



Phytochemical, Antioxidant, and Antimicrobial Activity of *Psidium guajava* Leaves Against Oral Dental Pathogens

KEYWORDS

Psidium guajava leaves, phytochemical, antioxidant, antimicrobial activity, oral dental pathogen

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ABSTRACT Chemical substances used for prevention of dental caries are known to have many side-effects. Thus, natural products should be explored for their anticaries action. This study screened the antimicrobial effect of alcoholic and aqueous extracts of *Psidium guajava* leaves against oral dental pathogens *Streptococcus mutans*, *Staphylococcus aureus* and *Lactobacillus acidophilus*. The aqueous extract showed greatest bacterial inhibition against *Streptococcus mutans* and *Staphylococcus aureus* strains, but methanolic extract was found more effective in inhibiting *Lactobacillus acidophilus*. Phytochemical and antioxidative capacity analysis was also performed on the aqueous and ethanolic extracts for the presence of phenols, alkaloids, saponins, terpenoids, flavonoids, tannins and reducing sugar. The alcoholic leaf extracts showed higher anti-oxidative capacity and phytochemicals content like alkaloids and steroids which are active antimicrobial components in plants. This investigation provides preliminary information for using guava leaf extracts to control dental problems.

INTRODUCTION

Dental caries is a widespread human disease that has affected many populations all over the world. It is a microbial disease that results in the dissolution and destruction of the calcified tissues of the teeth. Early stage of dental caries is characterized by the destruction of superficial dental structures caused by acids which are by-products of carbohydrates metabolism by *Streptococcus mutans*, a cariogenic bacterium (Loesche WJ, 1986). As drug resistant microorganisms are increasing and artificial drugs have unpleasant side effects, researchers are now trying to pay more attention to herbal plant based alternatives.

P. guajava belongs to the family *Myrtaceae*. The leaves and bark of *P. guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi OC et al, 2008). Guava leaves has shown a significant antibacterial activity against *Staphylococcus*, *Shigella*, *salmonella*, *Bacillus*, *E.coli* and *Pseudomonas*. It also has a potent antifungal, antiyeast (Candida), anti-amaebic and antimalarial actions (Pandey & Shweta, 2012). *S. mutans* is the primary causative agent and the pathogenic species responsible for dental caries (tooth decay or cavities) specifically in the initiation and development stages. *Lactobacillus* acts after tooth decay is initiated, by binding to the biofilm and producing lactic acid, further aggravating damage to the enamel of teeth (Hoshino T et al., 2012). Due to the complex composition of phytochemicals, the total antioxidant activities of plant extracts cannot be evaluated by using one single method. The present study was designed to determine the phytochemical constituents, antioxidant capacity and antimicrobial activity of ethanolic and aqueous extracts from leaves of *Psidium guajava* on oral dental pathogens.

MATERIALS AND METHODS

Collection of Plant material and Extract Preparation:

The fresh and tender guava leaves were collected from a local garden in Kalyan. The authentication of plant leaves was done at the Blatter Herbarium, (Herbarium specimen no

NYL 1) St. Xaviers college, Mumbai. The leaves were thoroughly washed, shade dried and then crushed by electric grinder. 30 gm of guava leaves powder was subjected to soxhlet extraction. The extracted solutions were concentrated in a rotary evaporator and stored at 4 °C for further use.

Procurement of the microorganisms

Freeze-dried forms of the microorganisms *Streptococcus mutans* (MTCC 890) and *Lactobacillus acidophilus* (MTCC 10307) were obtained from Microbial Type Culture Collection MTCC, Chandigarh, and a glycerol stock of *Staphylococcus aureus* was obtained from School of Biotechnology and Bioinformatics, D. Y. Patil University.

