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Health Impact of Chocolates on Dental caries

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ABSTRACT

Dental caries is the most common among the spectrum of oral diseases and are still continuing to be a major public health issue in developed as well as in the developing countries, affecting 60%-90% of children and adults worldwide. *Streptococcus mutans* is major organism responsible for dental caries. The research aimed to investigate the antibacterial effectiveness of chocolates with varying cocoa contents against *Streptococcus mutans*, and it also involved investigating the correlation between chocolate consumption with different cocoa contents and the occurrence of caries. The study was conducted during July 2023 to April 2024 at Sathyabama Dental college and hospital. The antibacterial activity of the chocolates was assessed using the agar well diffusion method, with chlorhexidine and distilled water serving as positive and negative controls, respectively. A questionnaire was designed for the online survey. Inclusion criteria of the survey study were between 18-60 years who consumed chocolates. Demographic parameters such as age and sex of the participants were considered for this study. Online survey was conducted from 403 peoples. One way ANOVA post hoc Duncan test was used to determine the significance of different cocoa content chocolates for the antibacterial activity. Paired t test and Pearson correlation was used to analyze the online survey study. P-value less than 0.05 was considered as a statistically significant. The zone of inhibition for chocolates with different cocoa contents followed the order: up to 100% (12.7±0.4) > 80% (12±0.6) > 60% (11.33±0.3) > 40% (10.86±0.3) > 20% (9.96±0.5) > 0% (no zone). Among the chocolates, those with higher cocoa content exhibited greater antibacterial effects against *Streptococcus mutans*, the primary organism responsible for dental caries. According to the online survey findings, individuals consuming dark chocolate or chocolates with cocoa content exceeding 60% had a lower incidence of dental caries. The study concluded that the antibacterial activity against caries-causing organisms depended on the cocoa content, with high cocoa content chocolates displaying significant antibacterial properties. The survey results indicated that individuals consuming chocolates with higher cocoa content were associated with a lower prevalence of dental caries. Both aspects of the study emphasized that an increase in cocoa content in chocolates correlated with a reduced risk of dental caries.

Keywords: *Streptococcus mutans, Dental caries, Cocoa, Antibacterial activity*

1. INTRODUCTION

Dental caries is the most common among the spectrum of oral diseases and are still continuing to be a major public health issue in developed as well as in the developing countries, affecting 60%-90% of children and adults worldwide (Hiremath et al., 2016). It is a chronic bacterial disease that cause the decalcification of enamel due to acid produced by the bacteria (Roberts et al., 2022). Streptococcus mutans is major organism responsible for dental caries. S. mutans attaches to the tooth surface and causes an acidic environment around the tooth by breaking down the sugars into lactic acid. Thus, demineralization of the enamel occurs leading to dental caries. Bacteria in the dental plaque and sugar containing diet are

the factors for dental caries (Lemos et al., 2019). Dental caries may be characterized by tooth pain, difficulty in chewing food, inflammation of the tissues around the tooth, abscess formation, problem with smiling and communication due to damaged teeth (Khan et al., 2024).

A common misconception of chocolates is that chocolates is one of the main reasons for dental caries as being one of the junk foods. Exceptional statement is that dark chocolate prevents caries and harden the tooth enamel due the presence of theobromine in cocoa. Theobromine is a compound present in cocoa which is a water-insoluble crystalline bitter powder and is found in high concentration in dark chocolates than milk chocolates (Lakshmi et al., 2019). However, not all kinds of chocolate are tooth friendly but the cocoa content in it make the oral health benefits (Nimbulkar et al., 2020). Chocolates high in sugar content causes tooth decay (Sheiham, 1983).

