Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[4] March 2020 : 31-38

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Anticariogenic Efficacy of *Psidium Guajava* extracts *and Azadirachta indica*

Arya S[1,2], Smitha C[1,] and Usha R[2*]
Department of Microbiology, PMS College of Dental Science and Research , Thiruvananthapuram, Kerala, India.
Department of Microbiology, Karpagam University, Coimbatore, Tamil Nadu, India
*Email: ushaanbu09@gmail.com

ABSTRACT
Dental caries and periodontal diseases are considered as the most important global oral health burdens. Dental caries is the localized destruction of susceptible dental hard tissues, which is a biofilm-dependent manifestation. Periodontal diseases are also associated with bacterial biofilm in the oral cavity. Natural anti-biofilm agents are promising candidates who could provide novel strategies for biofilm-associated infections. A detailed literature review of plants suggested potent antibacterial activity of many traditional medicinal plants, of which Psidium guajava depicted exceptional activity along with anti-inflammatory and antioxidant activity. In this context, a comparative analysis was performed to evaluate the anti-cariogenic potential of *Psidium guajava and Azadirachta indica extracts. The study was carried out to investigate the antibacterial activity and biofilm inhibition activity* of *Psidium guajava plant leaf extracts against oral pathogens. Antimicrobial assay of ethanolic extract of Psidium guajava leaves showed a wider zone of inhibition for Enterococcus faecalis with a zone of 11 mm in 1mg/ml concentration and MIC of 461.86µg/mL, and Azadiracta indica showed MIC value of 401.522µg/mL. Antibiofilm activity was confirmed by CV assay, and lethality recorded by EtBr/AO staining.*
Keywords: *Dental caries, Periodontal disease, Biofilm, Quorum sensing, Phytochemicals*

Received 09.01.2020 Revised 26.02.2020 Accepted 11.03.2020

INTRODUCTION

Dental caries and periodontal disease contributes to the most important oral health burdens. As per WHO and NHS reports - Prevalence studies on dental caries in India have shown as results ranging from 31.5% to 89%. Dental caries and Periodontal disease is prevalent in 20-50% of the population around the globe. Dental caries being a multifactorial disease is due to the demineralization and destruction of hard tissues of teeth like dentine and enamel due to the acid production by oral bacteria on the fermentation of food. Robust evidence shows the association of periodontal diseases with systemic diseases such as cardiovascular disease, diabetes, and adverse pregnancy outcomes[1]. The key factor contributing to dental caries is the oral flora residing in dental plaque[2]. *Streptococcus mutans* is considered as the principal etiologic agent in dental caries. It has adopted various strategies to colonize the tooth surface and has a pivotal role in caries associated dental infection. *Enterococcus faecalis* is an endodontic biofilm pathobiont, which plays a significant role in initiating biofilmformation, leading to dental caries and eventual tooth loss. The key caries-associated microbial virulence traits include acidogenesis and acid tolerance, intracellular polysaccharide storage, and extracellular glucanformation, which promotes the attachment of oral pathobionts to the tooth surface and increases plaque's pH-lowering ability.

Biofilm is a dense microcommunity having different bacterial colonies embedded in extracellular polymeric matrix which is generally impervious to antimicrobial agents [3]. Dental biofilm is a complex, organized microbial community that is the primary etiologic factor for the most frequently occurring oral diseases, dental caries, and periodontal diseases. Although dental biofilm cannot be completely eliminated, its pathogenicity can be lessened through effective oral hygiene measures[4]. The initial attachment of bacterial cells to the tooth surface is the critical stage in biofilm formation. Progressing biofilms contributes to various odontological infections due to enhanced virulence of various pathobionts

