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Research Article

SCREENING OF *PSIDIUM GAUJAVA* FOR EFFECTIVE PHYTOMEDICINES AND STUDY ON ITS ANTIBACTERIAL EFFECT AGAINST DENTAL CARIES BACTERIA

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ABSTRACT

This study focused on checking antibacterial effect of *Psidium gaujava* against dental caries forming oral flora. The antimicrobial activity of *Psidium guajava* was checked with ethanol, acetone, chloroform, methanol and water extracts against selected bacterial isolates. The acetone and methanol extract showed maximum inhibitory activity but ethanol extract inhibit only the growth of *Pseudomonas aeruginosa* and the water extract revealed high activity against both *Streptococcus viridans* and *Bacillus megaterium.* In phytochemical screening, the acetone and ethanol extracts gave positive results for steroids, terpenoids and flavonoids. Phenolic compounds were there only in acetone extract. Saponins were absent in ethanol extract and tannins simply present in acetone and methanol extract. Phytochemical analysis was done by Thin Layer Chromatography (TLC). Acetone solvent system produced three spots with highest Rf value 0.722. The chloroform solvent system produced three spots with highest Rf value 0.780. But the water, ethanol and methanol solvent system developed only two spots.

Keywords: Dental caries, Phytomedicines, Phytochemical screening, *Psidium gaujava,* Guava, Oral flora

INTRODUCTION

Dental caries is also known as tooth decay or a cavity, a disease where bacterial processes change carbohydrate like sugar in food left on teeth to acid that demineralises hard tooth structure (enamel, dentin, and cementer)[1]. If demineralization exceeds saliva and other demineralization like from calcium, these tissues progressively break down, producing dental caries (cavities, holes in the teeth). Two groups of bacteria are responsible for initiating caries: *Streptococcus mutans* and *Lactobacillus*. If left untreated, the disease can lead to pain, tooth loss and infection[2].Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. It is a specific type of drug resistance. The patterns of antibiotic usage greatly affect the number of resistant organisms which develop. Overuse of broad-spectrum antibiotics, such as second- and third-generation greatly hastens the development of resistance[3]. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals[4].This study was conducted to check the antibacterial effect of *Psidium gaujava* against some of the dental bacterial flora. Most phytochemical analyses investigated the properties of guava leaf products, revealing more than 20 isolated compounds, including alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triterpenes, and vitamin C[5-6]. Leaf and bark extracts have in vitro antimicrobial activity mostly associated with flavonoids, such as morin glycosides, quercetin, and quercetin glycosides [7-9]. This study aimed to derive remedy for dental caries using phytoderivatives from *Psidium guajava.*

MATERIALS METHODS

Selection of Bacterial Strains

Bacterial strains of six different species (*Pseudomonas aeruginosa, Streptococcus salivarius, Streptococcus viridans, Streptococcus mutans, Bacillus megaterium, and Neisseria catarrhalis*) with enhancing activity in caries formation were selected from Microbial Technology Laboratory, Malankara Catholic College, Mariagiri, Kaliakkivilai, Tamil Nadu.

Collection of Medicinal Plants

The medicinal plant sample was collected from the Maruthuvarmalai region of Western Ghats of Kanyakumari district. The different parts such as root, stem, leaves and inflorescence of *Psidium guajava* were selected for testing its antibacterial studies and characterization of secondary metabolites of effective ones.

Preparation of Plant Extracts

Plant sample was shade dried and ground well. 10 gram of powdered sample was filled in screw cap bottles with 10 ml of different solvent systems (acetone, ethanol, chloroform, methanol and water). It was kept at 22^0 C for fifteen days.

Antibacterial Effect Checking of Medicinal Plant Extracts

Antibacterial effect of medicinal plant extracts were checked by Well- diffusion method.

Well Diffusion Method

The bacterial isolates were effectively swabbed on the prepared Mueller-Hinton agar plates. After allowing the inoculums to dry at room temperature, six mm diameter wells were bored on it. The extract was introduced (50 µl of a 100mg/ml concentration) into three duplicate wells. The plates were allowed to stand at room temperature for one hour for the diffusion of extract into the agar and then they were incubated at 37^0 C for 18 hours. After incubation, the plates were observed for the results.

Phytochemical Screening

A preliminary phytochemical analysis was conducted for the detection of steroids or terpenoids (*Liebermann-Burchard Test),* flavonoids (*Shinoda's Test*), Carbohydrates (Molisch's Test), saponins, tannins and phenolic compounds[10].