Cocoa beans consist of certain phytochemicals such as tannins, polyphenols and flavonoids which are antioxidant. Tannins are responsible for the dark color of the chocolates and bitter taste which helps to prevent dental caries by inhibiting the bacteria that deteriorates the teeth (Sánchez et al., 2023). Polyphenols have the ability to neutralize microorganisms that cause bad breath and prevent from turning sugar into acids by bacteria. Flavonoids help to reduce tooth decay (Sims et al., 2020). Compounds in cocoa possessed antibacterial property and fight against plaque (Lakshmi et al., 2019). There is a myth that chocolate causes caries. This study was carried out to verify the extend of correctness of the statement. Therefore, the present study is to investigate the antibacterial effectiveness of chocolates with varying cocoa contents against cariogenic bacteria, Streptococcus mutans and it also involved investigating the correlation between chocolate consumption with different cocoa contents and the occurrence of caries.

2. MATERIALS AND METHODS

The antibacterial activity was assessed and online survey was conducted from 403 peoples and the correlation was analyzed. It was conducted at Sathyabama Dental College and Hospital during the month of July 2023 to April 2024. The study was approved by Human Ethical Clearance Committee (Ref: 290/IRB-IBSEC/SIST Dated 1st March 2023). All the participant's consent was taken for the survey study.

Inoculum preparation

Streptococcus mutans (MTCC 497) culture was used for the experiment for the antibacterial sensitivity test. The bacterial culture from Mutan Sangius (MS) agar plate was inoculated in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hrs anaerobically. The turbidity was adjusted to 0.5 McFarland standard of 10^6 CFU/ml (Wassel and Khattab, 2017).

Antibacterial Activity Assay

The antibacterial activity of different cocoa content chocolates (0%, 20%, 40%, 60%, 80%, up to 100%) was performed by agar well diffusion method. To the freshly prepared autoclaved Mueller Hinton agar, 5% blood was added and poured into the sterile petri plates. The plates were allowed to cool and solidify. Lawn culture of *S. mutans* bacterial inoculum was made on individual Mueller Hinton Blood agar plates. Using a gel puncture, wells of 8mm diameters were created on the plates. The chocolates used in the study were heated, melted in sterile test tubes and then loaded to the corresponding wells. 0.2% Chlorhexidine and distilled water were used as positive and negative controls respectively. The plates were incubated at 37°C for 24-48hrs anaerobically. After 24 hours of incubation, the petri plates were observed for zone of inhibition and diameters were measured. The procedure was repeated in triplicates (Dakshinamoorthy *et al.* 2016).

Online Survey Study

This study was conducted based on the consumption of different cocoa content chocolates and its prevalence on caries by providing online google form questionnaire. Inclusion criteria of the study were between 18-60 years who consumed chocolates whereas exclusion criteria were below 18 years and those who don't consume chocolates. Online Survey questionnaire in (Fig.1.) was prepared based on the relationship between chocolate consumption and its impact on dental caries. The questions were about the frequency of chocolate consumption, cocoa content in the chocolate, awareness about high cocoa content prevents caries and whether they have/had caries. Online survey was conducted from 403 peoples. Pearson correlation and paired t- test were used to analysed the data using SPSS software.

Online Survey Questionnaire

Survey on Chocolate Eating Habit and its Prevalence on Dental Caries

Are you consuming chocolates?

○Yes

○No

Name _____

Email ID _____

Age _____

Gender: Male/Female/Other

I hereby inform that I'm willing to participate in the survey entitled "Survey on Chocolate Eating Habit and its Prevalence on Dental caries"

○Yes

○No

Frequency of consuming chocolate

○ Less than 4 days a week

○ 4 days a week

○ 5 days a week

○ Everyday

Cocoa content in the chocolate you consume

○ 0%

○ 20 %

○ 40 %

○ 60 %

○ 80 %

○ Upto 100 %

○ Not aware

Do you know Cocoa present in chocolate prevents Dental caries?

○Yes

○No

Do you have/had Dental Caries?

○Yes

○No

Your consent to share your inputs for our survey study

○Yes

○No

(Fig.1.): Online survey questionnaire on chocolate eating habits and its prevalence on dental caries.