ANTIMICROBIAL ACTIVITY TESTS

Antibacterial effect of medicinal plant extracts were checked by Well-diffusion method. The ampoules containing freeze-dried forms of the micro-organisms were opened and revived in Brain heart infusion Broth (hi-media) for *Streptococcus mutans*, Mueller Hinton broth (hi-media) for *Lactobacillus acidophilus* and Nutrient broth (hi-media) for *S. aureus*. 20, 40, & 60 micro liters of the ethanolic and aqueous extracts of Guava leaves were incorporated in the 8 mm wells which were bored onto the Mueller hinton agar plates which have been inoculated separately with *S. mutans*, *L. acidophilus* and *staphylococcus aureus* using the spread plate technique (1x10⁸cfu/ml). Streptomycin for *S. aureus*, Vancomycin for *Streptococcus mutans*, Penicillin for *L. acidophilus* were used as control Antibiotics. After 24 hrs at 37 °C incubation, the plates were observed for the results and zone of inhibitions measured. The experiment was done in triplicate and values are presented as Mean ± S.D.

ESTIMATION OF PHYTOCHEMICAL CONSTITUENTS

1. Estimation of total phenol content (TPC)

The total phenol content was determined by Folin-Ciocalteu method. (McDonald S et al, 2001) and expressed in terms of Gallic acid equivalent (mg/g). (Chandha & Dave, 2009).

2. Estimation of total flavonoids (TF)

The total flavonoid content was determined by Aluminum chloride method and expressed in terms of quercetin equivalent (mg/g). (Chang C *et al*, 2002)

3. Estimation of sugars

Estimation of sugars in the extract was done by DNSA method.(Mohun&Cook,1962) and expressed in terms of maltose equivalent (mg/g).

EVALUATION OF ANTIOXIDANT ACTIVITY

1. α , α -diphenyl- β -picryl-hydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1-diphenyl-2- picrylhydrazyl by the method of McCune and Johns.(McCune & Johns,2002) and expressed in terms ascorbic acid equivalent (mg/g).(Chandha & Dave, 2009)

2. Nitric oxide (NO) radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reagent at 540 nm (Green *et al*. 1981; Chandha & Dave 2009). NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

4. Estimation of reducing power (RP)

The reducing power was determined by the method of Athukorala Y *et al* (2006) and expressed in terms of standard ascorbic acid (mg/g).

5. Superoxide anion (SO) radical scavenging assay

The superoxide anion scavenging activity was measured as described by Robak & Gryglewski (1998) and expressed in terms of Gallic acid equivalent (mg/g).

6. Hydrogen peroxide (H₂O₂) radical scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide was determined according to the method of Ruch&Klaunig(1989) and expressed in terms of ascorbic acid equivalent (mg/g).

STATISTICAL ANALYSIS:

Analysis of the antibacterial action of the extracts of guava leaves was carried out at different concentrations, by comparing the mean diameter of the inhibition holes as a variable. For antioxidative activity Mean \pm SD for samples in triplicates was used for comparisons.

RESULTS AND DISCUSSION

Plant essential oils and extracts have been used for thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. This work showed that the guava extracts inhibited bacterial growth but their effectiveness varied.(Fig.1) Aqueous extract was found to be very effective in inhibiting the growth of *S.aureus* and *S.mutans* compared to ethanolic extracts and both extracts are less effective against *L.acidophilus* compared to other two pathogens. All the gram positive microorganisms viz., *S.aureus*, *S.mutans* and *L.acidophilus* were more susceptible bacteria to all plant extracts. This may be due to differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram-negative outer membrane acting as a barrier to

many environmental substances including antibiotics (Burt S,2004). Effective antimicrobial activity of aqueous extract may be because of active compounds which are flavonoids with different levels of antibacterial activity. The structure elucidation study reveals that five flavonoidal compounds are quercetin, quercetin-3-O- α -L-arabinofuranoside, quercetin -3-O- β -D- arabinopyranoside, quercetin-3-O- β -D glucoside and quercetin-3-O- β -D-galactoside.(Metwally AM *et al*, 2010) similar trend was also observed in our results that the total phenolic content and the total flavonoid content is higher in aqueous extracts of Guava leaves than the ethanolic extracts (Table 1).Reducing power of plant extract was reported to be directly correlated with its antioxidant activity and is based on the presence of reductants like Quercetin-3, 5-diglucoside and cyanidin-3-sophoroside-5-glucoside which exert antioxidant activity by breaking the free radical chain and donating a hydrogen atom.(Duh PD *et al*, 1999;Garg D *et al*, 2012) Reducing power is highest in both extract and this can also be linked with higher content of reducing sugar in extract.(Table 2)

The H₂O₂, NO, DPPH scavenging activity in guava leaves was estimated to be higher in the ethanolic extract. This can be associated to the presence of total phenols in the extract (Elmastas M *et al*, 2006) Aqueous extract has high SO scavenging activity which is associated to the coexistent higher total flavanoids in the same extract.(Chen & Yen, 2006).

