Original Article

In Vitro Evaluation of Antimicrobial Activity of Psidium guajava Leaf Extract against Selected Pathogenic Bacteria

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Abstract

Guava production in India, which accounts for 25 million metric tonnes or 45% of global production, is the highest. China (7%) and Indonesia (5%) are the second and third largest producers. Guava is a rich source of vitamin C which is most effective to cure UTI diseases. Leaf extracts in different solvents (Water, Benzene and methanol) prepared by Soxhlet extraction method were tested against two gram positive and two gram negative bacteria (Isolated from patient infected with UTI) by agar well diffusion method. Results indicated that methanol extract was most effective against Staphylococcus aureus, followed by Enterococcus faecalis (Gram positive)(17mm) in methanol extracts. In benzene extracts zone of inhibition were of 17 mm against Staphylococcus aureus (Gram positive), Enterococcus faecalis Gram positive) and similar pattern was in water extracts. In case of gram negative bacteria zone of inhibition was 13mm against Pseudomonas aeruginosa (Gram negative) in methanol extract and similar pattern was observed in benzene and water extracts. Gram positive bacteria were more sensitive than gram negative bacteria. This antimicrobial property in guava leaf is due to phenolic compounds present in it.

Keywords: Guava, Phenols, Staphylococcus aureus, agar well diffusion method, methanol extract.

INTRODUCTION

The Guava (Psidium guajava) is a fruit growing in India and a member of the Myrtaceae family. It is still farmed for commercial use in Myanmar, South Asia, the Hawaiian Islands, Cuba, Sri Lanka, and India. It was first domesticated in tropical America, from Mexico to Peru. Andhra Pradesh, Bihar, Maharashtra, Uttar Pradesh, Karnataka, Gujarat, Tamil Nadu, Orissa, and Chhattisgarh are the principal states producing guava. The state that produces the most guavas is Uttar Pradesh. The highest grade guava is said to be produced in Allahabad, both in India and internationally. Vitamins C and A are abundant in it (Rai et al., 2007). Vitamin C-rich meals help to fight UTI by preventing the growth of E. coli, making urine less acidic, and lowering the risk of recurrent UTI. It has traditionally been used to treat gingivitis, toothaches, ulcers, wounds, sore throats, vomiting, coughs, and diarrhea (Vieira et al., 2001; Teixeira et al., 2003; Venkatesan et al., 2005). Guava leaf extract has antidiabetic (Birdi et al., 2010;Mazumdar et al., 2015), anticancer (Bamosa et al., 2010; Ryu et al., 2012; Bontempo et al., 2012), anti-inflammatory (Roy et al., 2006; Matsuzaki et al., 2010; Denny et al., 2013), antioxidant (Masuda et al., 2003; He and Venant, 2004; Musa et al., 2022), anti-spasmodic (Dicarlo et al., 1996; Lozoya et al., 2002), and antimicrobial activity (Gonçalves et al., 2008; Pelegrini et al., 2008; Castro-Vargas et al., 2010; Banu and Sujatha, 2012; Puntawong et al., 2012; Chen et al., 2015; Samiha at al., 2017) For its medicinal properties, anthocyanins, alkaloids, flavonoids, tannins, and terpenoids all play a role (Ojewole , 2005; Shao et al., 2010; Ghosh et al.,2010; Castro-Vargas et al., 2010). The present investigation was performed to look the antimicrobial property of Psidium guajava leaf extracts against two gram-negative bacteria, two gram-positive bacteria.

Materials and Methods

Extraction: Shade-dried leaf pieces were ground into a fine powder, and 10 g of the powder were extracted using a Soxhlet apparatus at a 1:10 ratio in 100 ml of water, benzene and methanol. The extraction time for the solvent was set at 4 hours every

day, for a total of 36 hours. The resulting extract was extracted from the solvent chamber, and the surplus solvent was rotary evaporated. For use in subsequent experiments, the resulting residue was kept in a refrigerator.

Bacterial culture: A total four microorganisms were used to assess the anti bacterial properties, it includes two gram–positive bacteria, two-gram negative bacterial strains-such as Escherichia coli (Gram negative), Pseudomonas aeruginosa (Gram negative), Staphylococcus aureus (Gram positive), Enterococcus faecalis (Gram positive), which were isolated from patients infected with UTI.

Agar well diffusion method: The Perez et al., (1990) reported agar well diffusion assay was used to find out antimicrobial activity. On Muller-Hinton agar plates, 0.1 ml of diluted inoculums (2 x 108 CFU/ml) of the test organism was applied. Wells with a 6 mm diameter were drilled into the agar and filled with 10 µl each with solvent blank (DMSO) and plant extract at a concentration of 10 mg/ml. Overnight, the plates were incubated at 37 °C. As a positive control, amoxicillin were employed. Each well's zone of inhibition of test organism growth was measured in millimetres. Each test was performed in triplicate.

