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RESEARCH ARTICLE

Evaluation of the Impact of some Plant Extracts against *Streptococcus* Spp. Isolated from Dental Decay Infection

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Abstract:

Aims:

This study aimed to isolate and identify the *Streptococcus* spp. bacteria from patients with dental caries infection. Furthermore, the antibacterial activity of new plant extracts on isolated *Streptococcus* spp. was also evaluated.

Methods:

A total of 150 samples were obtained at random from people of various ages and genders who were suffering from dental caries infection. Four different culture media were used for isolation: nutrient agar, MacConkey agar, blood agar, and *Streptococcus* selection agar. The identification of bacterial isolates was distinguished by macroscopic examination and the VITEK 2 system.

Results:

Only 120 cases (80%) showed positive culture, and these were distributed as follows: *Streptococcus sanguinis* had the highest rate and accounted for 38.33%, and *Streptococcus pseudoporcinus* represented only 29.16%. A 20% decline was reported for *Streptococcus salivarius*, with the lowest proportion being 12.5% for *Staphylococcus warneri*. The MIC for 16 tested plant extracts ranged from 0.97 to 125 µg/mL, whereas the MBC values ranged from 3.9 to 500 µg/mL. Imipenem was a positive control, with MIC values ranging from 3.9 µg/mL to 15.6 µg/mL; the MBCs varied from 31.2 to 125 µg/mL against all isolated species.

Conclusion:

Among the isolated bacterial species from tooth decay, *Streptococcus sanguinis* had the highest rate of isolated bacteria and accounted for 38.33%, while *Staphylococcus warneri* had the lowest percentage at 12.5%. Sargassum, Proskia, and Cicer arietinum were three distinct extracts that demonstrated superior antibacterial activity against all of the tested bacterial species. Their MIC values ranged from 0.97 µg/mL to 15.6 µg/mL, and the MBC values were between 3.9 µg/mL and 31.2 µg/mL.

Keywords: New plant, Extracts, Dental caries, *Streptococcus* spp., Antibacterial activity, Dental decay infection.

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1. INTRODUCTION

One of the most prevalent dental conditions that affect people is dental caries, which destroys the tooth enamel permanently and can lead to problems with food intake, thus causing severe discomfort [1]. Oral microbial infections, particularly cariogenic bacteria, are primarily responsible for dental caries [2]. Several researchers have confirmed that *Streptococcus mutans* is a major causative agent of dental caries [3 - 6]. Glucosyltransferase is an enzyme found in the main cariogenic pathogen *S. mutans*, which produces water-insoluble glucan from sucrose and deposits it on the surface of tooth enamel, where it acts as an adhesive for *S. mutans* and other Streptococci species [2]. Dental plaque is created by the colonisation and aggregation of bacteria that can make acid (acidogenic) and withstand an acidic environment (aciduric), which ultimately results in localised loss of teeth due to acid accumulation and mineral decalcification [4, 7]. Dental caries will eventually cause tooth loss if left untreated.

Natural bacterial flora of caries-prone teeth is dominated

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by species like *Streptococcus parasanguinis*, *Abiotrophia defectiva, Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus sanguinis* [8]. It has been reported in several studies [9] that the healthy oral cavity has a unique microbiota that differs from that linked to oral disease. Dental professionals often prescribe a variety of antibiotics for dental infections, including tetracycline, erythromycin, penicillin, and cephalosporin [10]. The evolution of antibiotic resistance in oral infections results from exposure to a wide range of medications. In addition, most antibiotics include a risk of allergic reactions of varying degrees of severity [11].

Due to the rise of antibiotic resistance in oral microbial pathogens against synthetic antibiotics, plant-derived treatments that are becoming more effective in treating oral illnesses have gained more attention in recent years [12 - 15]. Natural plant products have no side effects and can be used in the oral cavity for an extended period of time [16]. According to the World Health Organization (WHO), up to 80% of people worldwide utilize traditional medicine for basic health care [17]. This medicine is based on the natural ecosystem or community knowledge [18]. Conventional medicines also use herbal plant extracts as antibacterial agents, and they have gained significant attention in the fight against tooth caries [19 - 22]. In the current study, we evaluated 16 different plant extracts, 14 of which are recognized as traditional Iranian medicines. These plants have a potent odour and have been used for many years as antiseptic carminatives to treat many diseases. Polysaccharide extract from pomegranate peel and protein from *Cicer arietinum* were the other two extracts that were tested.