residing in the biofilm. As stated by Gomashe *et al*, biofilms are impervious to antimicrobial agents, which is a matter of concern. In recent years, most of the research has been focusing on identifying various alternative medicines to treat infections caused by drug-resistant bacteria. These pathobionts contribute to carious lesions, thus the study of the emergence of particular biofilm is relevant in the control of plaque formation. The biofilm-forming bacteria are resistant to antimicrobial agents due to the lack of penetration of antimicrobial agents[5]. Biofilm being impervious to various drugs and chemicals poses a great challenge in the therapeutic scenario of medical and odontological infections. Antiplaque agents are effective against biofilm formation[6]. Various compounds have been tested for their antibiofilm activities. While effective in plaque removal, these chemical agents containing chlorhexidine gluconate, as well as those that contain essential oils, have unwanted side effects. Tooth discoloration and occasional loss of taste are some side effects associated with the use of chlorhexidine gluconate. Natural, herbal-based antiplaque agents are also available to combat oral infections. These phytochemical based products due to its anti-inflammatory and antimicrobial properties are effective against dental plaque build-up[7]. The present study is designed to evaluate the antibiofilm activity of certain plant extracts.

In a bacterial micro-community, the pathobionts communicate with each other by producing, detecting, and responding to certain signal molecules called auto inducers. Cell to cell communication between various bacterial species is generated via chemical signaling molecules called auto inducers. Quorum sensing is seen among same bacterial species or different species. Quorum sensing signals enable bacterial species to alter gene expression for better survival in the prevailing environment[8]. Recent studies suggest the relevance of quorum sensing among bacterial communities. There are many small molecules dependent interaction among various microbes and between microbes and hosts. As stated by Whiteley *et al,* [9], there is certainly more to be discovered, and there is an opportunity to sort out fundamental differences between these diverse systems. Understanding these issues will be critical as we move towards translating necessary studies of QS to meet future needs, including functional studies of the human microbiome [9].The quorum-sensing signalling ability of bacteria plays a crucial role in biofilm formation. These two factors contribute to the enhanced virulence among bacterial communities owing to increased drug resistance, causing difficulties in the therapeutic scenario. Anti-QS agents can abolish the QS signalling and prevent the biofilm formation, therefore reducing bacterial virulence without causing drug-resistance to the pathogens, suggesting that anti-QS agents are potential alternatives for antibiotics[10], Many plant-derived natural products possess inhibitory action on quorum sensing. Natural anti-biofilm agents are promising candidates who could provide novel strategies for biofilm-associated infections.

Surgical treatment of the demineralised tooth structures remained the treatment strategy for the past decades. The de merit of the method is that the oral pathogens are not completely eliminated. So the next treatment method to eliminate the pathobionts will be the use of antimicrobial agents. But the emergence of drug resistance possess serious threats in controlling the infection, and worsened the treatment regimens. Natural drugs could represent a remarkable approach to limit the emergence and spread of multi-drug resistant organisms. Phytochemical based herbal extracts find great application in dentistry as anti plaque agents, analgesics, antimicrobials etc. and hence can reduce caries, gingivitis and periodontitis[11].

A detailed literature review of plants suggested potent antibacterial activity of many traditional medicinal plants, of which *Psidium* guajava depicted exceptional activity along with anti-inflammatory and antioxidant activity. In this context, a comparative analysis was performed to evaluate the anti-cariogenic potential of *Psidium guajava* and *Azadirachta indica* extracts. The study was carried out to investigate the antibacterial activity and biofilm inhibition activity of *Psidium guajava* plant leaf extracts against ***oral*** pathogens. *Azadiracta indica* extract was taken in the present study to compare the efficacy pattern.

MATERIALS AND METHODS

The test organism used was *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 10231). Growth of bacterial and fungal culture adjusted according to McFarland Standard, 0.5%. The test organisms were maintained in BHI medium and PDA medium under sterile conditions.

Preparation of plant extracts

Ethanolic extract was prepared by cold percolation method[12]. The plant material (Leaves) were dried in open air protecting the area from direct exposure to sunlight.20-gram dried plant leaves crushed in sterile pestle & mortar and suspended in 50 ml 80% Ethanol overnight in rotary shaker in sterile conditions. The suspended extract was then filtered using Whatman filter paper no.1.

Phytochemical analysis

Phytochemicals, chemical compounds that occur naturally in plants, are responsible for color and biological properties. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients. The phytochemical analysis was carried out by method as described by Harborne *et al* [5] on alcoholic extract of plant.

