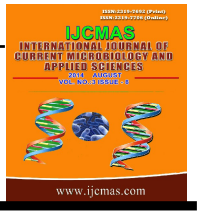
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 335-351**
http://www.ijcmas.com



Original Research Article

Investigation of Biofilm Inhibition Activity and Antibacterial Activity of *Psidium guajava* Plant Extracts against *Streptococcus mutans* Causing Dental Plaque

Ashok V.Gomashe, Anjali A. Sharma* and Ashwini Kasulkar

Department Of Microbiology, Shri Shivaji Science College Congress Nagar, Nagpur-12, Maharashtra, India
**Corresponding author*

Keywords

Psidium guajava, Biofilm, *Streptococcus mutans*, Dental caries, Plaques, Bacterial adhesion, Exopolysaccharide

A B S T R A C T

Dental caries is a localized, transmissible pathological infectious disease which results in destruction of hard dental tissue. Dental plaque when allowed to accumulate may lead to caries formation and discomfort due to the inflammation of the gingival area. Both conditions are direct consequences of poor oral hygiene measures of an individual. This begins with the formation of dental plaques which is a structurally and functionally organized biofilm. *Streptococcus mutans* is a pivotal bacterium in the formation of dental plaque and dental caries. Several antibiotics are available to treat oral infections but these have several side effects. An anti-plaque agent is an agent that causes an effect on plaque which results in a reduction in caries and/or gingivitis. Thus there is an urgent call for alternative prevention and treatment options that are safe equally effective and economical as well. Hence the search for alternative products continues which diverts our interest in natural phytochemicals isolated from plants used in traditional medicines and considered as good alternatives to synthetic chemicals. Decoctions of various plant extracts have long been used in folklore practices to maintain the hygiene of the oral cavity since ancient times. The extracts of various parts of *Psidium guajava* plant were tested for their antibiofilm activity against the oral plaque forming bacteria *Streptococcus mutans.* It was found to significantly inhibit biofilm formation. In the present study it was found that the extract from *Psidium guajava* showed strong activity against *Streptococcus mutans.* The extract also prevents the formation biofilm by the bacteria. The study suggested the possible benefits of this herbal preparation which inhibit the biofilm formation by *Streptococcus mutans* an oral pathogen. The three solvents (acetone, ethanol and methanol) based extracts of *Psidium guajava* L. showed good activity but the acetone extract was highly active against *Streptococcus mutans.* The acetone extract and ethanol extract (*Psidium guajava)* is highly active against *Streptococcus mutans*.

Introduction

Over time the irrational use of the antibacterial agents has produced strains of multiple antimicrobial resistant bacteria. Increased prevalence of antibiotic resistance has led to the introduction of combination therapy which has increased treatment

efficacy and contained drug resistance to some extent, although combination therapy provided the answer to antibiotic resistance for a while, there have been reports of emerging resistance to drugs in combination and multi-drug resistance in some bacteria . Oral biofilms are compositionally and structurally are complex bacterial communities. Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth [3]. *Streptococcus mutans* are believed to be the principal etiological agent of human dental caries. It has developed multiple mechanisms to colonize the tooth surface and has become a significant species in cariogenic biofilm.Microbial biofilm cause a variety of microbial infections on human health. Dental plaque is a biofilm that grows in the oral cavity. It is the primary cause of dental caries and other oral infections and the less common peri-implantitis (similar to periodontitis, but with dental implants), however biofilms are present on healthy teeth. Effective plaque removal procedures are expected to prevent the development of these diseases.

Despite the ever increasing knowledge in oral health care, the average adult in the country has between 10 and 17 decayed, missing or filled permanent teeth. The majority of the population has at least minor gingivitis, an inflammation of the gums, with a much smaller percentage suffering from moderate to severe periodontitis. (16) Dental plaque, which exists not only on the tooth surface but also under the gums, is a diverse community of microorganisms in the form of a biofilm. The microorganisms bind tightly to one another and to the solid surface of tooth by means of an extracellular matrix consisting of polymers of both host and microbial origin. (12) It is more prevalent in the Asian and the Latin countries. In India, nearly 60-70% of the child population is affected by dental caries. (19) The biofilm is an extracellular matrix that surrounds microbial cells and is comprised of biological polymers such as exopolysaccharide (EPS), protein, and DNA. The role of the biofilm is to attach to abiotic surfaces, the epithelia of multicellular organisms, and interfaces such as that between air and water. Surface adhesion of bacteria is an essential step and is required for the bacteria to arrange themselves favourably in their environment.

Fermentation of carbohydrate by acidogenic oral bacteria is the key factor in development of dental caries. The acid released through microbial action leads to demineralization cavitation of tooth. Streptococcus spp. can colonize the tooth surface and initiate the plaque formation by synthesizing extracellular polysaccharides from sucrose.