2. MATERIALS AND METHODS

2.1. Media Preparation

All culture media were prepared in accordance with the manufacturer's instructions, and the media was sterilized by autoclaving at 121°C for 15 minutes.

2.2. Samples Collection

The study was approved by the research ethics committee at the Department of Medical Laboratory Techniques, Al Maarif University College in Iraq (Code 2022 - 128). A total of 150 samples were collected at random from patients ranging in age from 22 to 75 years old, including 65 females and 85 males suffering from dental carries in Ramadi Dental Hospital in Al-Anbar Province between December, 2021 to June, 2022. The type of swabs used could prevent the growth of bacteria for 72 hours (sterile swab with transport media). All collected swabs were shipped to the laboratory within two hours. The samples from the swabbing across the root caries of the patient's tooth were all streaked directly and separately on the solid media, including blood agar, MacConkey agar, nutrient agar, and *Streptococcus* selection agar (HiMedia, Mumbai, Maharashtra, India). The Petri dishes were incubated aerobically and anaerobically for 24 to 72 hours at 37°C, and the plates were then checked visually for any bacterial growth. After incubation, isolated colonies were selected and cultured to purify the samples.

2.3. Plant Extracts Preparation

The aerial parts of the plant (leaves, roots, and stems) were collected from different Iranian provinces starting in 2021. All plant materials were washed and dried at room temperature in the shade. The powdered plant material was extracted (50 g) three times in 48 hrs (3 x 48 hrs) at room temperature using the maceration method and pure methanol. The extracts were lyophilised with a freeze dryer after being extracted with a rotary evaporator. The concentrated extracts were stored in opaque containers at 4°C in cold and dry conditions until testing. The extracts were made in solutions by weighing 10 mg of extract powder and dissolving it in 9 mL of deionized water with 1 mL of methanol as a co-solvent; the final concentration was thus 1 mg/mL.

Polysaccharides and proteins were extracted using distilled water rather than methanol. A total of 100 mg of *Cicer arietinum* protein was ground separately using an electric mill and subjected to the Soxhlet apparatus to defat the sample using hexane for 9 hours. To extract protein, the defatted powder was then sonicated with sodium hydroxide solution (1 N, pH 8.5) with 10% w/v for 1 hour x 2. The extract was centrifuged at 8000 g for 15 minutes. Protein was precipitated by reducing the pH of the solvent to 4.5 with HCl (1 N). The mixture was then centrifuged twice at 8000 g for 10 minutes to precipitate protein, which was then washed twice with distilled water. The mass obtained was freeze-dried for further analysis. Pomegranate peel was dried at the shade and crushed to extract its polysaccharide content. The powder was boiled with distilled water for 3 hours. The aqueous extract was then filtered and subjected to concentration using a rotary evaporator. In the next step, the concentrated extract was freeze-dried to obtain a powder.

2.4. Identification of the Bacterial Isolates

Colonies were sub-cultured on the surface of *Streptococcus* selection agar plates for further purification. They incubated anaerobically for 48 hours at 37°C. The isolates were primarily defined by using the following methods:

2.4.1. Morphology of Colony

- Shape, size, colour, elevation, and margin of colony and appearance were observed in an overnight plate culture on *Streptococcus* selection agar.
- Gram staining and microscopic examination: The bacteria suspensions were applied to clean slides and allowed to dry by air. The slides were exposed to crystal violet solution for 1 minute and then washed with water. The slides were then flooded with Gram's iodine for another minute, washed with water, and decolorized with 95% ethyl alcohol for 30 seconds. The slides were washed again with water before being counterstained with safranin stain for 1 minute. Finally, the slides were air-dried prior to examination with 100x magnification. The color of the cells was then used to identify bacteria: purple for Gram-positive cells and pink or red for Gram-negative cells.
- We next performed a range of biochemical tests, including catalase, oxidase, urease, motility, methyl-red, Voges Proskauer, and slide coagulase.