Estimation of Alkaloids

Dragandroff's test

8gm of Bi(No3)3. 5H2O was dissolved in 20 ml HNO_3 and 2.72g of potassium iodide in 50 ml H2O. These were mixed and allowed to stand. When KNO_3 crystals out, the supernatant was discarded off and made up to 100 ml with distilled water. The alkaloids were regenerated from the precipitate by treating with Na_2CO_3, followed by extraction of the liberated base with ether.

To 0.5ml of alcoholic solution of extract added to 2.0 ml of HCl. To this acidic medium, 1.0 ml of reagent was added. An orange red precipitate produced immediately indicates the presence of alkaloids.

Estimation of flavanoids

In a test tube containing 1 ml of alcoholic extract, 5-10 drops of dilute HCl and a small piece of $ZnCl_2$ or Mg were added, and the solution was boiled for a few minutes. In the presence of flavonoids, reddish-pink, or dirty brown color was produced.

Estimation of Phenol

To 2 ml of alcoholic solution of extract, 2 ml of distilled water followed by drops of 10% aqueous solution of FeCl3 solution were added. The formation of blue or green color indicates the presence of phenols**.**

Antibacterial Assay

The antimicrobials present in the samples were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Petri plates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Enterococcus faecalis* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter, and different concentrations of the sample such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin (10mg/ml) was used as a positive control.

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Potato Dextrose agar plates were prepared, and lawn culture was made with an overnight grown culture of *Candida albicans***.** Wells of approximately 10mm was bored using a well cutter, and test samples of different concentrations such as 250µg/mL, 500µg/mL, and 1000µg/mL were added into the well.

Determination of Minimal Inhibitory Concentration

The MIC of the test compound was determined in accordance with the NCCLS guidelines. Minimal inhibitory concentration (MIC) was determined by using two-fold serial dilution method[13]. The growth of stock inoculum was adjusted to 1% McFarland Standard. The broth dilution assay was done in 96 well microtiter plates. Each wells in the plate were added with 100µl of the diluted (two times) conidial inoculum suspensions and bacterial suspension, the final volume in each well was 200 µl.

The sample was dissolved in DMSO to a final concentration of 10mg/mL and was added in increasing concentration such as 125µg, 250µg, 500µg, 1000µg to the wells respectively and incubated overnight at room temperature. A control well was kept with the organism alone. Growth was observed by visual inspection and by measuring the optical density (OD) at 630 nm using an ELISA plate reader[14].. The OD was measured immediately after the visual reading. The formula determined the growth inhibition for the test wells at each extract dilution:

Percentage of inhibition = [(OD of control - OD of test)/ (OD of control)]× 100

Bacterial biofilm preparation

Bacterial inoculum of *Enterococcus faecalis* was prepared, and growth was adjusted to 0.5% McFarland standard. Nutrient broth was prepared, sterilized by autoclaving at 121°C 15lbps for 15mins and was dispersed to each well in a 12 well plate. 20µl of inoculum was added to each well, and the plate was kept for incubation at 37°C for 72 hours for the formation of biofilm After 72 hours, the biofilm was added with different concentration of the sample (Ethanolic extract of Neem) such as 250µg, 500µg, and 1000µg. The control well was kept without the addition of sample. The plate was again kept in incubator for 24 hours.

Crystal violet assay

Biofilm inhibition activity detected by Crystal violet assay[15]. The micro titre wells were rinsed three times with sterile distilled water to remove unattached bacterial cells. They were air-dried at room

temperature and adherent bacteria were stained with 200 µl of 1% w/v of aqueous solution of crystal violet for 20 minutes. The wells were then rinsed three times with distilled water to remove the dye and dried. Stained adherent cells were detached from the walls of the wells using 300 µl of DMSO (Dimethyl sulphoxide) and the absorbance was read at 600nm.

Live Dead Assay Using Ethidium Bromide (Etbr) /Acridine Orange (Ao) Double Staining

Lethality was confirmed by Live Dead Assay Using Ethidium Bromide (Etbr) /Acridine Orange (Ao) Double Staining [16].The biofilm formed on wells were washed by cold PBS and then stained with a mixture of AO (100µg/ml) and EtBr (100µg/ml) and incubated in dark at 37ºC. The stained biofilms were washed twice with PBS and observed by a fluorescence microscope in blue filter of fluorescence microscope (Olympus CKX41 with Optika Pro5 camera).