Biofilms enhance the virulence of the pathogen and have their potential role in various infections. The biofilm forms those are more resistant to antimicrobial agents and therefore more difficult to control, remain largely unexplored. Their inherent resistances to antimicrobial agents are at the root of many persistent and chronic bacterial infections. Biofilms have been reported to be less susceptible to antimicrobial agents and have reduced sensitivity to inhibitors. The resistance shown by biofilm to various antibiotics is a matter of concern. In recent years, much of research has been focused in identifying various alternative medicines to treat infections caused by the drug resistant bacteria. Various chemicals have been tested for their antibiofilm activities. Unfortunately, those chemicals cannot be used as drug molecules to treat the diseases associated with the biofilm forming bacteria.(6) The bacterial biofilms are

creating problems as they attach to surfaces of some dental alloys, impression materials, dental implants, restorative and cement materials. Biofilms are difficult or impossible to destroy, particularly those cells that form the deeper layers of a thick biofilm. The insertion of orthodontic appliances tends to create new surfaces available for plaque formation and to increase the level of microorganisms in the oral cavity. Gaps between synthetic and natural elements are dangerous sites which may be colonized by bacteria. [5] Orthodontic patients with fixed appliances frequently have prevalence of *Streptococcus mutans* in plaque compared with untreated orthodontic patients [6].

These microorganisms have an important role in cariogenesis processes and consequently the study of the growth of this 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. The EPS is a major feature of biofilms and is believed to play a major role in their resistance. [4] Biofilm formation is said to be under genetic control. HtrA gene has a significant role in surface protein expression and biofilm formation by *Streptococcus mutans*. The formation of dental plaque biofilms includes a series of steps that begins with the initial colonization of the pellicle and ends with the complex formation of a mature biofilm.

They communicate via small diffusible signaling molecules (e.g. competence-stimulating peptide, CSP; auto inducer); CSP induces both genetic competence and acid tolerance in recipient sessile cells. The bacteria that become part of a biofilm engage in quorum sensing [14] Communication can occur between cells in biofilm communities in a variety of ways, including gene expression, cell-cell signaling (ex. quorum sensing) [10] and antibiotic resistance. These specific cell-cell interactions have proven to be important in dental plaque biofilm. [13] Biofilm communities provide their members easy access to food and nutrients and allow the cells inside, becoming more resistant to the body's natural antimicrobials as well as the antibiotics which are administered. During inhospitable conditions such as nutrient starvation, microbes in biofilm communities have the ability to enter into latent stages in which organisms are viable but in non culturable state.

Several agents are commercially available, for example the antibiotics commonly used to treat oral infections i.e. Penicillins and Cephalosporins, Erthromycin, Tetracycline and derivatives, and Metronidazole have been documented [1]. These chemicals can alter oral microbiota and have undesirable side effects such as vomiting, diarrhea, and tooth staining [9]. Other antibacterial agents used in the prevention and treatment of oral diseases including Cetypridinium chloride, Chlorhexidine, amine fluoride or products containing such agents are reported to exhibit toxicity, cause staining of teeth or in the case if ethanol (commonly found in mouth washes) have been linked to oral cancer[5]. Given the incidence of oral disease, increased resistance by bacteria, to antibiotics adverse effects of some antibacterial agents currently used in dentistry and financial considerations in developing countries, there is a need for alternative prevention and treatment options that are safe, effective and economical. Hence the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals [11]. It has been well documented that medicinal plants confer

antimicrobial activity towards oral bacteria. Plant extracts or phytochemicals that inhibit the growth of oral pathogens, reduce the rate of biofilms and dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases can serve as alternatives in prevention and treatment of dental caries.

Psidium guajava belongs to family *Myrtaceae* with tough dark leaves that are opposite, simple, elliptic to ovate and 5–15 centimeters (2.0–5.9 in) long. The flowers are white, with five petals and numerous stamens. Medical research in laboratory models, extracts from guava leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain. [(5)] It is a small tree, various parts of which exhibit antioxidant, hepatoprotective, antimicrobial and anti diabetic properties.[4] Present study is therefore focusing on identifying plants that can be used as an herbal alternative to chemical drugs to treat dental infections. We are studying the efficacy of *Psidium guajava* plant parts extracts against dental plaque forming *Streptococcus mutans*.

Materials and Methods

Media used for antibacterial assay was Muller Hinton Agar (HiMedia) and Media for biofilm formation and inhibition assay was Brain Heart Infusion medium which was purchased from HiMedia Laboratories, Mumbai, India. Test organism used for the study was *Streptococcus mutans* MTCC 890 procured from Institute of Microbial Technology, Chandigarh, India. The activity of various parts (Leaves, Flowers, Bark, and Roots) of *Psidium guajava* plant was studied in different solvents such as Hot water, Acetone, Methanol, Ethyl acetate and Ethanol.

The present research work was designed to evaluate biofilm inhibition and antibacterial activity of medicinal plant *Psidium guajava*. The plant material was authenticated from the Department of Botany of the college. The study was carried out in the duration of August 2013 to February 2014 in The Department of Microbiology, Shivaji Science College Nagpur, Maharashtra, India. In this study, there were following steps which are as follows:

Preparation of plant extracts

All the plant materials (Leaves, flowers, barks and roots) were dried in open air protecting that area from direct exposure to sunlight. The dried plant parts were crushed in sterile pestle mortal and suspended in Hot water, Acetone, Methanol, Ethyl acetate, Ethanol overnight in sterile conditions. The suspended extract was then filtered using Whatman's filter paper no.1.
The extracts were prepared as follows:

Preparations of Hot water extract (HWE)

Hot water extract was prepared by plain decoction method. For this 30 gm of powdered of each plant materials (leaves, flowers, barks and roots) were taken into beaker containing 300 ml of sterile distilled water. This was heated moderately in water bath till menstrum reduced to less than $1/4^{th}$ of its original volume (approx. to 75ml). The water content from extract was evaporated off completely to achieve dried form of extract and the resulting liquid was filtered using Whatman's filter paper no.1.