Antioxidative capacity of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Rice E C ,2004). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation (Benavente GO *et al*,1997)

CONCLUSION

Dental caries is a global oral health problem. Proper and regular hygiene is required to prevent dental problems. In allopathy, the treatment of dental problems is expensive hence restricted. So, this study was designated in the process of developing a plant based substitute for dental treatment against conventional chemical agents.The results of our study evidenced that *Psidium guajava*, has the ability to inhibit the growth of the common oral flora with its abundant source of secondary metabolites and anti-oxidant property. Hence Guava leaf extracts can be an alternative preventive therapy and minimize the excessive use of antibiotics for the prevention of dental caries.

Table 1. Phytochemical constituents of *Psidium guajava* leaf extracts.

Tests	Standard equivalent in Aqueous extract (μ g/g)	Standard equivalent in Ethanolic extract (μ g/g)
Total phenol content	247.24 \pm 14.39	181.86 \pm 3.3
Total flavonoids	498.24 \pm 14.37	340.93 \pm 56.82
Sugar content	1218.84 \pm 88.71	1391.7 \pm 105.78

Table 2. Antioxidant activity of *Psidium guajava* leaf extracts.

Tests	Standard equivalent in Aqueous extract (µg/g)	Standard equivalent in Ethanolic extract (µg/g)
DPPH scavenging assay	1529.12±33.38	1635.81±69.55
No radical scavenging assay	258.5±44.15	619.33±63.95
Reducing power assay	892.02±28.25	478.28±10.88
SO radical scavenging assay	567.4±9.73	457.57±12.19
H2O2 Radical scavenging assay	1955.79±139	2888.55±126.31

Fig 1: Effect of various concentrations of *Psidium guajava* leaf extracts on *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*.

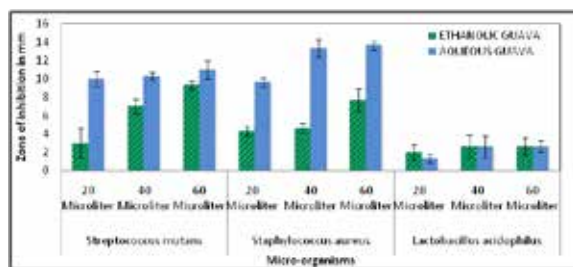


Fig 2: Zone of inhibitions exhibited by Guava extracts in *Streptococcus mutans*

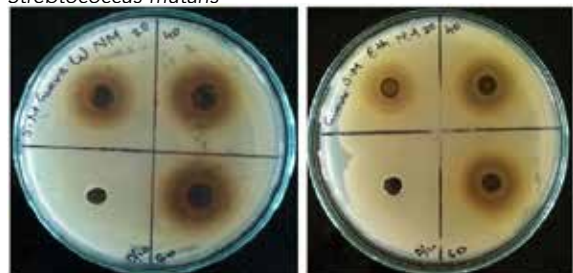
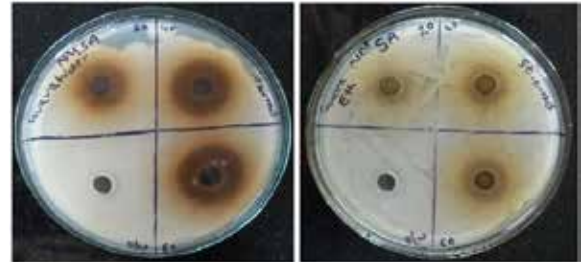


Fig3. Zone of inhibitions exhibited by Guava extracts in *Lactobacillus acidophilus*



Fig 4. Zone of inhibitions exhibited by Guava extracts in *Staphylococcus aureus*



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