For more confirmation, the VITEK 2 system was used to perform additional identification for bacterial isolates. Manual

biochemical tests for bacterial identification can take several days to weeks. However, using the VITEK 2 system technique dramatically reduces time and contamination while providing higher results accuracy. A vacuum device automatically filled the card, sealed it, and put it into the VITEK-2 reader-incubator module (incubation temperature 35.5°C), where it was exposed to kinetic fluorescence measurements every 15 minutes. The results were interpreted using the ID-GPC database.

2.5. Determining the Minimum Inhibitory Concentration (MIC)

To investigate the impact of plant extracts' antimicrobial activities on the growth of bacterial species isolated from dental caries, the MIC for all extracts was identified using 96-well microtiter plates against all isolated bacterial species as described [23]. The test organisms were prepared using six hours of Muller-Hinton Broth (MHB) growth, and the suspensions were calibrated to 0.5 McFarland turbidity standards (10^8 CFU/mL). All extracts were diluted two-fold with a DMSO concentration of ≤1% while varying the concentration from 1000 to 0.48 µg/mL (80 µL as final volume). Next, microtiter plates were filled with 20 µL of bacterial suspensions and 100 µL of MHB, and the plates were then incubated at 37°C for 24 hours. Microtiter plates were examined with a spectrophotometer at 620 nm after the incubation time. A standard antibiotic, imipenem, was utilised as a positive control. The negative control was MHB + DMSO. The plant extract with the lowest concentration was defined as the MIC, thus demonstrating complete prevention of visible growth.

2.6. Determining the Minimum Bactericidal Concentration (MBC)

The MBC value was determined after the MIC assay. Wells with no visible growth received 5 µL of a sample spread onto Muller Hinton Agar plates and incubated for 24 hours at 37°C. The MBC value was then defined as the concentration at which the least amount of bacteria growth/colony occurred.

3. RESULTS

3.1. Isolation and Identification of Bacterial Species from Dental Caries Samples

Of the 150 samples of dental caries, only 120 (80%) bacterial isolates were found; four different species of bacteria were identified by the VITEK 2 system from these isolates. *Streptococcus sanguinis* had the highest rate of isolated bacteria, accounting for 38.33%, followed by *Streptococcus pseudoporcinus*, representing only 29.16%. *Streptococcus salivarius* accounted for 20.0%, and *Staphylococcus warneri* was reported to be 12.5% (Table **1**).

Gram stain was found to be positive for all isolated bacterial species, with purple color for bacterial cells with different shapes. *Staphylococcus warneri* showed catalase negative and urease positive, whereas the other bacterial isolates were catalase positive. All isolates were methyl red-positive and were negative for oxidase, urease, and coagulase; they were non-motile, as shown in Table **2**.

3.2. Determination of MIC and MBC

The MIC and MBC values against isolated bacterial species were used to evaluate the plant extracts' antibacterial activity. The antibiotic imipenem was selected as a positive control as it is often used to treat infections caused by the tested bacterial species. Most plant extracts assessed demonstrated a broad spectrum of inhibitory capability and were effective against *Streptococcus* spp. and *Staphylococcus warneri.* They were isolated from dental caries infection. The lowest concentration of an antibacterial agent that significantly inhibits growth is known as the MIC, whereas the minimum concentration of the antibacterial agent that induces bacterial death is defined as the MBC. The MIC and MBC values are shown in Table **3**. The MIC concentrations for all extracts tested ranged from 0.97 to 125 µg/mL, whereas the MBC values ranged from 3.9 to 500 µg/mL. Imipenem was a positive control and has MIC values ranging from 3.9 µg/mL to 15.6 µg/mL. The MBCs varied from 31.2 to 125 µg/mL against all isolated species. Three different extracts were tested, including Sargassum, Proskia, and Cicer arietinum, which demonstrated strong antibacterial activity against all evaluated bacterial species. Their MIC values ranged from 0.97 µg/mL to 15.6 µg/mL and MBC values were from 3.9 µg/mL to 31.2 µg/mL.