RESULT

The present study was done to investigate the anti-cariogenic potential of *Psidium guajava* plant leaves extract against the oral bacteria *Enterococcus faecalis*, which is a prominent bacterium in the formation of endodontic biofilms and dental caries. A comparative evaluation of the antibacterial and antibiofilm activity of Ethanolic extracts of *Psidium guajava* plant leaf extract and *Azadiracta indica* plant leaf extract were carried out against the oral pathobionts: *Enterococcus faecalis* and *Candida albicans*. The plant leaf extracts showed good activity against the oral pathobionts, and significant inhibition of biofilm was recorded.

Phytochemical analysis of the plant extract was carried outto analyse the presence of bioactive compound*.* In the present study, a primary phytochemical analysis was carried out to identify the active constituents such as alkaloids, flavonoids, and phenols present in the leaves of plant extracts. The dried and powdered leaves of *P. guajava& Azadiracta indica* were dissolved in DMSO, and the extracts thus obtained were analyzed for the presence or absence of secondary metabolites[17],[18]. The experiments conducted in plant leaf extracts for the analysis of phytochemicals showed the following results.

Phytochemical analysis of plant extracts revealed a higher percentage of alkaloids in *Psidium guajava* plant extract, and *Azadiracta indica* extract showed a higher percentage of flavonoids. The medicinal value of these plants lies in some bio active substances that produce certain biological action on the human body. The most significant of these bioactive components of plants are alkaloids, tannins, flavonoids, and phenolic compound[19]. Table 1 shows the phytochemical analysis of herbal extracts used in the present study.

In order to assess the antibacterial activity, ethanolic extract of *Psidium guajava* and *Azadiracta indica* leaves were tested against test organism *Enterococcus faecalis and Candida albicans* **.** *Psidiumguajava plant* leaf extract showed potent antibacterial activity against *Enterococcus faecalis***,** studied by agar diffusion assay*.* The Table-2, 3 shows the antibacterial & antifungal activity of ethanolic extracts of *Psidium gujava and Azadiracta indica* leaves against *Enterococcus faecalis and Candida albicans.* The zone of inhibition was found to be more in *Psidium gujava* leaf extract, 12mm, compared with 11mm zone for *Azadiracta indica* extract for *Enterococcus faecalis* and more or less similar zone of inhibition for *Candida albicans* ranging between 11mm and 12mm. Antibacterial assay suggests the potent ability of *Psidium gujava* plant leaf extract against the oral pathogens, which can be a promising aid in the therapeutic scenario of orodental infection.

The MIC of the test compound was determined in accordance with the NCCLS guidelines[20]. The standard protocol to evaluate the antibacterial activity is MIC. The minimum inhibitory concentration is the lowest concentration of a chemical, which prevents the visible growth of a bacterium. The minimal inhibitory concentration of plant extracts was calculated using ED50 PLUS V1.0 software. Guava leaf extract showed a minimum inhibitory concentration of 461.86µg/mL, and *Azadiracta indica* showed a MIC value of 401.522µg/mL. Table 4shows the MIC value of plant extracts against the test organism. From the above results, it is evident that the ethanolic extract of *Psidium gaujava* plant leaf possess significant antibacterial activity and can be used as an alternative source in treatment strategies.

The active ingredient of the ethanolic extract of *Psidium gujava* leaves showed positive anti- adherence effect on the *Enterocuus faecalis* biofilm formation[5]. This active plant extract was found to inhibit the biofilm formation, and the absorbance was read at 600nm. Percentage of inhibition of biofilm at a concentration of 1mg/ml of ethanolic extract was found to have appreciable activity against *Enterococcus faecalis.* Both plant extracts showed significant biofilm inhibition activity. Table 5 shows the biofilm inhibition activity of plant extracts against test organisms*. Enterococcus faecalis* is a common endodontic biofilm forming oral bacteria which plays a major role indental caries and persistent endodontic infections[17]. Inhibition of biofilm formation is the primary step in controlling persistent endodontic infections. Lethality was confirmed by a live dead assay using EtBr/AO staining (Figure 3).