Statistical Analysis

Data were analyzed using IBM SPSS version 25. One way ANOVA post hoc Duncan test was used to determine the significance of different cocoa content chocolates. Paired t-test and Pearson correlation was used to analyze the survey study. P-value less than 0.05 was considered as a statistically significant.

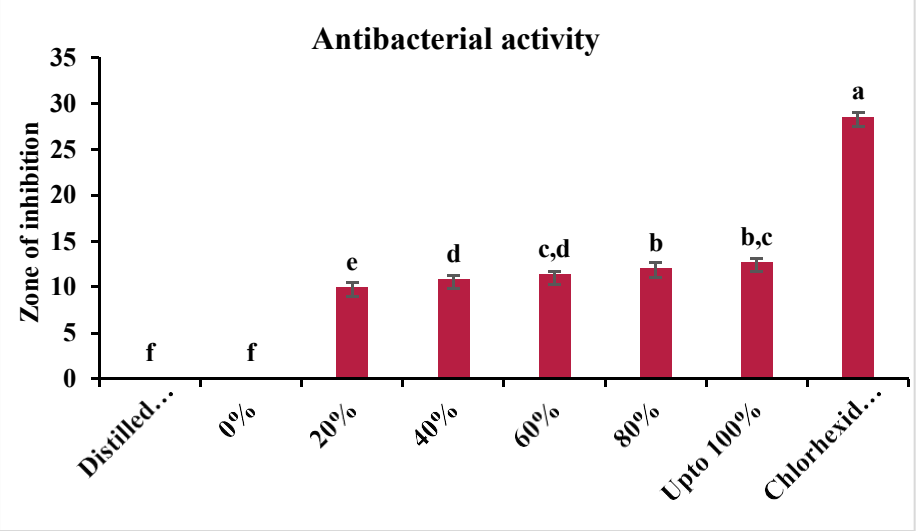
3. RESULTS

Antibacterial Activity Assay

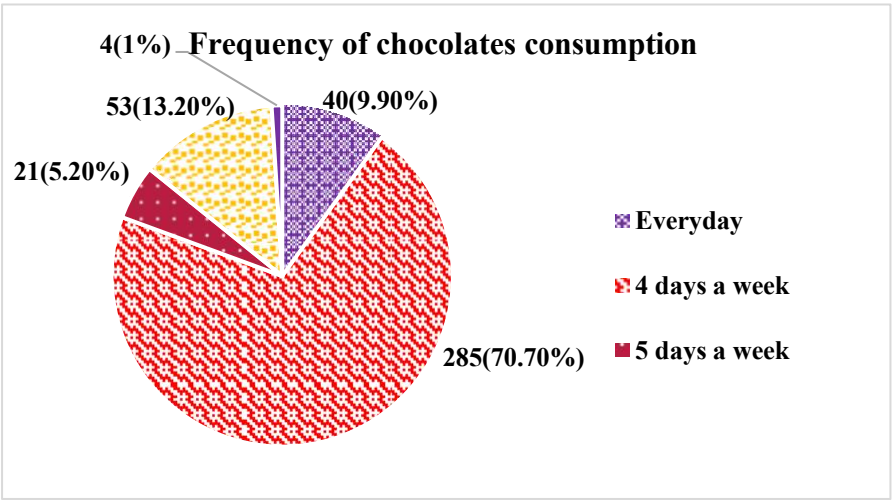
Chocolates of different cocoa content (0%, 20%, 40%, 60%, 80%, up to 100%) were used in the study to demonstrate the antibacterial activity against the dental caries causing organism viz. *S. mutans.* Chocolates of different cocoa content such as 20%, 40%, 60%, 80%, up to 100% exhibited the antibacterial activity against *S. mutans* except 0% cocoa content. The zone of inhibition of different cocoa content chocolates and chlorhexidine against S. mutans were expressed in mean±SD which was shown in (Table 1.). Among the chocolates, those with higher cocoa content exhibited greater antibacterial effects than lesser cocoa content against S. mutans (Fig.2.).