4. DISCUSSION

In this study, 150 samples were taken randomly from patients of various ages and genders, who were suffering from dental caries infection. The samples yielded 120 isolates with positive bacterial culture (80%), whereas 30 samples gave negative bacterial culture at a rate of 20%. *Streptococcus sanguinis* was found in the highest percentage of isolates at 38.33%, followed by *Streptococcus pseudoporcinus* at 29.16%, *Streptococcus salivarius* at 20%, and the lowest rate of detected bacteria was recorded at 12.5% for *Staphylococcus warneri*. The results of the current study were found to be in agreement with the findings of a previous study [24] conducted in 2012 at Nationwide Children's Hospital Dental Clinic in Columbus, Ohio, which found that *Streptococcus pseudoporcinus* and *Streptococcus sanguinis* correlated with dental caries infection at higher levels.

Another study [25] conducted in 2017 at the central laboratory of a Diyala Health Governorate Hospital showed that, among 150 samples obtained from dental caries infection, only 68 isolates (45.33%) were positive; *Streptococcus mutans* is the primary causative agent for dental caries infection, accounting for 13.3% of bacteria (0.97 µg/mL to 125 µg/mL). These outcomes contradicted the isolation and identification results obtained in this study; the difference could be due to the isolation method and the laboratory conditions used. *Streptococcus* species were found in dental caries infection, including *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Streptococcus mutans* [26].

All 16 plant extracts were employed at 1 mg/mL and indicated great activity against the four bacterial species isolated from patients with dental carries infection versus positive control imipenem. The MIC concentrations for all extracts tested ranged from 0.97 µg/mL to 125 µg/mL; the MBC values observed from 3.9 µg/mL to 500 µg/mL. Imipenem was a positive control, with MIC values ranging from 3.9 µg/mL to 15.6 µg/mL against all isolated species. The MBCs varied from 31.2 µg/mL to 125 µg/mL. These results

were in agreement with findings of previous research [27] that tested the plant extract mixture and its individual constituent of plant extracts (*Psidium* sp., *Mangifera* sp., *Mentha* sp.) on the formation of biofilms, including *Streptococcus sanguinis* and *Streptococcus mutans*. The authors reported that the plant extracts mixture compared with its respective constituent plants showed the lowest MIC towards *S. sanguinis* (3.81 mg/mL) and *S. mutans* (1.91 mg/mL). Furthermore, synergistic effects were also reported. The *Psidium* sp. at 15.24 mg/mL and plant extracts mixture and *Psidium* sp. at 30.48 mg/mL demonstrated the lowest MBCs against *S. sanguinis* and *S. mutans*, respectively. Another study [28] evaluated the effect of *Zingiber officinale* extract on the growth of *Streptococcus mutans* and *Streptococcus sanguinis*, and the findings revealed that the MIC was 0.02 mg/mL for *S. mutans* and 0.3 mg/mL for *S. sanguinis*. The MBC was 0.04 mg/mL for *S. mutans* and 0.6 mg/mL for *S. sanguinis*; these results were consistent with those obtained in the current study.

Table 1. The distribution of bacterial species isolated from patients with dental caries.

S.No.	Bacterial Species	Number of Bacteria Isolated	Percentage %
1-	*Streptococcus sanguinis*	46	38.3%
2-	*Streptococcus pseudoporcinus*	35	29.2%
3-	*Streptococcus salivarius*	24	20.0%
4-	*Staphylococcus warneri*	15	12.5%
	Total number of bacterial isolates	120	100.0%

Table 2. Biochemical observations of the different bacterial isolates obtained from patients with dental caries.

Biochemical Test	*Streptococcus sanguinis*	*Streptococcus pseudoporcinus*	*Streptococcus salivarius*	*Staphylococcus warneri*
	Result	Result	Result	Result
Catalase	+	+	+	-
Oxidase	-	-	-	-
Urease	-	-	-	+
Coagulase	-	-	-	-
Methyl-red	+	+	+	+
Voges Proskauer	-	-	-	-
Motility	-	-	-	-

Table 3. The antibacterial activity of several plant extracts against different isolates obtained from dental caries infection.

