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Antimicrobial activity of ethanol and water extracts for root of *Psidium guajava* against pathogenic bacteria

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Abstract:

India is the original home of the guava (*Psidium guajava L*.), which is grown both wild and domestically. Guava has been utilized for food and as a source of pharmaceutical basic ingredients. Determining the antimicrobial efficacy of ethanol and water extracts from guava roots against particular pathogenic bacteria was the goal of this investigation. Samples for the study were gathered from Chhattisgarh. Using ethanol and water, 100 grams of ground root powders were extracted one at a time. After being evaporated and 0.2 grams dissolved in the extraction solvent. The extracts were tested against gram-positive (Streptococcus oralis) and gram-negative (Escherichia coli, Pseudomonas) bacteria. Inhibition zone data was measured in millimeters and examined. Since water is more polar and absorbs more bioactive chemicals than ethanol, it exhibits stronger inhibition when used as an extracting solvent. The most inhibited bacteria were Streptococcus oralis, E. coli, and Pseudomonas, in that order. The gram-positive and gram-negative bacteria did not significantly differ from one another. Comparing certain ethanol and water root extracts to certain commonly used commercial antibiotics revealed some surprising and considerable suppression against bacteria. The study's findings suggest that Chhattisgarh guava roots, when extracted using ethanol and water, may provide valuable active chemicals for treating gram-positive and gram-negative bacterial infections.

Keywords:

Psidium guajava; Streptococcus oralis; Pseudomonas; Escherichia coli

1. Introduction:

The guava, or *Psidium guajava L*., is indigenous to India, where it grows both wild and under cultivation. Guavas can be grown successfully up to 2,000 meters above sea level. After planting, the tree usually begins to give fruit one or two years later and continues to do so for 30 years (Beentje, 1994). The fruit is a superb source of vitamin C, calcium, potassium, and iron. It is eaten raw or cooked into juices, and the leaves are used in conventional treatments for diabetes and diarrhea. As certain germs evolve in ways that diminish or completely eradicate the effectiveness of medications, chemicals, or other therapeutic and preventive agents, resistance has grown. More damage is caused by the germs as they persist and grow. Bacteria can cause harm by rapidly pumping out antibiotics, neutralizing antibiotics before they lose their effectiveness, or changing the antibiotic's attack site to prevent it from affecting the bacteria's ability to function (Valdes-Infante et al. 2007). There is a long history of medicinal use for guava leaves and bark, and this usage continues today. Guava herbal medicines are used in Peru to treat vomiting, diarrhea, coughing, and vaginal discharges. As of right now, western and central Africa, as well as Latin America, use the leaves to treat diarrhea (Gutierez et al., 2008). Chemicals like tannins, phenols, triterpenes, flavonoids, saponins, carotenoids, lectins, vitamins, fiber, and fatty acids are present in guava, which increases its potency as a therapeutic herb (Begum et al., 2002). Quercetin, morin, quarcertin-3-0-glucopyranoside, guaijaverin, and quercetin are isolated chemicals from guava leaves that have herbal effects (Arima and Danno, 2002; Suganya et al., 2007). Quercetin is the main active ingredient. (Gamal et al. 2011), the crude components of guava seeds are 11.52% proteins, 0.54% oil, and 79.62% crude fiber. The present research on guava extracts is a result of the guava's herbal heritage. Numerous chemical tests have supported the use of this herb in the treatment of various ailments. Adult patients with acute diarrhea responded well to a plant medication that was developed from the leaves. Additionally, guava leaves and fruit juice have been tested for treating infantile diarrhea. The findings of the study indicate that guava has a good curative effect on infantile rotaviral enteritis, with those treated with guava recovering at a rate shorter than that of the controls in just three days (Wei et al., 2000). By preventing the release of molecules from the gastrointestinal space during episodes of acute diarrhea, an alcoholic leaf extract has also been shown to exhibit morphine-like effects. The quercetin-related action is similar to that of morphine. Guava's lectin components have been demonstrated to bind to E. coli, a common diarrhea-causing agent, and inhibit the bacteria's ability to adhere to the intestinal wall, hence avoiding diarrheal infection (Coutino et al., 2001). It has been demonstrated that bark and leaf extracts are poisonous in vitro against a variety of microorganisms. Studies have demonstrated the activity of guava leaves and bark against S. aureus. The bacteria that cause diarrhea are Shigella, Salmonella, Bacillus, and E. coli (Mohammad et al., 2011). Ethanolic extracts of guava and pomegranate considerably raised serum albumin concentrations in comparison to carbon tetrachloride during in vivo experiments to determine the therapy of serum contraction in hepatoxic rats. In analogous investigations, ethanolic extracts of pomegranate and guava considerably decreased the hepatoxic rats' liver weight in contrast to carbon tetrachloride (Mohieiden et al., 2011). Studies have demonstrated antifungal, antiyeast, anti-amebic, and antimalarial properties (Somwyas et al., 2013). In a 2003 study using guinea pigs, Brazilian researchers found that leaf extracts had a variety of effects on the cardiovascular system, which may be helpful in treating irregular arrhythmia (Yamashiro et al., 2003). The heart is shielded by antioxidants found in guava leaves, which also enhance cardiac function. In two randomized studies, guava consumption for 12 weeks resulted in an average 8-point drop in blood pressure, a 9% decrease in total cholesterol, and an 8% decrease in triglycerides. There may be bioactive substances in guavas that have therapeutic applications.