Table 1: Phytochemical Analysis of plant extracts

TEST	INFERENCE
Sample 1: Ethanolic Extract of *Psidium guajava*	
1. ALKALOIDS	(+ + +)
2. FLAVANOIDS	(-)
3. PHENOLS	(+ +)
Sample 2: Ethanolic Extract of *Azadirachta indica*	
1. ALKALOIDS	(+ +)
2. FLAVANOIDS	(+ + +)
3. PHENOLS	(-)

Table 2: Antibacterial Activity Of Plant Extracts against *Enterococcus faecalis*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Ethanolic extract of *Psidium guajava* extract	Streptomycin (100µg)	23
	250	NIL
	500	NIL
	1000	12
Ethanolic extract of *Azadirachta indica*	250	NIL
	500	NIL
	1000	11

Table 3: Antifungal Activity of Plant Extracts against *Candida albicans*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Ethanolic extract of *P.guajava*	Clotrimazole(100µg)	27
	250	NIL
	500	NIL
	1000	11
Ethanolic extract of *A.indica*	250	NIL
	500	NIL
	1000	12

Table 4: Minimal Inhibitory Concentration

Organism: *Enterococcus faecalis*	
Concentration (µg/mL)	**Percentage of Inhibition**
Control	0.00
Sample 1: Ethanolic extract of *P.guajava*	
125	32.85
250	37.73
500	53.17
1000	77.69
Sample 2: Ethanolic extract of *A.indica*	
125	21.91
250	37.87
500	58.64
1000	72.08

Table 5: Antibiofilm Activity- Crystal Violet Assay

Concentration (µg/mL)	Percentage of Inhibition
CONTROL	0.00
Sample 1: Ethanolic extract of *P.guajava*	
250	18.00
500	37.94
1000	43.26
Sample 2: Ethanolic extract of *A.indica*	
250	11.81
500	18.74
1000	45.40

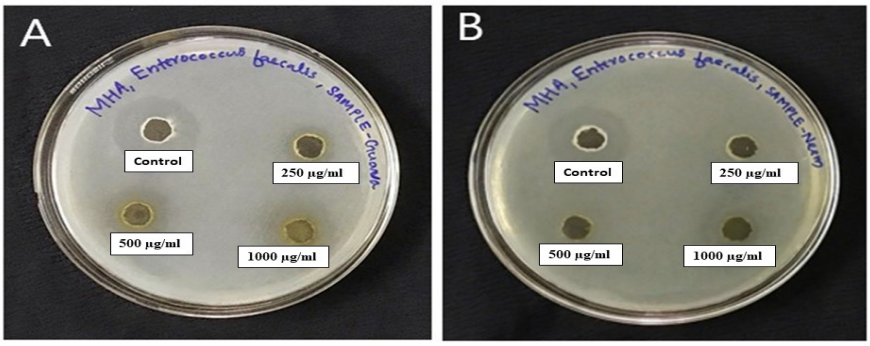

Figure 1: antibacterial assay for Enterococcus faecalis
A) zone of inhibition due to *Psidium gujava* extract
B) zone of inhibition due to *Azadiracta indica* extract