Preparation of Acetone / Methanolic/ Ethyl acetate/ Ethanol Extract

All the plant material was dried in open air protecting that area from direct sunlight. The dried plant parts were crushed in sterile pestle mortal as stated above. 100 mg of

each powdered plant material was extracted with Acetone/ Methanolic/Ethyl acetate/ ethanol by dipping in it for 24 hrs, resulting liquid was filtered using Whatman's filter paper no. 1.

Antibacterial assay of plant extracts by agar well diffusion method

The extracts (Hot water, Acetone, Methanol, Ethyl acetate, Ethanol) were tested for antibacterial activity using agar diffusion on Mueller Hinton Agar. The Media was sterilized by autoclaving at 121^{o} C at 15 lbs pressure for 15 minutes. The molten agar was allowed to cool to 45 ˚C and then 20ml of Mueller Hinton agar was poured aseptically into Petri plates. The agar was allowed to set and harden. Agar test plates of each test organism were prepared. Using a sterile swabs lawn of the test organism was spread onto the Mueller Hinton agar plates. The wells were punctured in the centre by using a sterile cork borer. Then the wells were filled with various extract containing leaves, flowers, bark, and roots. The plates were incubated at 37^{o}C for 24 hrs. After incubation the plates were observed for the zone of inhibition. The zones were measured using zone measuring scale.

Detection of biofilm formation

Brain Heart Infusion broth containing 1% D-glucose was prepared and 3 ml of the broth was transferred into screw cap tube. The broth was then sterilized by autoclaving at 121^{o} C at 15 lbs pressure for 15 minutes. The screw cap tubes were then inoculated with 30 µl of overnight grown culture of *Streptococcus mutans*. The tubes were then tilted at an angle of 30^{o} and incubated at 37^{o}C for 18 hrs. After incubation the supernatant was carefully decanted without disturbing the adhering cells. The tube containing biofilm were washed with saline solution i.e., 0.85% NaCl 2-3 times to separate the cells adhered to glass surface. 1 % Crystal violet solution was added to observe for the adhered cells.

To study the biofilm inhibition activity

Brain Heart Infusion broth containing 1% D-glucose was prepared and 30 µl of the broth was transferred into the microtitre plates. The broth was then sterilized by autoclaving at121^{o} C at 15 lbs pressure for 15 minutes. The microtitre plate was then inoculated with 3 µl of overnight grown culture of *S. mutans* MTCC 890 strain. The extracts (Hot water, Acetone, Methanol, Ethyl acetate, Ethanol) containing leaves, flowers, bark, roots were added into each wells. The plate was then incubated at 37^{o}C for 18 hrs. After incubation the supernatant (non adherent cells) was carefully decanted without disturbing the adhering cells. The tube containing biofilm were washed with saline (0.85% NaCl). Then 30 µl saline was added and mixed well to separate the cells adhered to surface and to remove loosely attached cells. 1 % Crystal violet solution was added to observe for the adhered cells. The optical density was measured at 550 nm using a spectrophotometer.

Results and Discussion

Streptococcus mutans is the important bacterium in the formation of dental plaque and dental caries. Various extracts of a plant *Psidium guajava* were tested for their antibacterial activity against the oral plaque forming bacteria *Streptococcus mutans.* They were found to significantly inhibit biofilm formation. In present study it was found that the extract from *Psidium guajava* showed strong activity against *Streptococcus mutans.* The extract also prevents the formation of biofilm by *Streptococcus mutans*. The study was carried out to

investigate the antibacterial activity and biofilm inhibition activity of *Psidium guajava* plant parts extracts against *Streptococcus mutans.* For this purpose, various extracts such as Hot water extracts, Acetone extracts, Methanol extracts, Ethyl acetate extracts, and Ethanol extracts of parts of plant *Psidium guajava* were tested against test organism *Streptococcus mutans.* Extracts of *Psidium guajava* showed the potent antibacterial activity against *S. mutans* studied by an agar diffusion assay. The Table-1 showing the antibacterial activity of Hot water extract against *S. mutans.* The zone of inhibition was found to be more in leaves (20mm), in flowers (17mm), but less zone in bark (13mm) and in roots (12mm) (Graph-1) (Figure 1-2)

The active plant extracts of the plant showed positive anti- adherence effect on the *S. mutans* biofilm formation on the glass surface in the presence of 1 % dextrose. These active plant extract was found to inhibit the biofilm formation on the glass surface and showed decrease in the turbidity when the OD was taken at 550 nm. (Table 6-10) (Graph 6-10)