Chocolates with different cocoa content	Zone of inhibition (in mm) against *S. mutans*
0% cocoa content	0.00 ± 0.00^{f}
20% cocoa content	9.97 ± 0.50^{e}
40% cocoa content	10.87 ± 0.35^{d}
60% cocoa content	$11.33\pm0.35^{c,d}$
80% cocoa content	12.00 ± 0.66^{b}
Upto 100% cocoa content	$12.70\pm0.40^{b,c}$
Chlorhexidine	28.50 ± 0.50^{a}
Distilled water	0.00 ± 0.00^{f}

(Table 1.): Antibacterial activity of different cocoa content chocolates and chlorhexidine against *S. mutans.* N=3. Mean±SD. Different letter denotes significantly different according to Duncan's test ($p<0.05$).



(Fig.2.): Comparison of antibacterial activity of different cocoa content chocolates as well as chlorhexidine against *S. mutans.* N=3. Mean±SD. Different letter denotes significantly different according to Duncan's test ($p<0.05$)



(Fig.3.): Frequency of chocolates consumption.

Online Survey study

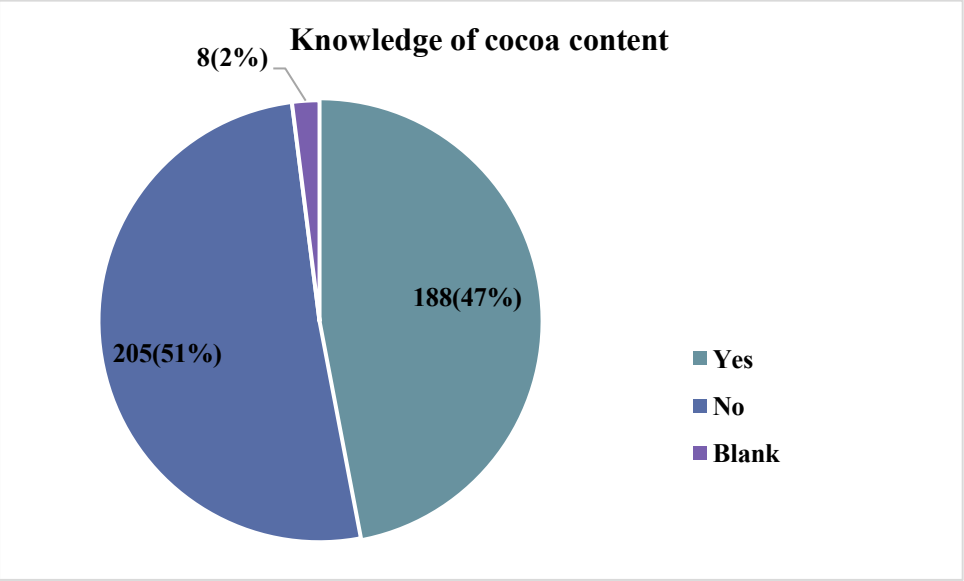
Online Survey was conducted based on the consumption of different cocoa content chocolates and its prevalence on caries. Total number of participants in the survey was 403 of which 276 (68.5%) were female participants and 127 (31.5%) were male participants. Frequency of chocolate consumption was depicted in (Fig.3.). 40 (9.9%) of the participants consumed chocolates everyday, 285 (70.7%) consumed four days a week, 21 (5.2%) consumed five days a week,53 (13.2 %) consumed less than four days a week and 4 (1%) of participants were not responded. No. of participants those who have / not have dental caries based on the different cocoa content in the chocolates were shown in (Table 2). All the 6 (1.5%) participants consuming 0% cocoa content chocolates found to have dental caries (1.5%). Out of 73 (18.1%) participants consuming 20% cocoa content chocolates, 71 (17.6%) found to have dental caries and 1 (0.2%) found dental caries. Of 54 (13.4%) participants consuming 40% cocoa content chocolates, 30 (7.4%) found to have dental caries and 23 (5.7%) were found no sign of dental caries. Of 64 (15.9%) of participants consuming 60% cocoa content chocolates, 11 (2.7%) found to have dental caries and 53 (13.2%) found no sign of dental caries. Of 29 (7.2%) participants consuming up to 80% cocoa content chocolates, 1 (0.2%) found to have dental caries and 27 (6.7%) found no sign of dental caries. Of 27 (6.7%) participants, none of the participants (0%) found to have dental caries and 27 (6.7%) found no sign of dental caries. Of 150 (37.2%) participants who were not aware of cocoa content of the chocolates, 82 (20.3%) found to have dental caries and 67 (16.2%) found no sign of dental caries. Comparison of dental caries and no sign of dental caries based on percentage of cocoa content in the chocolates as shown in (Table 3). Presence and absence of dental caries were found to be more or less similar to the people who consumed less than 60% of the cocoa content. Interestingly, no. of people with caries and no. of people without dental caries were found to be significantly different ($p>0.05$) from the people who consumed more than 60% of the cocoa content. Percentage of the knowledge about cocoa content to the participants was shown in (Fig.4.). 205 (51%) of the participants were found to have no knowledge about the cocoa content of the chocolates and 188 (47%) of them were found to have a knowledge about the cocoa content of the chocolates. However, 8 (2%) of the participants were not responded. Thus, from the survey we found that awareness about cocoa content to the participants was less and people consumed cocoa content above 60%do not have caries. Correlation analysis of Cocoa content of chocolates and its impact on dental caries was shown in (Table 4).