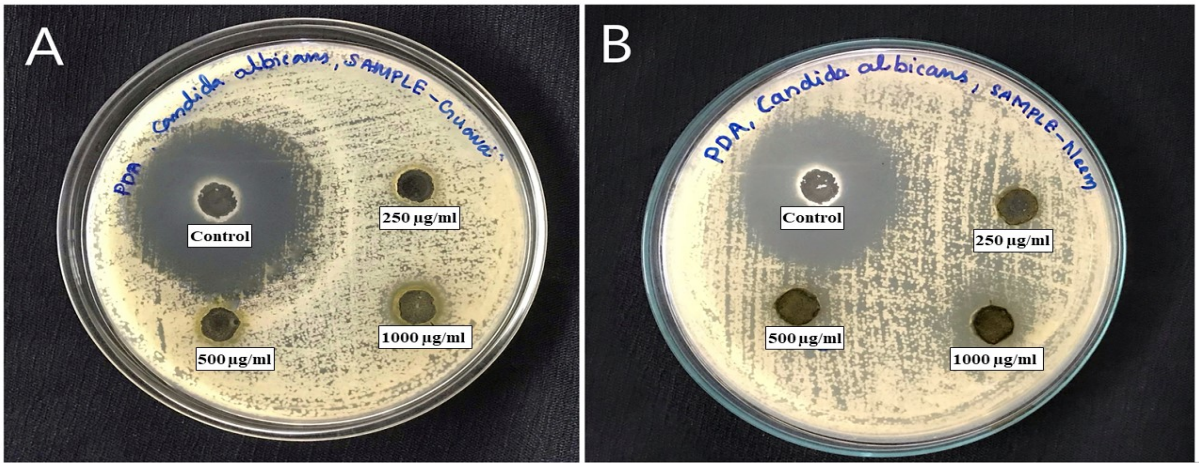

Figure 2: Antifungal assay of plant extract against *Candida albicans* a.zone of inhibition by *Psidium gujava* *extract* ; b.zone of inhibition by *Azadiracta indica* *extract*

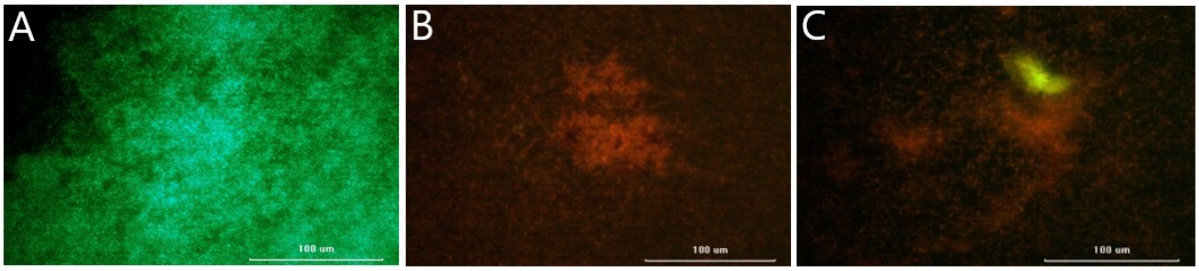

Figure 3: live dead assay of plant extract A: control B: live dead assay *of P.guajava* leaf extract C:live dead assay of *A.indica* leaf extract

DISCUSSION

The emergence of antibiotic resistance among pathogenic bacteria encourages us to search for new molecules as an alternative treatment[21]. The application of antibiotics for the prevention of dental caries is not recommended since there is a risk of development of MDR(multiple drug resistance)[5]. In this connection, the last few decades have witnessed wide investigations, focused to identify natural products with antibiofilm activity[22]. By following the previous studies, it is evident that over-usage of antibiotics has adverse side effects. Detailed literature review of plants suggested potent antibacterial activity of many traditional medicinal plants, of which *Psidium guajava* depicted exceptional activity along with anti-inflammatory and antioxidant activity. In the present study, ethanolic extract of *Psidium guajava* plant leaves showed significant antibacterial activity, confirmed by evaluating MIC and potent antibiofilm activity. Lethality was confirmed by alive dead assay using EtBr/AO staining. DNA binding dyes AO and EtBr purchased from Sigma, USA were used for the morphological detection of apoptotic and necrotic cells. AO is taken up by both viable and nonviable cells in biofilm and emits green fluorescence if

intercalated into double stranded nucleic acid (DNA). EtBr is taken up only by nonviable cells and emits red fluorescence by intercalation into DNA[16]. In the present study the lethality rate of test organism was high with ethanolic extract of *Psidium gujava* plant leaf extract. Hence the study suggests the use of *Psidium gujava* plant leaf extracts as an alternative antiplaque agent, and can be used to prevent dental caries.