Streptoccous mutans is the most important oral bacteria which plays a major role in dental caries, bacteremia and consequently bacterial endocarditis[14]. Application of antibiotics for prevention of dental caries is not recommended, since there is risk of development of MDR (multiple drug resistance) strains. The use of plants and their extracts in the treatment of diseases dates back to 460-370 BC when Hippocrates practiced the art of healing by use of plant based drugs. Different plants and their parts (flowers, bud, leaves, stem, bark, fruits, pulp and roots) have been used for thousands of years[8] . *S. mutans* were found to form biofilm on the surface of screw cap tube in the presence of 1% dextrose. In this study plant extracts of *Psidium guajava* has significant antibacterial activity and thus can be employed as an effective anti plaque agent and can be used in the prevention of dental caries. The plant extract was evaluated for antistreptococcal and antibiofilm activity against *S. mutans. Psidium guajava* was found to be active against *Streptococcus mutans* also has potential antibiofilm activity. Currently, there is an increasing interest to investigate the effect of natural compounds, especially plants extracts, on the residence of the oral cavity. It has been reported that *Morus alba*,[9]*Andrographis paniculata* and Chinese black tea[6], *cranberry* [15] and *Mikania* sp. [16] exhibited potentially useful antibacterial properties towards some oral pathogens. The present study has shown that the extract of *Psidium* guajava exhibited antibacterial activities. The selection of this plant was based on findings that their extracts exhibited antibacterial activities against oral microbes grown under the planktonic state [13].The planktonic state refers to the condition where the bacteria were allowed to grow as suspension in the test tubes. *P. guajava* can significantly disrupt the adhesion of the early plaque colonizers to the pellicle. This subsequently will interfere with the initial stage of biofilm development. Similar observations have also been reported by Percival et al [10]. who strongly acknowledged the importance of the salivary pellicle during the initial stages of biofilm formation. The property of the pellicle can be altered in the presence of certain plant extracts. The many positive antimicrobial activities on oral bacteria exhibited by plant extracts provide great support in the promotion of such extracts as oral healthcare agents. Their use may help to moderate the development of dental plaque so that its texture is always thin and porous. Besides, mouthwash sold in pharmacy stores or local supermarkets are either contains many chemicals or alcohol-based which

may cause unwanted side effects to the consumers.

Streptococcus mutans adheres by hydrophobic bond interaction to the enamel surface. Therapeutic agents, which help to prevent hydrophobic bond formation, would help to reduce the incidence of caries[7]. The leaf extract of *Psidium guajava* showed a broad spectrum antibacterial activity against Gram-negative bacteria. The previous findings that Gram-negative bacteria are hardly susceptible to the plant extracts in doses less than 2 × 105 µg/ml [61]. The variation of susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts. The three solvents (acetone, ethanol and methanol) based extracts of *Psidium guajava* L. showed good activity but the acetone extract was highly active against *Streptococcus mutans* [61]. The results in the present study has showed that the acetone extract and ethanol extract (*Psidium guajava)* is highly active against *Streptococcus mutans*. The similar antibacterial effect was described by Rathish Nair et al [12] where they reported that the two solvents acetone and methanol based extracts of *Psidium guajava* leaves showed good activity but acetone extract was highly active against *S. mutans*

Fathilah et. al. [2] has reported that the aqueous extracts of *P. guajava* have caused a reduction in the adhesion of the early plaque settlers. The present study showed that *Psidium guajava* has excellent antibacterial and biofilm inhibition activity against *Streptococcus mutans*.

Table-1: Antibacterial activity of Hot water extract against *S. mutans:*

Hot water extract	Zone of inhibition in mm
Leaves	20
Flowers	17
Bark	13
Roots	12

Graph-1 Antibacterial activity of Hot water extract against *S. mutans:*

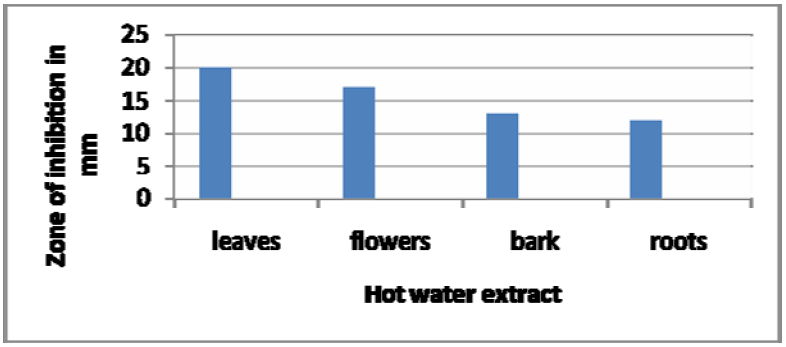


Figure-1
Antibacterial activity of Hot water extract of guava leaves against *Streptococcus mutans*

Figure-2
Antibacterial activity of Hot water extract of guava flowers against *Streptococcus mutans*

Table-2: Antibacterial activity of Acetone extract against *S. mutans:*

Acetone extract	Zone of inhibition in mm
Leaves	28
Flowers	15
Bark	12
Roots	R

Where, R= Resistant

Graph-2: Antibacterial activity of Acetone extract against *S. mutans*

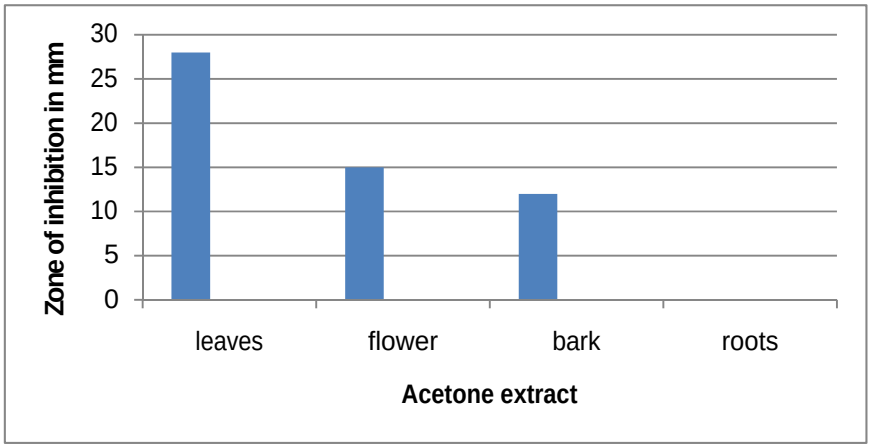


Figure-3 Antibacterial activity of Acetone extract of guava leaves against *Streptococcus mutans*