Cocoa content	**No. of people consuming chocolates**	**Dental caries**	**No sign of dental caries**	**Blank (participants who were not responded)**
0%	6 (1.5%)	6 (1.5%)	0 (0%)	4 (1%)
20 %	73 (18.1%)	71 (17.6%)	1 (0.2%)	
40 %	54 (13.4%)	30 (7.4%)	23 (5.7%)	
60%	64 (15.9%)	11 (2.7%)	53 (13.2%)	
80%	29 (7.2%)	1 (0.2%)	27 (6.7%)	
Upto 100 %	27 (6.7%)	0 (0%)	27 (6.7%)	
Not Aware	150 (37.2%)	82 (20.3%)	67 (16.2%)	
Grand total	403 (100%)	201 (49.9%)	198 (49.1%)	4(1%)

(Table 2): No. of participants those who have / not have dental caries based on the different cocoa content in the chocolates.

Consumption of Cocoa content	**Dental caries**	**No sign of dental caries**	**p-value**
Less than 60% cocoa content	107 (89.9 %)	24 (18.3%)	0.321
60% and above cocoa content	12 (10.08%)	107 (81.7%)	0.026

(Table 3): Comparison of dental caries and no sign of dental caries based on percentage of cocoa content in the chocolates. $p<0.05$was considered as a statistically significant according to Paired t-test.



(Fig.4.): Knowledge of cocoa content of chocolates.

Pearson Correlations					
		Cocoa Content	Dental Caries	No Dental Caries	Antibacterial Activity
Cocoa Content	Pearson Correlation	1	-.506	.660	.791
	Sig. (2-tailed)		.305	.154	.061
	N	6	6	6	6
Dental Caries	Pearson Correlation	-.506	1	-.456	.065
	Sig. (2-tailed)	.305		.363	.903
	N	6	6	6	6
No Dental Caries	Pearson Correlation	.660	-.456	1	.613
	Sig. (2-tailed)	.154	.363		.195
	N	6	6	6	6
Antibacterial Activity	Pearson Correlation	.791	.065	.613	1
	Sig. (2-tailed)	.061	.903	.195	
	N	6	6	6	6

(Table 4): Correlation matrix of Cocoa content, Dental Caries, no sign of Dental Caries, and Antibacterial Activity against *S. mutans* based on Pearson Correlation test ($p<0.05$).