A detailed review of plant products by Song *et al* [23], revealed clear evidences that plant derived natural bioactive compounds are an excellent source to provide preventative and therapeutic substance against biofilm-based infections. However, emergence of drug-resistance necessitates the need for identifying new antimicrobial substances, and herbal based bioactive compounds may act as an alternative for antibiotics[5] and chemotherapeutic agents[24]. The results showed that the ethanolic extract of *P. guajava* leaf was able to inhibit the bacteria and fungi used in this study with different degrees of inhibition. The information obtained from the study may provide validation for its reported medicinal uses in dentistry[25].

CONCLUSION

In conclusion, the *P.guajava* leaf ethanolic extract is most effective against the tested bacterial strains than the fungal strains[26]. As quoted by John et al., 2013, the data gathered in this study may assist in the formulation of these extracts as anti-plaque agents in oral care products. Such products that combine traditional therapy with the latest research will have broader markets due to its nature-based approach and low cost[27].

The study confirms the anti-cariogenic potential of *P. guajava* extracts, which can find applications in therapeutic regimens. The present study is only a qualitative analysis of the traditional medicinal plant *Psidium gujava*. The study recommends further investigations on the quantitative analysis of the various bioactive compounds present in guava which could contribute significantly to the therapeutic scenario of orodental infections[28].

COMPETING INTRESTS

The authors have declared that no competing interest exists.

REFERENCES

1. Nazir, M. A. (2017). Prevalence of periodontal disease, its association with systemic diseases and prevention. *International Journal of Health Sciences*, *11*(2), 72.
2. Tahir, L., & Nazir, R. (2018). Dental Caries, Etiology, and Remedy through Natural Resources. *Dental Caries: Diagnosis, Prevention and Management*, 19.
3. Sharma, D., Misba, L., & Khan, A. U. (2019). Antibiotics versus biofilm: An emerging battleground in microbial communities. *Antimicrobial Resistance and Infection Control*, *8*.
4. Gurenlian, J. R. (2007). The Role of Dental Plaque Biofilm in Oral Health. *Journal of Dental Hygiene*, *81*(5), 11.
5. Gomashe, A. V., Sharma, A. A., & Kasulkar, A. (2014). *Investigation of Biofilm Inhibition Activity and Antibacterial Activity of Psidium guajava Plant Extracts against Streptococcus mutans Causing Dental Plaque*.
6. Takenaka, S., Ohsumi, T., & Noiri, Y. (2019). Evidence-based strategy for dental biofilms: Current evidence of mouthwashes on dental biofilm and gingivitis. *The Japanese Dental Science Review*, *55*(1), 33–40.
7. Chenicheri, S., Usha, R., Ramachandran, R., Thomas, V., & Wood, A. (2017). Insight into oral biofilm: Primary, secondary and residual caries and phyto-challenged solutions. *The Open Dentistry Journal*, *11*, 312.
8. Jayaraman, A., & Wood, T. K. (2008). Bacterial quorum sensing: Signals, circuits, and implications for biofilms and disease. *Annu. Rev. Biomed. Eng.*, *10*, 145–167.
9. Whiteley, M., Diggle, S. P., & Greenberg, E. P. (2018). Correction: Corrigendum: Progress in and promise of bacterial quorum sensing research. *Nature*, *555*(7694), 126–126.
10. Jiang, Q., Chen, J., Yang, C., Yin, Y., & Yao, K. (2019). Quorum Sensing: A Prospective Therapeutic Target for Bacterial Diseases. *BioMed Research International*, *2019*, 1–15.
11. Pandita, V., Patthi, B., Singh, S., Singla, A., Vashishtha, V., & Malhi, R. (2014). Dentistry meets nature-role of herbs in periodontal care: A systematic review. *Journal of Indian Association of Public Health Dentistry*, *12*(3), 148.
12. Parekh, J., & Chanda, S. (2007). In vitro antibacterial activity of the crude methanol extract of Woodfordia fruticosa Kurz. Flower (Lythraceae). *Brazilian Journal of Microbiology*, *38*(2), 204–207.
13. Kang, D. H., & Siragusa, G. R. (2001). A rapid twofold dilution method for microbial enumeration and resuscitation of uninjured and sublethally injured bacteria. *Letters in Applied Microbiology*, *33*(3), 232–236.
14. Tan, H. T., Rahman, R. A., Gan, S. H., Halim, A. S., Hassan, S. A., Sulaiman, S. A., & Bs, K.-K. (2009). The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complementary and Alternative Medicine*, *9*(1), 34.
15. Shukla, S. K., & Rao, T. S. (2017). An Improved Crystal Violet Assay for Biofilm Quantification in 96-Well Microtitre Plate. *BioRxiv*, 100214.