2. Materials and methods:

2.1. Collection of plant part:

The root of Psidium guajava (family Myrtaceae) was collected from a rural area of Sarangarh District, Chhattisgarh, India.

2.2. Identification and authentication of collected plant:

The fresh parts were collected from a rural area of Sarangarh District, Chhattisgarh, and authenticated by Professor Dr. Ashwini Kumar, Dixit, Department of Botany, Guru Ghasidas Vishwavidhyalaya Central University Koni Bilaspur (C.G.). The plant's (Psidium guajava) roots were washed and shaded for two months. The dried root was then crushed into a fine powder and used for further study.

2.3. Test organisms (source): bacteria:

Microbes were collected such as Escherichia coli, Pseudomonas, Streptococcus oralis, collected from the biotechnology lab and sent to the laboratory for culture and sensitivity testing at

Department of Biotechnology, GGV Bilaspur. Standard antibiotics amoxicillin.

2.4. Soxhlet extraction:

The solid powder material containing some of the desired compound was placed inside a thimble 4 made of thick filter paper, which was loaded into the main chamber of the soxhlet extractor. The 2 soxhlet extractor was placed in a flask containing the extraction solvent. The soxhlet was then equipped with a condenser. The solvent was heated to reflux. The solvent vapor travels up a 4 distillation armed and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools and drips backed down into the chamber housing the solid material. The chamber containing the solid material slowly filled with a warm solvent. When the 9 soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side 2 armed, with the solvent running backed down to the distillation flask. The cycle allowed it to repeat many times, over hours or days. During each cycle, a portion of the non-volatile 2 compound dissolves in the solvent. After many cycles, the desired compound was concentrated in the distillation flask. The extracts were dried using a rotary evaporator at 30-40°C and 0.2 gms weighed then dissolved in 1 ml of each solvent and stored at 4°C.

2.5. Antimicrobial activity:

The ethanolic and aqueous extracts of the root were tested for their antimicrobial activities 10 against bacteria such as Escherichia coli, Pseudomonas, and Streptococcus oralis. Antimicrobial 3 activity was carried out by the agar-well diffusion method. Mueller Hinton Agar (MHA) was poured into plates of 9–10 cm; the depth of the agar was about 3–4 mm. With a sterile cotton swab, the test culture was spread evenly over the plate successively in three directions to obtain an even inoculum. The plate was allowed to dry for 3–5 minutes. Wells of 5 mm diameter were cut on the surface of the agar. Thirty microliters of 10%, 20%, 50%, and 7 100% solutions (v/v) of aqueous and alcoholic extracts of Psidium guajava were added to different wells, and in one well, normal saline and another well for the standard sample (amoxicillin) was used. The plates were the zone of inhibition was incubated at 37°C for 24 h. measured on a scale to the nearest mm, including disc diameter.