Extracts	Bacterial Species (The MIC and MBC are reported in µg/mL)							
Name	*Streptococcus sanguinis*		*Streptococcus pseudoporcinus*		*Streptococcus salivarius*		*Staphylococcus warneri*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ferula asafoetida	15.6	62.5	31.2	125	62.5	250	15.6	62.5
Zumeria majda	31.2	125	31.2	250	15.6	125	31.2	62.5
Thymus migricus	31.2	62.5	7.8	62.5	15.6	125	15.6	62.5
Artemisia santolina	15.6	62.5	31.2	125	7.8	62.5	31.2	62.5
Sargassum	3.9	7.8	1.95	7.8	3.9	7.8	0.97	3.9
Proskia	7.8	15.6	7.8	31.2	15.6	31.2	3.9	15.6
Roots of *Echinacea angustifolia*	31.2	125	15.6	250	15.6	125	31.2	62.5
Echinophora platyloba	15.6	62.3	7.8	125	31.2	62.3	15.6	250
Pimpinella tragiodes	125	250	62.5	125	31.2	62.5	62.5	250
Phlomis bruguieri	15.6	125	31.2	125	7.8	250	31.2	62.5
Phlomis kurdica	31.2	250	31.2	500	15	125	31.2	500
Phlomis olivieri	15.6	125	62.5	250	31.2	250	15.6	250
Satureja khuzestanica	15.6	62.3	31.2	125	7.8	250	3.9	62.3
Satureja spicigera	7.8	62.5	15.6	250	15.6	500	31.2	125
Polysaccharide extract of pomegranate peel	31.2	62.5	7.8	125	15.6	250	31.2	62.5
Cicer arietinum proteins	7.8	15.6	3.9	7.8	1.95	7.8	3.9	15.6
Imipenem as positive control	15.6	31.2	7.8	62.5	15.6	125	3.9	31.2

Abbreviations: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration

Previous research [29] examined the effect of *Allium sativum* bulb extract on pathogenic bacterial species, including *Lactobacillus acidophilus*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus mutans*, and *Staphylococcus aureus* isolated from periodontal and dental caries infections. The MBC values in this study ranged from 60 ± 5 mg/mL to 215 ± 7 mg/mL. The activity of *Allium sativum* bulb extract on the tested bacterial species in the cited study was higher than the MBC data obtained in our study. Several studies tested the effects of a variety of medicinal and herbal plant extracts, including *Arctii Fructus*, *Caryopteris incana*, *Aralia continentalis*, *Symplocarpus renifolius*, *Lamium amplexica, Artocarpus lakoocha Roxb, Albizia myriophylla, Eurycoma longifolia, Glycyrrhiza glabra,* and *Anacardium occidentale.* They were found to have superior antimicrobial efficacy against cariogenic and non-cariogenic bacterial species [29 - 35]. The current study findings are promising as an alternative treatment for dental caries infection, thus reducing the use of antimicrobial therapy for dental caries and decreasing antimicrobial resistance for cariogenic bacterial isolates that cause tooth infection.

Streptococcus mutans and *Streptococcus sobrinus* have been identified in several studies as the etiological agents of dental caries in humans. The cariogenic possibility of these bacteria is directly correlated with their acidogenic and aciduric capacity. Both species have evolved significantly over time in terms of a variety of changes adapted to cope with the acidic and occasionally harsh conditions found in the human oral cavity [36, 37]. The absence of *S. mutans* and *S. sobrinus* in this study could be attributed to a number of factors, including the location of the tooth from which the bacteria were isolated, the method used to isolate the bacteria, and the laboratory conditions.

