9.49 ± 0.005
Viscosity	5.37 ± 0.002	6.45 ± 0.003	5.39 ± 0.006	6.48 ± 0.004	5.45 ± 0.003	6.42 ± 0.003

Table 2: Mean scores of gingival index and plaque index at different time intervals in group I and group II

		Gingival index				*Plaque index*			
	Interval	*Mean ± SD*	*Diff from baseline*	*p value*	*T value*	*Mean ± SD*	*Diff from baseline*	*p value*	*T value*
Group I	Baseline	2.23 ± 0.62	–			3.55 ± 1.35	–		
	14th day	1.09 ± 0.51	1.14	<0.001	13.99	1.40 ± 0.54	2.15	<0.001	12.13
	21st day	1.1 ± 0.51	1.07	<0.0 01	6.9	1.40 ± 0.54	2.15	<0.001	12.13
Group II	Baseline	2.42 ± 0.64	–	–	–	4.2 ± 1.76	–		
	14th day	1.123 ± 0.35	1.3	<0.001	16.14	1.70 ± 94	2.5	<0.001	11.62
	21st day	1.16 ± 0.51	1.26	<0.001	8.93	1.70 ± 94	2.5	<0.001	11.62

$p > 0.05$ not significant

Table 3: Changes in *S. mutans* count at baseline, 14th day, and 21st day in group I and group II

		Low		*Moderate*		*High*	
Examination interval	*Interval*	*Number of children*	*Percentage*	*Number of children*	*Percentage*	*Number of children*	*Percentage*
Group I (30)	Baseline	8	26.6	22	73.3	0	0
	14th day	26	86.6	4	13.3	0	0
	21st day	28	93.3	2	6.6	0	0
Group II (30)	Baseline	5	16.6	24	80	1	3.3
	14th day	24	80	6	20	0	0
	21st day	28	93.3	2	6.6	0	0

Table 4: Changes in *Lactobacilli* count of group I and group II on the baseline, 14th day, and 21st day

Interval group I	*No. of Lactobacilli per mL saliva*	*Frequency*	*Percentage*	*Interval group II*	*No. of Lactobacilli per mL saliva*	*Frequency*	*Percentage*
Baseline	0–1,000			Baseline	0–1,000		
	>1,000				>1,000		20
	<10,000	5	80		<10,000	24	80
	<100,000	25	20		<100,000	6	
14th day	0–1,000	11	36.7	14th day	0–1,000	20	66.7
	>1,000	19	63.3		>1,000	10	33.3
	<10,000				<10,000		
	<100,000				<100,000		
21st day	0–1,000	30	100	21st day	0–1,000	30	100
	>1,000				>1,000		
	<10,000				<10,000		
	<100,000				<100,000		

Table 5: Intergroup comparison of percentage reduction% of *Streptococcus mutans* and *Lactobacilli* counts in group I and group II

	Streptococcus mutans		*Lactobacilli*	
	0–14 days	*0–21 days*	*0–14 days*	*0–21 days*
Group I	92.700	77.4000	88.8000	99.5667
Group II	81.9310	42.9000	91.5000	99.5667
Difference between groups				
p value	0.061	0.071	0.059	0.068
T value	1.314	0.095	1.029	0.937

exhibited that these positive effects were supplemented by side effects, the most upsetting being extrinsic tooth staining[34] and others such as disagreeable taste and burning sensation.[28] Hence, the hunt for products that can substitute continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good replacements to synthetic chemicals.[8]

The purpose of this study was to evaluate the effect of cocoa with honey mouthwash mouth wash on gingival inflammation, antiplaque activity, and on the levels of salivary SM and *Lactobacillus* in a group of children. Chlorhexidine digluconate was taken as a benchmark control in our study in a concentration of 0.2% since it was the most commonly prescribed concentration.[35–38]

The cocoa mouthwash formulation was found to be clear without any fibrous matter or clouding (Table 1). pH of the formulation was found to be >8.9 and <9.35 which indicates uniform pH without any significant deviation in pH. Viscosity ranged from 4.1 to 5.25. The addition of honey to the mouthwash increased the viscosity slightly, however, this was not a significant change. The stability study has been carried out as per ICH guidelines.[19–21]

Our study revealed a statistically significant decrease in both gingival and plaque indices from the baseline to the 14th day and further until the 21st day in both the groups ($p < 0.001$) (Table 2). However, the mean scores of the gingival and plaque indices on the 14th day and 21st day in both groups remained the same. Therefore, between the 14th and 21st day, no significant differences were seen in both indices among both groups. From this, we infer that both types of mouthwash had significant but comparable antiplaque and anti-inflammatory properties up to the 21st-day post rinse.

A previous study[39] found cocoa bean husk extract extremely effective in decreasing plaque accumulation and mutans streptococci count when it was used as a mouth rinse by children. These findings are consistent with the results of our study.