Estimation of minimum inhibitory concentration: 100 mg of each dried extract was dissolved in 5 ml of extracts to create a stock solution of extract (20 mg/ml), and from this stock, 2-fold serial dilutions of 10, 5, 2.5, 1.25, and 0.625 mg/ml were created. These concentrations were utilised to calculate the minimal inhibitory concentration along with the stocks. For the purpose of calculating the Minimum Inhibitory Concentration, the broth dilution method was used (MIC). Briefly, 2 ml of nutrient broth and 0. 1 ml of the prepared concentration of each extract were combined with the nutrient broth in six test tubes. After that, 0.1 ml of standardised inoculum was added to the test tube containing the extract and the suspension of nutritional broth. All test tubes were then securely corked and incubated for 24 hours at 37 °C. They were then checked to see if there was any visible growth, or not. The MIC was defined as the lowest concentration at which there was no discernible microbial growth (Esimone et al., 2012).

Results and Discussion

Antimicrobial activity of Guava leaf extracts in water, benzene and methanol was evaluated against four microorganisms such as Escherichia coli (Gram negative), Pseudomonas aeruginosa (Gram negative), Staphylococcus aureus (Gram positive), Enterococcus faecalis (Gram positive). The results shown in Table-1 indicates that zone of inhibition were larger against gram positive bacterial strains than gram negative bacterial strains. Staphylococcus aureus (Gram positive) showed 18 mm zone of inhibition followed by Enterococcus faecalis (Gram positive)(17mm) in methanol extracts. In benzene extracts zone of inhibition were of 17 mm against Staphylococcus aureus (Gram positive), Enterococcus faecalis (Gram positive) and similar pattern was in water extracts. In case of gram negative bacteria zone of inhibition was 13mm against Pseudomonas aeruginosa (Gram negative) in methanol extract and similar pattern was observed in benzene and water extracts. Antimicrobial activity of guava leaf is due to presence of isoflavonoids, gallic acid, carotenoids, ascorbic acid and specially phenolic compounds (Roy et al., 2006, Pelegrini et al., 2008; Castro-Vargas et al., 2010; Rahim et al., 2010; Ryu et al., 2012; Metwally et al., 2013). Leaves have Phenolic compounds, isoflavonoids, gallic acid, catechin, epicathechin, rutin, naringenin, kaempferol. Antibacterial screening has been done selectively by many researchers in guava essential oil and solvent extract against both gram positive and gram negative bacteria and yeast (Jairaj et al., 1999; Karawya et al., 1999; Gnan and Demello, 1999; Bauer et al., 1966, Olajide and Makinde, 1999; Vieira et al., 2001; Holetz et al., 2002; Abdelrahim et al., 2002, Kim and Fung, 2004; Sanches et al., 2005; Sacchetti et al., 2005 ; Qa'dan et al., 2005; Hoque et al., 2007; Ibrahim et al., 2011). Guava extract in aqueous form was reported to be efficient against Staphylococcus and Bacillus by Sanches et al., 2005. The growth of two isolates of Salmonella and enteropathogenic E. coli was significantly inhibited by the guava methanolic extracts, as reported by Lin et al., 2002. According to Biswas et al., (2013), guava (Psidium guajava) leaf extracts have the ability to fight off two gram-negative bacteria (Escherichia coli and Salmonella enteritidis) and two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus), antibacterial experiment, gram-positive bacteria were inhibited by the methanol and ethanol extracts of guava leaves, whereas gram-negative bacteria were resistant to all of the solvent. The antibacterial activity of each extract was evaluated using agar well diffusion, and it was discovered that the guava methanol extract had the largest and most significant zone of inhibition against all of the tested microorganisms. The Minimum Inhibitory Concentration (MIC) value against Escherichia coli (Gram negative), Pseudomonas aeruginosa (Gram negative), Staphylococcus aureus (Gram positive), Enterococcus faecalis (Gram positive), was also determined using methanolic extracts of all the samples. The test was conducted in accordance with the prescribed protocol, and the outcomes are displayed in Table 2. The findings showed that S. aureus had the lowest and most significant MIC value (1.05±0.03), whereas amoxicillin had a MIC of 0.25 mg/ml.

Table 1: Antibacterial activity of different leaf extracts of P. guajava

		Concentration of Plant Extracts										
		Water			Benzene			Methanol			DMSO	ANTIBIOTIC (Amoxicillin)
		Zone of Inhibition (in mm)										
S. No.	**Bacteria**	**25**	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**		
B1	*Escherichia coli* (Gram negative) .	-	6	10	-	8	12	-	10	12	0	18
B2	*Pseudomonas aeruginosa* (Gram negative)	-	8	12	-	8	12	-	11	13	0	18
B3	*Staphylococcus aureus* (Gram positive)	5	10	14	7	10	16	9	14	18	0	23
B4	*Enterococcus faecalis* (Gram positive)	5	9	14	7	10	16	9	14	17	0	23