Figure-4 Antibacterial activity of Acetone extract of guava flowers against *Streptococcus mutans*

Table.3 Antibacterial activity of Methanol extract against *S. mutans:*

Methanol extract	Zone of inhibition in mm
Leaves	15
Flowers	12
Bark	13
Roots	10

Graph.3 Antibacterial activity of Methanol extract against *S .mutans:*

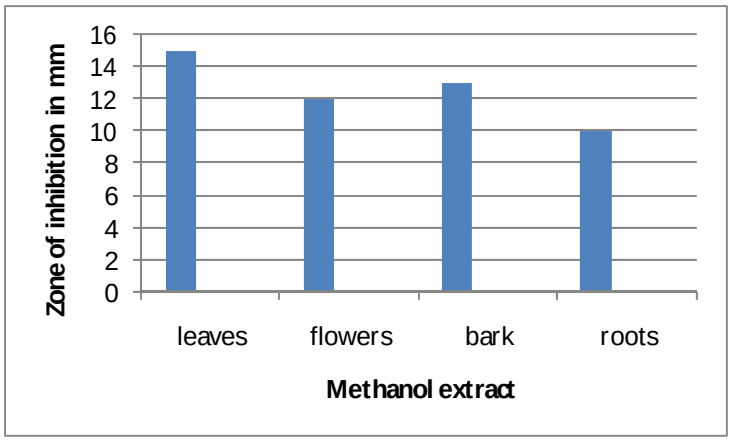


Figure-5 Antibacterial activity of Methanol extract of guava leaves against *Streptococcus mutans*

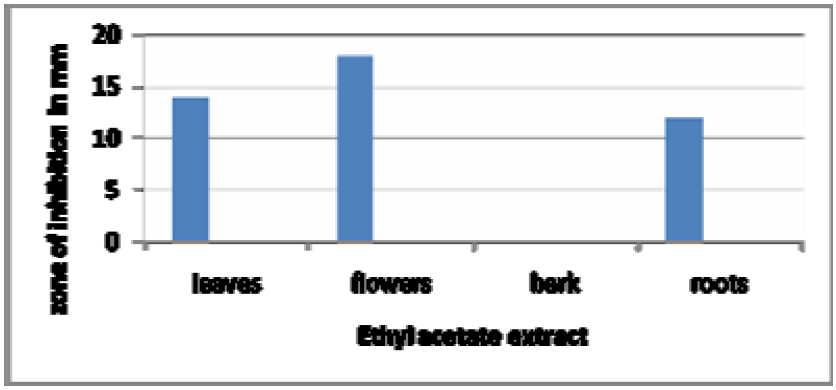
Figure-6 Antibacterial activity of Methanol extract of guava flowers against *Streptococcus mutans*

Figure-7 Antibacterial activity of Methanol extract of guava bark against *Streptococcus mutans*

Table.4 Antibacterial activity of Ethyl acetate extract against *S. mutans*

Ethyl acetate extract	Zone of inhibition in mm
Leaves	14
Flowers	18
Bark	R
Roots	12

Graph.4 Antibacterial activity of Ethyl acetate extract against *S. mutans*



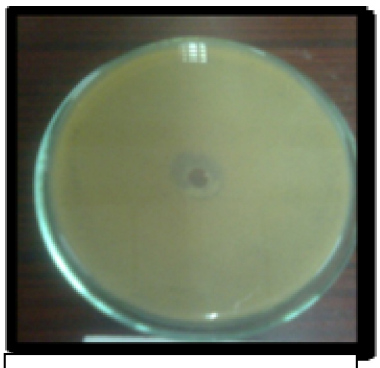


Figure-8 Antibacterial activity of Ethyl acetate extract of guava leaves against *Streptococcus mutans*

Figure-9 Antibacterial activity of Ethyl acetate extract of guava flowers against *Streptococcus mutans*

Figure-10 Antibacterial activity of Ethyl acetate extract of guava roots against *Streptococcus mutans*