The probable shielding effect of cocoa on dental caries is getting growing attention. The study of Kashket et al.[40] displayed the inhibitory effects of cocoa on plaque accumulation and caries formation were due to inhibition of bacterial polysaccharide production. Matsumoto et al.[41] and Osawa et al.[42] have also reported the cariostatic effects of cocoa bean husk due to glycosyltransferase enzyme inhibition, proposing that such inhibition could be caused by high molecular weight polyphenols, definitely by polymeric epicatechins with C-43 and C-8 intermolecular bonds. Another probable cause for the inhibition of plaque deposition was the reduction of the hydrophobicity on the cell surface of SM caused by polyphenols. Activity against SM due to the fatty acids contained in cocoa bean husk has also been proposed, mainly due to oleic and linoleic acids.

Table 6: Subjective criteria

Subjective criteria	*Taste acceptability*			*Burning*		*Dryness/soreness*	
	Acceptable	*Tolerable*	*Unacceptable*	*Present*	*Absent*	*Present*	*Absent*
Group I, *N*: 30	23	2	5	0	30	0	30
Group II, *N*: 30	30	0	0	0	30	0	30

Table 7: Objective criteria

Objective criteria	*Ulcer formation*		*Staining of teeth*		*Staining of tongue*		*Allergy*	
	Present	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*
Group I, *N*: 30	0		0		0		0	
Group II, *N*: 30	0		5		0		0	

Streptococcus mutans and LB species are the most common bacteria correlated with plaque formation.[43,44] In our study when SM counts were analyzed in both the groups, we found a statistically significant reduction in the bacterial counts from the baseline until the 21st-day post rinse [($p < 0.001$), Table 3]. These outcomes were similar to the results of a study done by Venkatesh Babu et al.[28] where the microbial count of SM in saliva was found to be reduced considerably.

When LB counts were assessed in group I and group II, we observed a steady decrease from the baseline to the 14th day and further up to the 21st day (Table 4). At the end of the intervention, we observed that all children exhibited 0 to 1,000 CFU of LB, which indicates light or no caries activity. No studies so far, have evaluated the effect of cocoa mouthwash on LB counts in saliva.

In the chlorhexidine mouthwash group, a statistically significant decrease in SM counts was detected (Table 3). Mali et al. found consistent results in their study where the microbial count of SM was found to have reduced considerably.[4]

No significant differences were observed between group I and group II at any of the time intervals when intergroup comparisons of the percentage reductions of SM and LB counts were made (Table 5). Therefore, we infer that microbiologically two types of mouthwash were equally effective and have highly significant antibacterial activity on SM and LB. The microbiological valuation strongly supports the ability of the experimental mouthwash.

In our study, due to the bitter taste associated with both cocoa, honey was added as a sweetener to increase patient compliance and acceptability. We assume that honey has been an additional factor for the efficacy of the antimicrobial activity of the cocoa mouthwash as honey has potent antibacterial activity[9,45,46] effective against a very broad spectrum of species, and to have antifungal[47,48] properties as well.

We found that, at the beginning of the study, due to the bitterness of the mouthwashes, children were initially reluctant to participate in the study. After an interactive session with the children and after the promise of material incentives, they willingly participated in the study. Three children dropped out of the study after a few days due to non-compliance and hence were excluded from the study.

In our study, we found that cocoa mouthwash with honey was acceptable in taste and biocompatible in the majority of the children (Tables 6 and 7). The subjective criteria scored by the children revealed that the bitter taste was experienced by 5 out of 30 children using a cocoa mouthwash with honey. Objective criteria revealed staining of teeth in five children in the chlorhexidine group.

However, continued research on the safety and efficacy of natural, non-toxic plant products on improving children's oral health is the need of the hour for their better economic and therapeutic utilization. Studies on the antiplaque and antimicrobial effects on cocoa are scarce and hence more studies with larger sample size needs to be planned to evaluate its efficacy, dosage, toxicity, exact concentrations, formulas for the patient recommendation, and lasting efficiency.

Clinical Significance

Currently, available mouth rinses in the market are chemically based, expensive, and have side effects, which limits their use, especially in India. Cost-effective and easily available herbs as an adjuvant to oral hygiene maintenance may have a far-reaching effect on the prevention as well as the prevalence of oral diseases. Our study indicated that cocoa with honey mouthwash can be used as a suitable alternative to chlorhexidine mouthwash in children, as an adjunct in their regular oral hygiene maintenance.

Conclusion

Cocoa with honey mouthwash is stable under permissible conditions and demonstrated effective antiplaque, and antimicrobial properties comparable with 0.2% chlorhexidine digluconate. Hence, cocoa mouthwash with honey is recommended in children as a substitute for chlorhexidine mouthwash.

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