Pearson Correlation

Cocoa Content vs. Dental Caries

Pearson Correlation: -0.506

Significance (2-tailed): 0.305

Interpretation: There is a moderate negative correlation (-0.506) between Cocoa Content and Dental Caries, suggesting that as Cocoa Content increases, the occurrence of Dental Caries tends to decrease. However, this correlation is not statistically significant ($p = 0.305$).

Cocoa Content vs. No sign of Dental Caries

Pearson Correlation: 0.660

Significance (2-tailed): 0.154

Interpretation: There is a moderate positive correlation (0.660) between Cocoa Content and the No sign of Dental Caries, indicating that higher Cocoa Content may be associated with a lower incidence of Dental Caries. However, this correlation is not statistically significant at the 0.05 level (p = 0.154).

Cocoa Content vs. Antibacterial Activity

Pearson Correlation: 0.791

Significance (2-tailed): 0.061

Interpretation: There is a strong positive correlation (0.791) between Cocoa Content and Antibacterial Activity. This suggests that an increase in Cocoa Content is associated with higher Antibacterial Activity. The correlation is marginally significant with a p-value of 0.061.

Dental Caries vs. No sign of Dental Caries

Pearson Correlation: -0.456

Significance (2-tailed): 0.363

Interpretation: There is a moderate negative correlation (-0.456) between the occurrence of Dental Caries and the absence of Dental Caries, but this correlation is not statistically significant (p = 0.363).

Dental Caries vs. Antibacterial Activity

Pearson Correlation: 0.065

Significance (2-tailed): 0.903

Interpretation: There is a very weak positive correlation (0.065) between Dental Caries and Antibacterial Activity, and this correlation is not statistically significant (p = 0.903).

No sign of Dental Caries vs. Antibacterial Activity

Pearson Correlation: 0.613

Significance (2-tailed): 0.195

Interpretation: There is a moderate positive correlation (0.613) between the no sign of Dental Caries and Antibacterial Activity. However, this correlation is not statistically significant at the 0.05 level (p = 0.195).

4. DISCUSSION

Dental caries is a major public health problem throughout the world and is the most common noncontagious disease. According to Global Burden of Disease Study in 2015, decay of secondary teeth and primary teeth ranked 1st (2.3 billion people) and 12th (560 million children) respectively (Subedi et al., 2024).

Dark chocolates contain theobromine that has anticariogenic property and even make the tooth strong (Hiremath et al., 2016). Theobromine in cocoa possessed antibacterial property and fight against plaque. Cocoa beans contain 1–4% theobromine. Quantity of theobromine can vary from 1.2%-2.4% in cocoa powder (Lakshmi et al., 2019).

In the present study, chocolates of different cocoa content (0%, 20%, 40%, 60%, 80%, up to 100%) were investigated for their antibacterial activity against the S. mutans. This work showed that higher cocoa content chocolates (upto 100%) produced maximum antibacterial activity than lesser cocoa content chocolates (80%) followed by 60%, 40%, and 20% respectively. However, 0% cocoa content chocolate doesn't produce antibacterial activity. This result agreed with the earlier literature reports (Janani et al., 2019). The dark chocolates produced greater zone of inhibition of S. mutans than the milk chocolate followed by plain chocolate (Bhat et al, 2016). Another study suggested that dark chocolate is good for teeth that prevents dental caries. Dark chocolate contains high theobromine than the milk chocolate. High cocoa content chocolates contain high theobromine than the less cocoa content (Nimbulkar et al., 2020). Corina et al. reported that dark chocolate exhibit bactericidal effect on S. aureus, S. mutans, S. pyogenes, P. aeruginosa, Serratia marcescens isolated from oral cavity (Corina et al., 2023). Osawa et al. reported that extracts from cocoa bean husks possess powerful anticariogenic potential [Osawa et al., 2001). Lakshmi et al. evaluated the antimicrobial activity of non-fluoridated chocolate-based theobromine toothpaste and reported that it produced significant zone of inhibition against the S. mutans (Lakshmi et al., 2019). Hence, high cocoa content chocolates are tooth friendly. Thus, the antibacterial activity of chocolates against S. mutans was depended on the cocoa content.