Table 2: Determination of MIC value of different leaf extracts of P. guajava

S. No.	Bacterial Strains	MIC value (mg/ml) of Methanol Extracts	Antibiotic
B1	*Escherichia coli*	2.7±0.06	1.50±0.05
B2	*Pseudomonas aeruginosa*	3.15±0.1	0.25±0.02
B3	*Staphylococcus aureus*	1.05±0.03	0.25±0.04
B4	*Enterococcus faecalis*	1.11±0.05	1.00±0.03

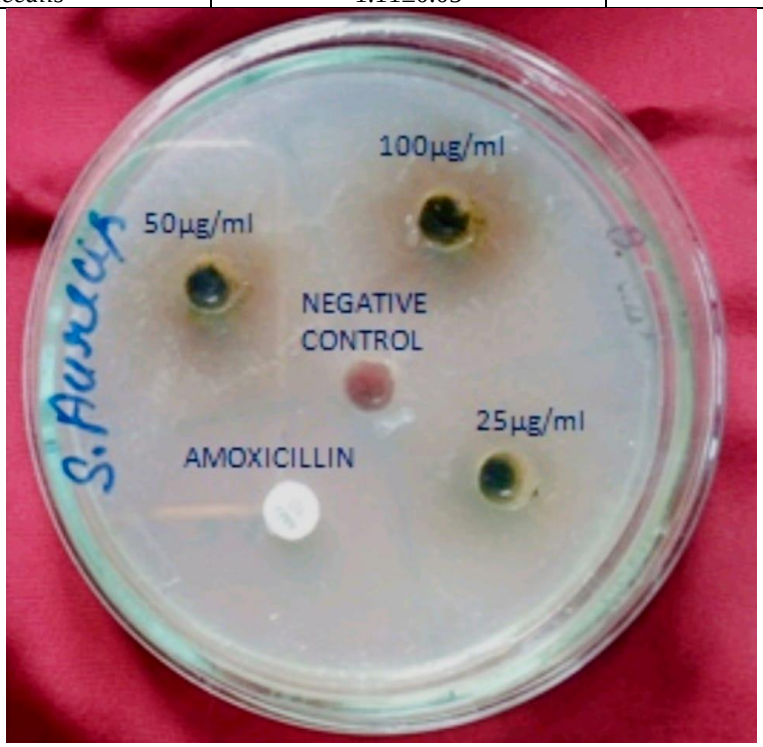

Fig. 1: Antibacterial activity of the methanolic extracts of P. guajava leaves against S. aureus

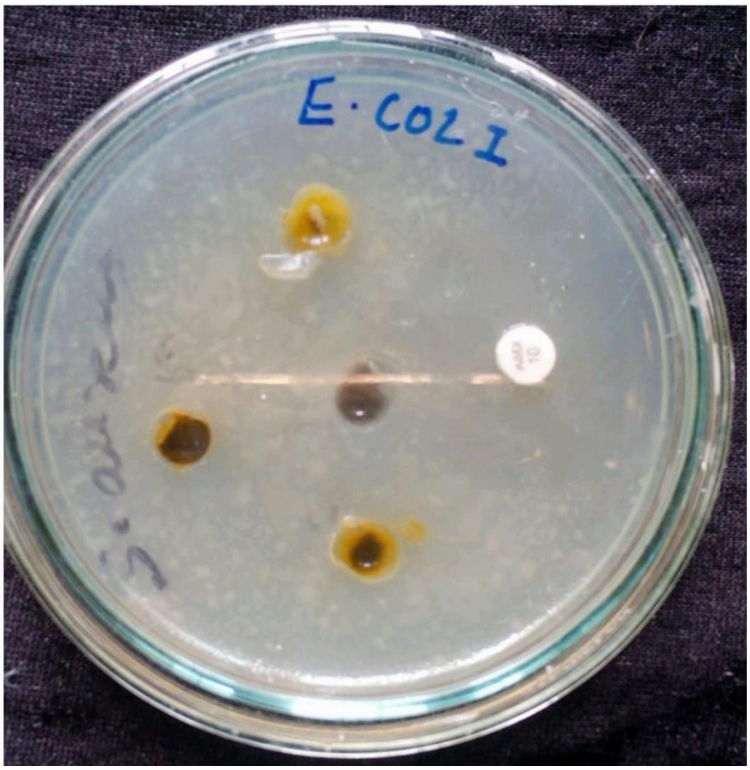

Fig. 2: Antibacterial activity of the methanolic extracts of P. guajava leaves against E. coli

Conclusion

In conclusion, guava leaf extracts and essential oil are very active against S. aureus, thus making up important potential sources of new antimicrobial compounds (Goncalves et al., 2008). Based on the findings of this study, P. guajava leaves have the potential to be a strong contender in the quest for a natural antibiotic that can treat conditions brought on S. aureus infections and/or disorders. To increase its use, additional research is required on its degree of toxicity and any potential antagonistic or synergistic interactions with other plants or medications.

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