Table.5 Antibacterial activity of Ethanol extract against *S. mutans*

Ethanol extract	Zone of inhibition in mm
Leaves	15
Flowers	17
Bark	10
Roots	11

Graph.5 Antibacterial activity of Ethanol extract against *S. mutans*

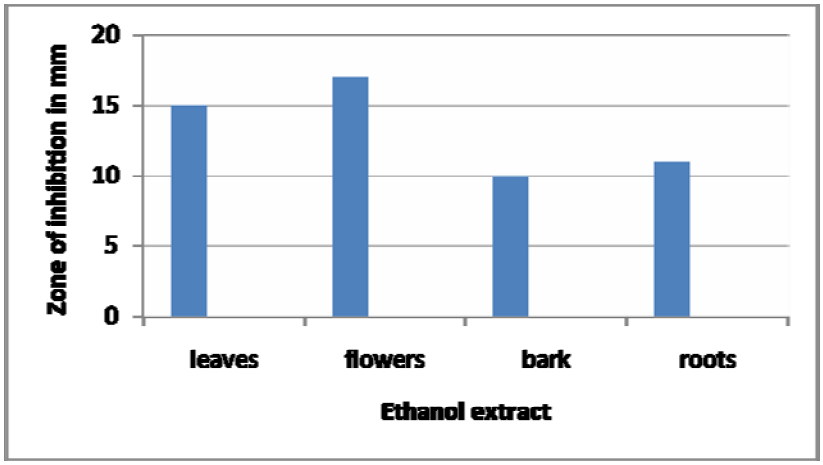


Figure-11
Antibacterial activity of Ethanol extract of guava leaves against *Streptococcus mutans*

Figure-12
Antibacterial activity of Ethanol extract of guava flowers against *Streptococcus mutans*

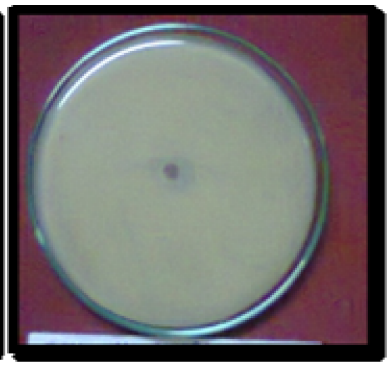


Figure-13
Antibacterial activity of Ethanol extract of guava roots against *Streptococcus mutans*

Figure-14 Formation of biofilm by *Streptococcus mutans*

Table.6 Comparison of OD before and after addition of Hot water extract

Well no.	OD before addition of extract (OD at 550nm)	After addition of Hot water extract	
		Part of plant used	OD at 550 nm
1	0.85	Leaves	0.40
2	0.65	Flowers	0.20
3	0.60	Bark	0.26
4	0.70	Roots	0.30

Table.7 Comparison of OD before and after addition of Acetone extract

Well no.	OD before addition of extract (OD at 550nm)	After addition of Acetone extract	
		Part of plant used	OD at 550 nm
1	0.18	Leaves	0.08
2	0.83	Flowers	0.18
3	0.91	Bark	0.31
4	1.02	Roots	0.58

Table.8 Comparison of OD before and after addition of Methanol extract

Well no.	OD before addition of extract (OD at 550nm)	After addition of Methanol extract	
		Part of plant used	OD at 550 nm
1	0.60	Leaves	0.27
2	0.67	Flowers	0.30
3	0.74	Bark	0.28
4	0.66	Roots	0.32

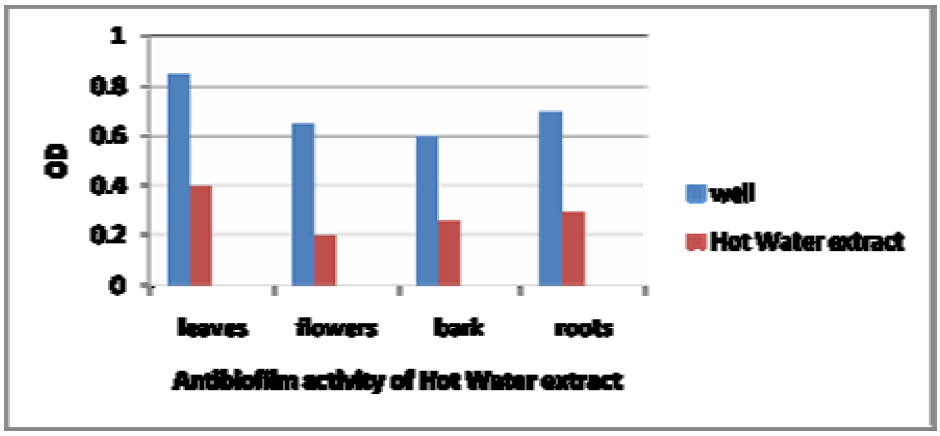
Table.9 Comparison of OD before and after addition of Ethyl acetate extract

Well no.	OD before addition of extract (OD at 550nm)	After addition of Ethyl acetate extract	
		Part of plant used	OD at 550 nm
1	1.30	Leaves	0.40
2	1.48	Flowers	0.35
3	0.65	Bark	0.47
4	1.40	Roots	0.18

