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In vitro comparison of the antimicrobial activity of two varieties of *Psidium guajava* against dental caries causing pathogen

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ABSTRACT

The antibacterial activity of two varieties of Psidium guajava were investigated by disc diffusion method against clinically isolated human cariogenic pathogen, Streptococcus mutans. The results indicated that the methanol flower extracts of both varieties have antibacterial activity against the tested dental caries causing bacterium. Among the two varieties, P. guajava berry showed potential inhibitory action than P.guajava variegata against S.mutans. HPLC analysis with methanol flower extract indicated the presence of five compounds .This study has shown the importance of guajava and indicated that the active compounds present in these two varieties could serve as a lead compound in the formulation of a new antibacterial herbal drug to cure dental caries.

Keywords: Antibacterial activity, Disc diffusion assay, Psidium guajava variegata, Psidium guajava berry, Streptococcus mutans, HPLC

INTRODUCTION

Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth .The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrates metabolism by *Streptococcus mutans*, a cariogenic bacterium [1].

Artificial drugs have unpleasant side effects, on the other hand, the number of drug resistant micro organisms is increasing, so researches are trying to pay more attention to herbal drugs. Mouthwashes for example, chlorhexidine have several adverse effects despite good plaque control and an antimicrobial effect [2] and [3].

Antibiotics such as penicillin and erythromycin have been reported to affectively prevent dental caries in animals and humans but they are never used clinically because of many adverse effects [4]. Natural products have shown to be a good alternative to synthetic chemical substance for caries prevention [5].

The literature survey of the folklore medicine reveals the use of *Psidium guajava* leaves to maintain oral hygiene ; dried fruits of *Terminalia chebula* as an anticaries agent; stem of *Achyranthus aspera* for the treatment of tooth-ache and stem of *Mimusops elengi* strengthens the gums[6,7,8,9].

Guava, known as the poor man's apple of the tropic, has a long history of traditional use, much of which is being validated by scientific research. The guava tree produces large quantities of fruit. The fruit is round, with a white or

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yellow skin and a pulp of the same color although the pulp is sometimes crimson. It has high vitamin C content. Leaf extract of guava have some pharmacological activities, such as anti-inflammatory, antidiarrheal, antioxidant, antimutagenic, besides antimicrobial activities.

Bark and leaf extracts have shown to have invitro toxic action against numerous bacteria. In several studies, guava showed significant antibacterial activity against Staphylococcus, Shigella salmonella, Bacillus, *E.coli* and Pseudomonas. It has also demonstrated antifungal, anti yeast (Candida), anti-amaebic and antimalarial actions.

The purpose of the present study was to compare the antibacterial properties of different parts (viz leaf, flower, bark). *P.Guajava variegata* and *P. gujava berry* against *S. mutans*. In this paper we report the results of such studies in order to orient future investigations towards the finding of new, potent and safe antibacterial compounds.

MATERIALS AND METHODS

Micro organisms used

Human dental caries pathogen, *Streptococcus mutans* used in the study was isolated from seven caries infected patients of the Rose Dental Clinic, Nagercoil, Kanyakumari district.

Identification of pathogen

Based on the colony morphology on the blood agar plates, the selected organism was identified according to the standard keys of Bergey's manual of determinative bacteriology[10].

Plant material collection

We selected two Indian medicinal plants for antibacterial assay based on their ethnomedicinal and traditional uses against infectious diseases based on literature survey and herbal healers. Leaf, bark and flower of two varieties of *Psidium guajava* were collected from a garden at Karungal, Kanyakumari district.

Crude extract preparation [11]

The plant parts such as leaf, bark and flower were washed well with water, shade dried for two weeks, powdered and stored in airtight container. Thirty grams of the leaf powder was mixed with 100ml of the solvents in a clean conical flask. The solvents used were methanol and hexane. The mixture was kept undisturbed for 72hrs in room temperature. Each mixture was stirred every 24hr using a sterile glass rod for perfect mixing. Extracts were filtered by using Whatmann No.1 filter paper and solvents were removed by placing in an incubator at 37°C for 48 hrs until all the solvents get evaporated. The residues under the bottom of the conical flasks were extracted with the solvents (methanol and hexane) respectively. The bark and flower were also extracted in the same way as mentioned above and used for antibacterial activity.

Antibacterial activity [12] Paper disc preparation

One hundred needle tips containing 10 discs were placed on a medicinal vial, sterilized by autoclaving and dried in an oven to remove moisture. In sterile condition each disc of 6mm (Himedia) diameter were loaded with solvent extracts using micropipette to obtain disc containing 100 µg, 200µg, 300µg and 400µg. A positive control disc (100µl) was prepared using Chlorhexidine.

Assay

The antibacterial activity was evaluated by agar disc diffusion method . A loopful of strain was inoculated in 30ml of nutrient broth in a conical flask and incubated at 37°C for 24hrs to activate the strain. A 100 μ l of test strain (10⁶ CFU/ml bacteria) was spread on the Muller Hinton agar media No.2. Then sterile disc containing 100 μ g, 200 μ g, 300 μ g, 400 μ g of each extracts was placed onto the surface of agar plate. For positive control, discs were impregnated with chlorhexidine. The plates were incubated at 37°C for 24hrs and the diameter (mm) of the inhibitory zones were determined. This test was repeated three times for each plant extract.