CONCLUSION

In this study, *Streptococcus sanguinis* was the most common bacteria isolated from dental caries (38.33%), while *Staphylococcus warneri* was the least common (12.5%). The MIC values for all tested extracts ranged from 3.9 µg/mL to 500 µg/mL, and the MBC values ranged from 3.9 µg/mL to 500 µg/mL against the four isolated species from patients suffering from dental caries infection. The MIC for positive control imipenem ranged from 3.9 µg/mL to 15.6 µg/mL, and MBCs ranged from 31.2 µg/mL to 125 µg/mL. This study suggested paying more attention to the causative factors for dental caries; people should see a dentist to get their teeth checked on a regular basis. To prevent dental caries from bacterial infection, a more effective plant extract is often used as a mouthwash and treatment for tooth decay diseases. Alternative therapies have been gaining great attention because they are safe and have few side effects compared to synthetic prescription medications. The focus is primarily on drugs that are derived from plants. Numerous medicinal and herbal plants are being examined for their possible antimicrobial activity and medical use as a substitute for synthetic drugs for use in dental care. Future research should examine the antibacterial activity of the newly tested plant extracts *in vivo*.

AUTHOR'S CONTRIBUTIONS

Anas Abdullah Hamad (A. A. H.), Maryam S. Alhumaidi (M. S. A.), and Azadeh Manayi (A. M.) contributed equally to this study. A. A. H., M. S. A., and A. M. contributed to the conceptualization. A. A. H. and A. M. contributed to the methodology. A. A. H. and M. S. A. participated in validation. A. A. H. and M. S. A. contributed to the formal analysis. A. A. H. and A. M. contributed to the investigation. A. A. H. participated in resource collection. A. A. H., A. M., and M. S. A. contributed to data curation. A. A. H., A. M., and M. S. A. contributed to writing the original draft. A. A. H., M. S. A., and A. M. participated in writing review and editing. A. A. H. and M. S. A. contributed to visualization. A. A. H. and A. M. participated in supervision. A. A. H., M. S. A., and A. M participated in project administration.

LIST OF ABBREVIATIONS

MIC	=	Minimum Inhibitory Concentration
WHO	=	World Health Organization
MHB	=	Muller-hinton Broth

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current study was approved by the research ethics committee at the department of Medical Laboratory Techniques, Al Maarif University College in Iraq (Code 2022 - 128).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all the participants.

STANDARDS OF REPORTING

The STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data that supported the findings of this study are available upon request from the corresponding author [A.A.H].

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Kouidhi B, Zmantar T, Bakhrouf A. Anticariogenic and cytotoxic activity of clove essential oil *(Eugenia caryophyllata)* against a large

number of oral pathogens. Ann Microbiol 2010; 60(4): 599-604. [http://dx.doi.org/10.1007/s13213-010-0092-6]

[2] Besra M, Kumar V. *In vitro* investigation of antimicrobial activities of ethnomedicinal plants against dental caries pathogens. 3 Biotech 2018; 8(5): 1-8. [http://dx.doi.org/10.1007/s13205-018-1283-2]

[3] Loesche WJ, Rowan J, Straffon LH, Loos PJ. Association of Streptococcus mutants with human dental decay. Infect Immun 1975; 11(6): 1252-60. [http://dx.doi.org/10.1128/iai.11.6.1252-1260.1975] [PMID: 1140847]

[4] Loesche WJ. Role of *Streptococcus* mutans in human dental decay. Microbiol Rev 1986; 50(4): 353-80. [http://dx.doi.org/10.1128/mr.50.4.353-380.1986] [PMID: 3540569]

[5] Clarke JK. On the bacterial factor in the aetiology of dental caries. Br J Exp Pathol 1924; 5(3): 141.

[6] Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001; 65(10): 1028-37. [http://dx.doi.org/10.1002/j.0022-0337.2001.65.10.tb03446.x] [PMID: 11699974]

[7] Hemlata R, Chandrabhan D, Renu B, Pradeep V. Isolation of dental caries bacteria from dental plaque and effect of tooth pastes on acidogenic bacteria. Open J Med Microbiol 2012; 2(3): 65-9. [http://dx.doi.org/10.4236/ojmm.2012.23009]

[8] Corby PM, Lyons-Weiler J, Bretz WA, *et al.* Microbial risk indicators of early childhood caries. J Clin Microbiol 2005; 43(11): 5753-9. [http://dx.doi.org/10.1128/JCM.43.11.5753-5759.2005] [PMID: 16272513]

[9] Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005; 43(11): 5721-32. [http://dx.doi.org/10.1128/JCM.43.11.5721-5732.2005] [PMID: 16272510]