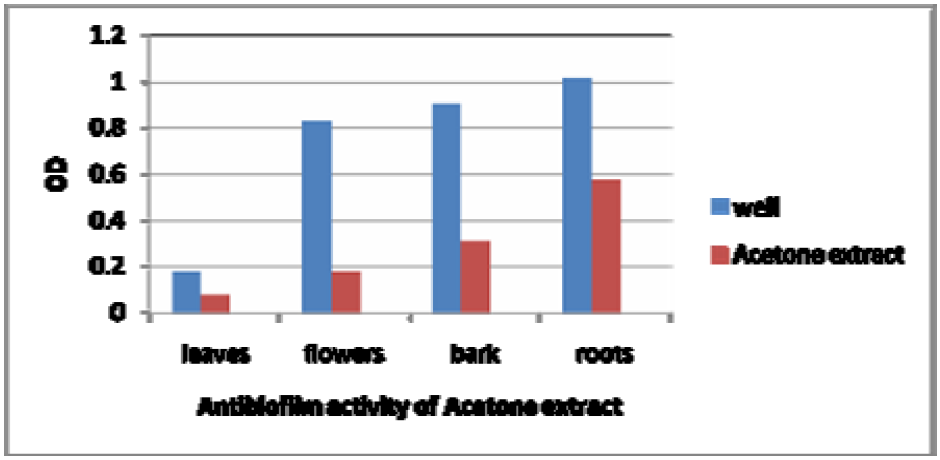
Table.10 Comparison of OD before and after addition of Ethanol extract

Well no.	OD before addition of extract (OD at 550nm)	After addition of Ethanol extract	
		Part of plant used	OD at 550 nm
1	0.78	Leaves	0.30
2	0.49	Flowers	0.23
3	0.94	Bark	0.20
4	0.77	Roots	0.37

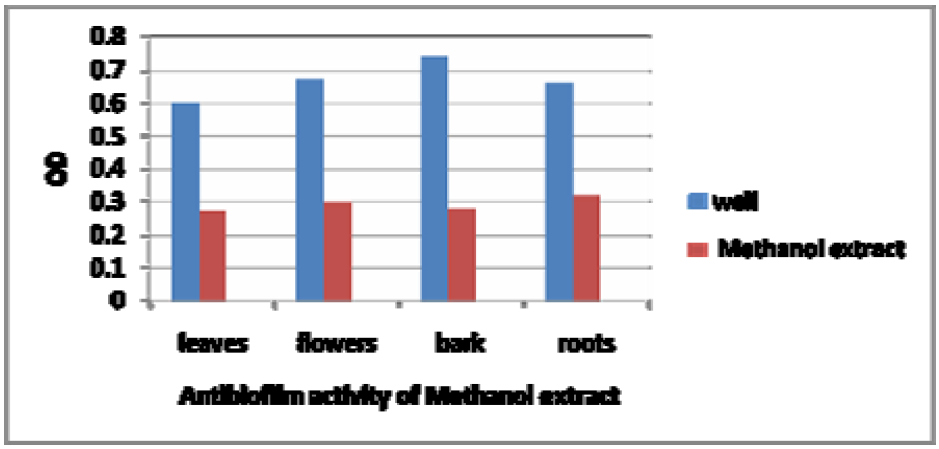
Graph.6 Comparative study of antibiofilm activity of Hot water extract



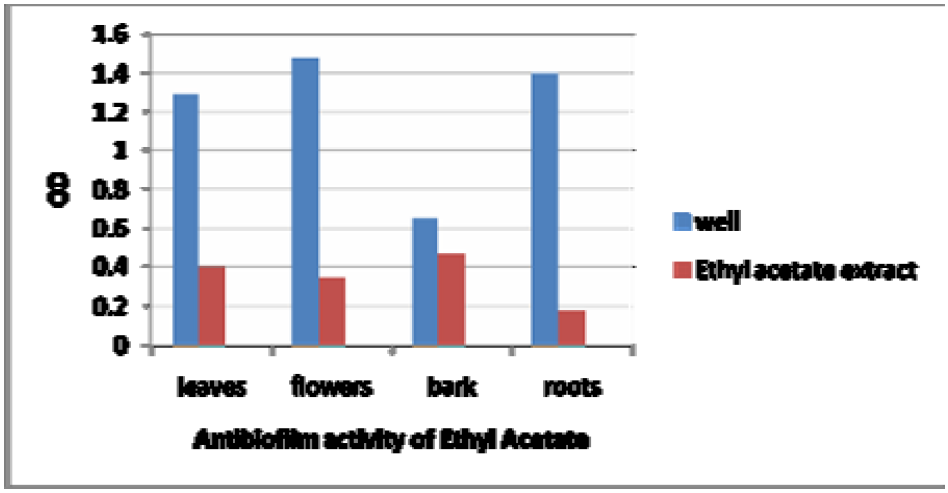
Graph.7 Comparative study of antibiofilm activity of Acetone extract



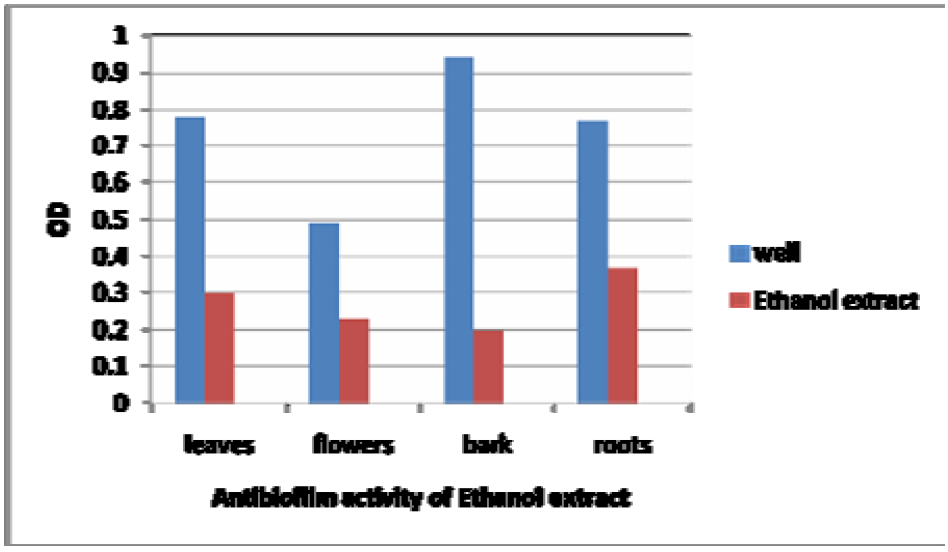
Graph.8 Comparative study of antibiofilm activity of Methanol extract