In this survey, most of the people consuming dark chocolates (cocoa content more than 60%) found to have less or no sign of dental caries. The dark chocolate lowers the risk of dental cavities with proper dental hygiene (Samanta et al., 2022). Summit dental health reported that dark chocolate was effective at fighting cavities, plaque and tooth decay (Summit, 2018). According to Hamasaeed et al. dark chocolate exhibits a protective effect on Enamel and Dentine Microhardness (Hamasaeed et al., 2024). On the contrary, people consuming milk chocolate or no cocoa content or cocoa content less than 60% were found to have caries. Milk chocolates were more cariogenic than other chocolates which, contributes to tooth decay and cavities whereas dark chocolates were less cariogenic than milk chocolates (Hedge et al., 2009).

The correlation analysis conducted on the variables—Cocoa Content, Dental Caries, No sign of Dental Caries, and Antibacterial Activity—revealed intriguing insights into potential relationships within the studied parameters. The negative correlation observed between Cocoa Content and Dental Caries (-0.506) suggests a potential protective effect of cocoa against dental caries. This aligns with existing literature highlighting the antimicrobial properties of cocoa, attributed to compounds like theobromine and polyphenols (Palombo, 2011). However, the correlation did not reach statistical significance ($p = 0.305$), emphasizing the need for larger sample sizes to confirm this trend. The robust positive correlation (0.791) between Cocoa Content and Antibacterial Activity supports the notion that cocoa may possess antibacterial properties. Previous studies have reported the antimicrobial potential of cocoa extracts against various bacteria, including Streptococcus mutans associated with dental caries (Jacosen et al., 2004). The marginally significant p-value ($p = 0.061$) suggests a promising avenue for further investigation. The lack of significant correlation between Dental Caries and Antibacterial Activity (0.065, $p = 0.903$) suggests that the presence or absence of dental caries may not directly correlate with the antibacterial efficacy observed (Su et al., 2023). However, the moderate positive correlation between No sign of Dental Caries and Antibacterial Activity (0.613) hints at a potential link between maintaining good oral health and enhanced antibacterial activity, although this correlation was not statistically significant ($p = 0.195$).

Thus, from this study, we found that chocolates with high cocoa content showed the best antibacterial activity than the lesser cocoa content chocolates against S. mutans as well as from the survey report most of the people consuming high cocoa content chocolates found to have no sign of dental caries. High cocoa content chocolates may prevent dental caries but also depends on the oral hygiene of person and further studies have to be carried out.

Limitations of the study

The study was conducted in a specific location, which was the only limitation and drawback of the research. The implication of the study was an effort to debunk the myth that chocolate consumption causes dental caries and to explore whether consuming high cocoa content chocolate helps to prevent dental caries. Further studies will be conducted in patients with the proper ethical approval.

5. CONCLUSION

The antibacterial activity assay as well as online survey revealed, people consuming cocoa rich chocolates are not affected by caries and the importance of cocoa content of chocolates on dental health. Among the chocolates, those with higher cocoa content exhibited greater antibacterial effects against *Streptococcus mutans*, the primary organism responsible for dental caries. The online survey revealed that individuals consuming chocolate with cocoa content exceeding 60% had a lower incidence of dental caries. The study proves that high cocoa content contain in the chocolates is safe for consumption and which lower the incidence of dental caries. It was proven by the antibacterial activity and further supported by the online survey study.

Apart from the consumption of Cocoa rich chocolates, oral hygiene practices must be followed to prevent caries. Further research with a larger and more diverse sample is warranted to validate these preliminary correlations. Additionally, investigating the specific bioactive compounds responsible for the observed effects would contribute to a more nuanced understanding of the mechanisms at play.

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Conflicts of interest

The authors report no financial or any other conflicts of interest in this work.

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