[10] Oberoi SS, Dhingra C, Sharma G, Sardana D. Antibiotics in dental practice: How justified are we. Int Dent J 2015; 65(1): 4-10. [http://dx.doi.org/10.1111/idj.12146] [PMID: 25510967]

[11] Poveda RR, José VB, José MSB, *et al.* Antibiotic use in dental practice. A review. Med Oral Patol Oral Cir Bucal 2007; 12(3): 186-92. [PMID: 17468711]

[12] Lynch AS, Robertson GT. Bacterial and fungal biofilm infections. Annu Rev Med 2008; 59(1): 415-28. [http://dx.doi.org/10.1146/annurev.med.59.110106.132000] [PMID: 17937586]

[13] Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evid Based Complement Alternat Med 2011; 2011: 680354. [http://dx.doi.org/10.1093/ecam/nep067]

[14] Nittayananta W, Limsuwan S, Srichana T, Sae-Wong C, Amnuaikit T. Oral spray containing plant-derived compounds is effective against common oral pathogens. Arch Oral Biol 2018; 90: 80-5. [http://dx.doi.org/10.1016/j.archoralbio.2018.03.002] [PMID: 29573647]

[15] Lugo-Flores MA, Quintero-Cabello KP, Palafox-Rivera P, *et al.* Plant-derived substances with antibacterial, antioxidant, and flavoring potential to formulate oral health care products. Biomedicines 2021; 9(11): 1669. [http://dx.doi.org/10.3390/biomedicines9111669] [PMID: 34829898]

[16] Gupta P, Shetty H. Use of natural products for oral hygiene maintenance: Revisiting traditional medicine. J Complement Integr Med 2018; 15(3) [http://dx.doi.org/10.1515/jcim-2015-0103] [PMID: 29584614]

[17] Khan AU. History of decline and present status of natural tropical thorn forest in Punjab. Biol Conserv 1994; 67(3): 205-10. [http://dx.doi.org/10.1016/0006-3207(94)90611-4]

[18] Pradhan BK, Badola HK. Ethnomedicinal plant use by Lepcha tribe of Dzongu valley, bordering Khangchendzonga Biosphere Reserve, in North Sikkim, India. J Ethnobiol Ethnomed 2008; 4(1): 22. [http://dx.doi.org/10.1186/1746-4269-4-22] [PMID: 18826658]

[19] Lim S-H. Effect of leaf-extract from *Camellia sinensis* and seed-extract from casia tora on viabillity of mutans *Streptococci* isolated from the interface between orthodontic brackets and tooth surfaces. Korean J Orthod 2003; 33(5): 381-9.

[20] Limsong J, Benjavongkulchai E, Kuvatanasuchati J. Inhibitory effect of some herbal extracts on adherence of *Streptococcus* mutans. J Ethnopharmacol 2004; 92(2-3): 281-9. [http://dx.doi.org/10.1016/j.jep.2004.03.008] [PMID: 15138013]

[21] Song JH, Yang TC, Chang KW, Han SK, Yi HK, Jeon JG. *In vitro* effects of a fraction separated from *Polygonum cuspidatum* root on the viability, in suspension and biofilms, and biofilm formation of mutans streptococci. J Ethnopharmacol 2007; 112(3): 419-25. [http://dx.doi.org/10.1016/j.jep.2007.03.036] [PMID: 17543483]

[22] Sampaio FC, Pereira MSV, Dias CS, Costa VCO, Conde NCO, Buzalaf MAR. *In vitro* antimicrobial activity of *Caesalpinia ferrea* Martius fruits against oral pathogens. J Ethnopharmacol 2009; 124(2): 289-94. [http://dx.doi.org/10.1016/j.jep.2009.04.034] [PMID: 19397986]

[23] Patel JB, Cockerill F, Bradford PA. Performance standards for antimicrobial susceptibility testing: Twenty-fifth informational supplement.M100-S25. 2015; 35: pp. (3)29-50.