Graph.9 Comparative study of antibiofilm activity of Ethyl acetate extract



Graph.10 Comparative study of antibiofilm activity of Ethanol extract



Acknowledgement

We are thankful to Department of Microbiology Shri Shivaji Science College Nagpur for giving us the opportunity to carry out this research work. We are obliged by the constant inspiration and support of all the colleagues and friends.

References

1. Bidault P., Chandad F.nd D. Grenier. (2007)., "Risk of bacterial resistance associated with systemic antibiotic therapy in periodontolgy". J. Can. Dent. Assoc. 73:721-5.
2. Fathilah, A.R., Othman, Y. and Rahim, Z.H.A. (1998) "Antibacterial properties of local plant extracts against selected plaque bacteria". Working together for Advancement of Dental Education and Research at 9th SEAADE and 13th IADR, 76.
3. Gamboa F., Estupinan M., and Galindo A. (2004) "Presence of streptococcus mutans in saliva and its relationship with dental caries: Antimicrobial susceptibility of the isolates",. Universitas Scientiarum. 9:23-27.
4. Joseph B and Minipriya R. (2011) "Review on nutritional, medicinal and pharmacological properties of guava (Psidium guajava Linn.)" Int J Pharm Bio Sci, 2 (1):53-69
5. Knoll-Kohler E. and Stiebel J. (2002) "Amine fluoride gel effects the viability and the generation of superoxide anions in humans polymorphonuclear leukocytes: an in vitro study". Eur. J. Oral Sci.110:296-301.
6. Limsong J, Benjavongkulchai E, Kuvatanasuchati J (2004) "Inhibitory effect of some herbal extracts on adherence of *Streptococcus mutans*". J Ethnopharmacol 92: 281-289.
7. Nostro, A., Cannatelli, M.A., Crisafi, A.D., Musolino, A.D., Procopio, F. and Alonzo, V. (2004) "Modifications of hydrophobicity, in vitro adherence and cellular aggregation of *Streptococcus mutans* by *Helichrysum italicum* extract". Lett Appl Microbiol 38, 423–427.
8. Nwez El., Okafor J. L., and Njokv O. "Antimicrobial activities of methanolic extracts of Trema guineensis (schumm and thorn) and Morindalucida Benth used in Nigerian Herbal Medicinal Plants". J. Bio. Res And Biotech. 2004; 2:39-46.
9. Park KM, You JS, Lee HY, Baek NI, Hwang JK (2003) Kuwanon G,. "An antibacterial agent from the root bark of *Morus alba* against oral pathogens". J Ethnopharmacol 84: 181-185.
10. Percival RS, Devine DA, Duggal MS, Chartron S, Marsh PD (2006) "The effect of cocoa polyphenols on the growth, metabolism, and biofilm formation by *Streptococcus mutans* and *Streptococcus sanguinis*". Eur J Oral Sci 114: 343- 348.
11. Prabu G. R., Gnanamani A. and Sadulla S. Guaijaverin. "A plant flavonoid as potential antiplaque agent against *Streptococcus mutans"*. J. Appl. Microbiol. 2006 101:487-95.
12. Rathish Nair, Sumitra Chanda, " In-vitro antimicrobial activity of *Psidium guajava* leaf extracts against clinically important pathogenic microbial strains", Brazillian Journal of Microbiology, 2007; 38:22-25.
13. Razak FA, Rahim ZH (2003) "The anti-adherence effect of Piper betle and *Psidium guajava* extracts on the

adhesion of early settlers in dental plaque to saliva-coated glass surfaces". J Oral Sci 45: 201-206.

14. Thomas B, Shetty S Y, Vasudeva A, Shetty V. "Comparative evaluation of Antimicrobial Activity of Triphala and commercially available Toothpastes: An in- vitro study"., Int. J. Pub. Health Dent. 2011; 2(1): 8-12.
15. Yamanaka A, Kimizuka R, Kato T, Okuda K (2004) "Inhibitory effects of cranberry juice on attachment of oral *Streptococci* and biofilm formation". Oral Microbiol Immunol 19: 150-154.
16. Yatsuda R, Rosalen PL, Cury JA, Murata RM, Rehder VL, et al. (2005) "Effects of Mikania genus plants on growth and cell adherence of *Streptococci mutans*". J Ethnopharmacol 97: 183-189.
17. Yu H. H., Seo S., Hur J., Lee H., Lee Y. and You Y. Asarum sieboldii (2006) "Extracts attenuate growth, acid production, adhesion, and water insoluble glucan synthesis of *Streptococcus mutans*". J. Med. Food. 9(4): 505-509.