Phytochemical Analysis (TLC)

Silica gel[G] slurry 1: 2 (W/V) with thickness of 0.25 mm was prepared on a head glass plate. It was dried for 15 to 30 min followed by hot treatment in an oven at 100^0 c for one to two hours. The samples were applied at one end (2.5 cm away from ends) of the gel plate with equal distance between them. The plates were dipped in solvent tanks to a depth of 1.5 cm from bottom and allowed to cover the solvent over the top. After that the plates were removed dried and processed for the identification of separated compounds (as colored spots) and the R_f values were calculated using the formula

$$Rf = \frac{\text{Distance (cm) moved by the solute (extract) from the origin}}{\text{Distance (cm) moved by the solvent from the origin}}$$

[Rf= Retention Factor]

RESULTS AND DISCUSSION

Antimicrobial effect of *Psidium guajava* extracts

The antimicrobial activity of *Psidium guajava,* ethanol, acetone, chloroform, methanol and water extracts against bacterial isolates (*Pseudomonas aeruginosa, Streptococcus salivarius, Strptococcus viridans, Strptococcus mutants, Bacillus megaterium, Neisseria catarrhalis)* were tabulated using well diffusion method. The acetone extract of *Psidium guajava* showed maximum inhibitory activity against *Bacillus megaterium*, *Streptococcus viridans* (29mm and 21mm respectively). Whereas its methanol extract showed high activity against *Neisseria catarrhalis* (20mm). Ethanol extract inhibit the growth of *Pseudomonas aeruginosa* (18mm) and the water extract revealed high activity against *Streptococcus viridans* and *Bacillus megaterium* both with a zone of 15mm.

Table 1: Zone of inhibition of different extracts of *Psidium guajava* against oral bacteria

S. no.	Bacteria	Acetone (mm)	Chloroform (mm)
1	*Neisseria catarrhalis*	18	10
2	*Streptococcus mutans*	15	13
3	*Streptococcus salivarius*	15	9
4	*Streptococcus viridans*	21	10
5	*Bacillus megaterium*	29	11
6	*Pseudomonas aeruginosa*	18	11

Phytochemical Screening

In *Psidium guajava*, test for steroids terpenoids and flavonoids were positive in acetone and ethanol extracts. Test for carbohydrates showed negative response with chloroform extract. Phenolic compounds were present in acetone extract and absent in other four extracts. Test for saponins gave negative result in ethanol extract and others gave positive results. It is observed that tannins were present in acetone and methanol extracts.

Table 2: Result of Phytochemical Screening of *Psidium guajava*

Experiment	Acetone Extract	Chloroform Extract	Ethanol Extract
Liebermann –Buchard test (steroids and terpenoids)	Present	Absent	Present
Shinodas test for flavanoids	Present	Absent	Present
Molisch's test for carbohydrates	Present	Absent	Present
Test for Phenolic compounds	Present	Absent	Absent
Test for saponins	Present	Present	Absent
Test for tannins	Present	Absent	Absent

Phytochemical Analysis by Thin Layer Chromatography (TLC)

Acetone solvent system of *Psidium gujava* produced three spots with highest Rf value 0.722. In water, ethanol and methanol solvent system of *Psidium gujava* each developed two spots. The chloroform solvent system produced three spots with highest Rf value 0.780.

The variation occurred in zonation of different extracts may be due to the polarity of solvents which determines the type of reaction and solubility of compounds. The acetone and methanol have better extracting capacity which may be attributed to the ability to extract the natural antimicrobial compounds such as alkaloids, flavanoids, terpinoids and phenolic compounds from the plant. Phytochemical screening of *Psidium guajava* revealed the presence of many vital secondary metabolites. Acetone extract of *Psidium guajava* contained most of all secondary metabolites (terpinoids / steroids, flavanoids, carbohydrates, saponins and tannins). This may be due to the high polarity of the solvent acetone or by selective solubility of the metabolite. The high antimicrobial properties in *Psidium guajava* may due to high phenolic composition. It gave positive results in the test for flavanoids. The extracts used were taken with polar and non-polar solvents and further resolved with TLC with minimum trial of solvent system, whose results also evidenced the presence of numerous secondary metabolites, as spots on TLC gel plate.

Table 3: Results of Thin Layer Chromatography of *Psidium guajava*

Solvent system	No of spots obtained	R_f value
Acetone	Compound 1	0.244
	Compound 2	0.360
	Compound 3	0.722
Water	Compound 1	0.480
	Compound 2	0.890
Ethanol	Compound 1	0.411
	Compound 2	0.722
Chloroform	Compound 1	0.366
	Compound 2	0.691
	Compound 3	0.780
Methanol	Compound 1	0.570
	Compound 2	0.780

CONCLUSION

The results evidenced that *Psidium gaujava* have the ability to inhibit the growth of the common oral flora with its abundant source of secondary metabolites. This also helps to become an alternate and minimize the excessive of antibiotics for the prevention of dental caries.

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