[24] Gross EL, Clifford JB, Stacey RK, *et al.* Beyond Streptococcus mutans: Dental caries onset linked to multiple species by 16S rRNA community analysis. PLoS One 2012; 7(10): e47722. [http://dx.doi.org/10.1371/journal.pone.0047722]

[25] Torki SH, Mubaruk KI. Bacteriological study of dental caries infection in baquba. J Sci 2019; 30(3): 19-27. [http://dx.doi.org/10.23851/mjs.v30i3.407]

[26] Aas JA, Griffen AL, Dardis SR, *et al.* Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol 2008; 46(4): 1407-17. [http://dx.doi.org/10.1128/JCM.01410-07] [PMID: 18216213]

[27] Shafiei Z, Haji Abdul Rahim Z, Philip K, Thurairajah N. Antibacterial and anti-adherence effects of a plant extract mixture (PEM) and its individual constituent extracts (*Psidium sp.*, *Mangifera sp*., and *Mentha sp*.) on single- and dual-species biofilms. PeerJ 2016; 4: e2519. [http://dx.doi.org/10.7717/peerj.2519] [PMID: 27761322]

[28] Azizi A, Aghayan S, Zaker S, Shakeri M, Entezari N, Lawaf S. *In vitro* effect of zingiber officinale extract on growth of *Streptococcus* mutans and *Streptococcus sanguinis*. Int J Dent 2015; 2015: 1-5. [http://dx.doi.org/10.1155/2015/489842] [PMID: 26347778]

[29] Bin C, Al-Dhabi NA, Esmail GA, Arokiyaraj S, Arasu MV. Potential effect of *Allium sativum* bulb for the treatment of biofilm forming clinical pathogens recovered from periodontal and dental caries. Saudi J Biol Sci 2020; 27(6): 1428-34. [http://dx.doi.org/10.1016/j.sjbs.2020.03.025] [PMID: 32489278]

[30] Chandra Shekar B, Nagarajappa R, Suma S, Thakur R. Herbal extracts in oral health care - A review of the current scenario and its future needs. Pharmacogn Rev 2015; 9(18): 87-92. [http://dx.doi.org/10.4103/0973-7847.162101] [PMID: 26392704]

[31] Pumpaluk P, Sritutarak B, Likhitwitayawuid K, *et al.* Antibacterial effect of herbal plants against three cariogenic microorganisms. M Dent J 2017; 37(1): 71-80.

[32] Lee YC, Cho SG, Kim SW, Kim JN. Anticariogenic potential of Korean native plant extracts against *Streptococcus* mutans. Planta Med 2019; 85(16): 1242-52. [http://dx.doi.org/10.1055/a-1013-1364] [PMID: 31597167]

[33] Hayhoe E, Palombo E. Extracts of Eremophila longifolia inhibit the cariogenic activities of *Streptococcus* mutans and *Streptococcus sobrinus*. Planta Med 2011; 5(12): 2476-82.

[34] Yadav G, Tripathi AM, Saha S, Dhinsa K, Rai A, Jain B. Comparison of antimicrobial efficacy of four different plant extracts against cariogenic bacteria: An *in vitro* study. Int J Clin Pediatr Dent 2020; 13(4): 361-7. [http://dx.doi.org/10.5005/jp-journals-10005-1796] [PMID: 33149409]

[35] de Araújo JSC, de Castilho ARF, Lira AB, *et al.* Antibacterial activity against cariogenic bacteria and cytotoxic and genotoxic potential of *Anacardium occidentale L.* and *Anadenanthera macrocarpa* (Benth.) Brenan extracts. Arch Oral Biol 2018; 85: 113-9. [http://dx.doi.org/10.1016/j.archoralbio.2017.10.008] [PMID: 29054025]

[36] Conrads G, de Soet JJ, Song L, *et al.* Comparing the cariogenic species *Streptococcus sobrinus* and *S. mutans* on whole genome level. J Oral Microbiol 2014; 6(1): 26189. [http://dx.doi.org/10.3402/jom.v6.26189] [PMID: 25475081]

[37] Berlutti F, Catizone A, Ricci G, *et al. Streptococcus* mutans and *streptococcus sobrinus* are able to adhere and invade human gingival fibroblast cell line. Int J Immunopathol Pharmacol 2010; 23(4): 1253-60. [http://dx.doi.org/10.1177/039463201002300430] [PMID: 21244775]