High Performance Liquid Chromatography [13]

The active flower extract of *P. guajava berry* was quantified using HPLC. A calibration curve was constructed and their linear ranges were determined. The curve was plotted by the peak area against concentration of each analytes.

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RESULTS AND DISCUSSION

The clinical isolate of *S. mutans* was identified according to the colony morphology, microscopic properties and biochemical tests. Pathogen in blood Agar plates showed pinpointed colonies and greenish discoloration with partial haemolysis around the colonies represents the alpha haemolytic Streptococci. S. Hamada et al.,[14] in his study, isolated *S.mutans* from dental plaque of sound teeth and carious dentin of the 10 subjects known to harbor *S. mutans* in the feces.

The antibacterial activity of *Psidium guajava berry* against cariogenic *S. mutans* is shown in the Table (1). All the extracts showed inhibitory, guajava berry activity. The maximum inhibitory zone was observed in methanol flower extract of *Psidium guajava berry* (11-20mm). Chanchal.K Roy and Amit Kumar Das [15] concluded that the methanolic extract of leaves of *Psidium guajava* plant possesses better hepatoprotective activity compared to other extracts. Anuja Dhiman et al., [16] tested the inhibitory effect of methanolic extract of *P.guajava* against *E.coli* with minimum inhibitory concentration, 0.78µg/ml. minimum bactericidal concentration of 50µl and appreciable antifungal activity with minimum inhibitory concentration of 12.5µg/ml.

Table 1. Antibacterial	activity of solvent e	stracts of <i>Psidium</i> g	guajava berry	against Streptococcus m	iutans
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Plant parts	Solvent used	Zone of inhibition (mm)					
		100mg	200mg	300mg	400mg	100µl(control)	
Flower	Methanol	++	++	++	++	+++	
	Hexane	+	+	+	++	+++	
Bark	Methanol	+	+	+	++	+++	
	Hexane	+	+	+	+	+++	
Leaf	Methanol	+	++	++	++	+++	
	Hexane	+	+	+	+	+++	

Table 2. Antibacterial activity of solvent extracts of Psidium guajava variegata against Streptococcus mutans

Plant parts	Solvent used	Zone of inhibition (mm)					
		100mg	200mg	300mg	400mg	100µl(control)	
Flower	Methanol	+	+	+	+	+++	
	Hexane	+	+	+	+	+++	
Bark	Methanol	+	+	+	++	+++	
	Hexane	+	+	+	+	+++	
Leaf	Methanol	+	+	++	++	+++	
	Hexane	+	+	+	+	+++	

+ represents the zone of inhibition about 1-10mm

++ represents the zone of inhibition about 11-20mm

+++ represents the zone of inhibition about 21-30mm

Table 3. Methanol flower extract of *Psidium guajava berry* showing peak value of fractions in HPLC

Sl.No.	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)
1	0.687	4.701	0.246	0.1	0.1
2	1.910	955.371	157.180	25.4	43.2
3	2.107	2273.666	197.370	60.5	54.2
4	2.930	489.389	7.935	13.0	2.2
5	5.597	34.031	1.337	0.9	0.4
	Total	3757.158	364.068	100.0	100.0

The inhibitory effect of *P.guajava variegata* against cariogenic *S.mutans* is shown in the Table (2). All extracts showed inhibitory effect. Among the leaf, bark and flower extracts, the methanol leaf extract showed potential inhibitory effect against S. mutans (11-20mm) diameter.

The positive control chlorhexidine represent maximum zone of inhibition (20-30mm) in both the varieties of plant extracts. Fani et al., [17] studied the inhibitory activity of the aqueous extract of garlic on *S.mutans* comparable with chlorhexidine by disc diffusion method. Dalirsani compared chlorhexidine with ten herbal extracts on *S.mutans*. In another research S.Saraya et al., [18] formulated guava chewable tablets which support the traditional use of *P.guajava* for the treatment of dental caries.

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In the present study the antibacterial activity of *P.guajava berry* flower extract was significantly more or less related to the zone of inhibition of chlorhexidine discs.

HPLC was done using active flower extract of *P.guajava berry* for the quantitative determination of compounds. Five peaks of compounds were observed and according to the height, compound 3 showed highest peak formation. This method was validated by determining relative standard deviation (RSD) analysis (Table 3). The RSD value for retention time and peak areas were 2.107 and 60.5 respectively.

The bioactive compounds should further be evaluated for its anticariogenic properties and should be subjected to invitro trials before using as a preventive mouth rinse and components of toothpaste. This natural therapeutant can meet out the challenges faced in dental caries management and can be an efficient community based healthcare system.

CONCLUSION

The results indicate the promising antibacterial activity of *P.guajava berry* against dental caries pathogen *S.mutans* isolated from caries infected patients.

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