3. Results:

Table. 1: Percentage yield of extract of Psidium guajava root by Soxhlet Apparatus

Sr. No.	**Plant**	**Colour**	**Amount of extract (gm)**	**% Yield**
1	*Psidium Guajava* Etnanolic Extract	Brownish black	2.211	11.05
2	*Psidium Guajava* Aqueous extract	Brown	0.948	4.74

3.1. Antimicrobial activity of *psidium guajava* root extracts:

Root extracts of *Psidium guajava* prepared in solvents (ethanol and aqueous) were tested for antimicrobial activity against bacteria. The antimicrobial activity of the extract was assessed by the presence or absence of a zone of inhibition. The roots of both extracts of *Psidium guajava* exhibited significant antimicrobial activity, as is evident by a clear zone of inhibition around the wells.

Zone of inhibition (mm) of various extracts against 3 different bacteria.

Table. 2:

Plant	***Extract***	***Concentration***	***E.coli***	***Pseudomonas***	***Streptococcus oralis***
Psidium guajava	Ethanol	Standard Drug Amoxicillin	3.9	3.98	4.12
		Saline	2.26	2.28	2.1
		10 %	3.7	3.5	3.92
		20 %	3.22	3.42	3.58
		50 %	2.7	2.66	2.66
		100 %	2.36	2.3	2.3
Psidium guajava	Aqueous	Standard Drug Amoxicillin	4.56	4.42	4.38
		Saline	0.6	0.48	0.58
		10%	4.08	3.28	3.34
		20%	3.62	3.1	2.8
		50%	2.52	2.4	2.0

		100%	NZ	NZ	NZ

NOTE- NZ- No Zone

The antimicrobial activity of *Psidium guajava* (root ethanol and aqueous extract with DMSO) was assayed in vitro by the agar-well diffusion method against clinical isolates of E. coli, Pseudomonas, and Streptococcus oralis. The above-given table no. 1 shows the significant microbial growth inhibition of ethanolic root extracts of *Psidium guajava* among the varying concentrations of root extracts. Higher concentrations exhibited maximum antimicrobial activity against the three clinical isolates of E. coli, Pseudomonas, and Streptococcus oralis.

The root aqueous extract of Psidium guajava in 10 µl demonstrated a higher zone of inhibition (4.08) against E. coli in the antimicrobial activity sequence. *Psidium guajava* ethanolic extract in 10 µl demonstrated a greater zone of inhibition (3.92) against Streptococcus oralis. Aqueous extract of 10µl concentration of *Psidium guajava* root extract exhibited significant antimicrobial activity when compared with standard drug Amoxicillin. There was no zone of inhibition in the 100µl concentration of the aqueous extract of the plant extract. Experiment revealed that the higher the concentration, the greater the zone of inhibition of plant extracts in antimicrobial activity. And the low concentration of extract showed that the zone of inhibition was decreased.

4. Discussion:

The result obtained might be considered sufficient for further studies for the isolation and identification of active principles and for the evaluation of the possible antimicrobial activity of other extracts from other parts of *Psidium guajava*.

It was supported by K. Simon Neethu et al. (2016), who stated that the extracts from other parts of *Psidium guajava* are used against microbial infections due to the presence of secondary metabolites such as phenols, essential oils, terpenoids, alkaloids, and flavonoids. Flavonoids were present in *Psidium guajava*, which was earlier studied by Fernandez M.R.V. et al. (2014) and reported that flavonoids exhibit strong antimicrobial activity.

5. Conclusion:

In conclusion the present work was carried out and showed the antimicrobial activities of the root of Psidium guajava. Our investigation revealed that ethanolic extracts of Psidium guajava root was more effective inhibit the bacterial growth as compared to aqueous extracts. The antimicrobial activity exhibited due to presence of flavonoids and alkaloids. This study may help in the development of antimicrobial drugs in future. The plant harbors many pharmacologically important compounds. It can be used as antimicrobial supplement in the developing countries towards the development of new antimicrobial agents.

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