16. Ribble, D., Goldstein, N. B., Norris, D. A., & Shellman, Y. G. (2005). A simple technique for quantifying apoptosis in 96-well plates. *BMC Biotechnology*, *5*(1), 12.
17. John, G., Kumar, K. P., Gopal, S. S., Kumari, S., & Reddy, B. K. (2015). Enterococcus faecalis, a nightmare to endodontist: A systematic review. *African Journal of Microbiology Research*, *9*(13), 898–908.
18. Kumar, S., Gayathri, K., Kripa, K. G., & Prathyusha, T. (2018). Preliminary Phytochemical Analysis and In vitro Pharmacological Evalua-tion of Phytosterol rich fraction from Vetiveria zizanioides Nash. *International Journal of Research in Pharmaceutical Sciences*, *9*(3), 922–930.
19. Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, *4*(7), 685–688.
20. Swenson, J. M., Ferraro, M. J., Sahm, D. F., Clark, N. C., Culver, D. H., & Tenover, F. C. (1995). Multilaboratory evaluation of screening methods for detection of high-level aminoglycoside resistance in enterococci. National Committee for Clinical Laboratory Standards Study Group on Enterococci. *Journal of Clinical Microbiology*, *33*(11), 3008–3018.
21. Noumi, E., Snoussi, M., Merghni, A., Nazzaro, F., Quindós, G., Akdamar, G., Mastouri, M., Al-Sieni, A., & Ceylan, O. (2017). Phytochemical composition, anti-biofilm and anti-quorum sensing potential of fruit, stem and leaves of Salvadora persica L. methanolic extracts. *Microbial Pathogenesis*, *109*, 169–176.
22. X. Song, Y.-X. Xia, and Z.-D. H. and H.-J. Zhang*, "A Review of Natural Products with Anti-Biofilm Activity," *Current Organic Chemistry*, Mar. 31, 2018. http://www.eurekaselect.com/153451/article (accessed Mar. 06, 2020).
23. Song, X., Xia, Y.-X., He, Z.-D., & Zhang, H.-J. (2018). A review of natural products with anti-biofilm activity. *Current Organic Chemistry*, *22*(8), 789–817.
24. Qa'dan, F., Thewaini, A.-J., Ali, D. A., Afifi, R., Elkhawad, A., & Matalka, K. Z. (2005). The Antimicrobial Activities of *Psidium guajava* and *Juglans regia* Leaf Extracts to Acne-Developing Organisms. *The American Journal of Chinese Medicine*, *33*(02), 197–204..
25. Mahfuzul Hoque, M. D., Bari, M. L., Inatsu, Y., Juneja, V. K., & Kawamoto, S. (2007). Antibacterial activity of guava (Psidium guajava L.) and neem (Azadirachta indica A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathogens and Disease*, *4*(4), 481–488.
26. Dhiman, A., Nanda, A., Ahmad, S., & Narasimhan, B. (2011). In vitro antimicrobial activity of methanolic leaf extract of Psidium guajava L. *Journal of Pharmacy and Bioallied Sciences*, *3*(2), 226.
27. John, N. R., Gala, V. C., & Sawant, C. S. (2013). Inhibitory effects of plant extracts on multi-species dental biofilm formation in-vitro. *Int J Pharm Bio Sci*, *4*(2), 487–495.
28. Arya, V., Thakur, N., & Kashyap, C. P. (2012). Preliminary phytochemical analysis of the extracts of Psidium leaves. *Journal of Pharmacognosy and Phytochemistry*, *1*(1), 1–5.

CITATION OF THIS ARTICLE

Arya S, Smitha C, and Usha R· Evaluation of Anticariogenic Efficacy of *Psidium Guajava* extarcts *and Azadirachta indica*. Bull. Env. Pharmacol. Life Sci., Vol 9[4] March 2020 : 31-38