Antibacterial activity of *Psidium guajava* L. against certain multidrug resistant gram-negative and gram-positive bacteria

Authors

Beulah Edwin¹*, Sony Paul², Iyanar Kannan³

¹,³Associate Professor, Department of Microbiology, Tagore Medical College and Hospital, Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai -600127, India

²Assistant Professor, Department of Microbiology, Tagore Medical College and Hospital, Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai -600127, India

*Corresponding Author

Dr Beulah Edwin MD

Associate Professor, Dept of Microbiology, Tagore Medical College and Hospital, Chennai -600127, India

Email: beulahrobin@tagoremch.com

Abstract

Drugs derived from plants play a significant role in the prevention and treatment of human diseases. *Psidium guajava* L., Myrtaceae, is used widely in traditional medicine used for the treatment of diarrhoea, dysentery, gastroenteritis, stomach aches, and indigestion. The present study aimed at studying the antibacterial activity of the ethanolic extract of leaves of *P. guajava* against the standard strains of bacterium and the antibiotics resistant clinical strains of bacterium. The extraction of the *P. guajava* leaves was carried out. The screening of the extracts for antibacterial activities was conducted by the disc diffusion method. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) with a concentration of 0.1 g/mL. All the bacterial strains under study were inhibited. *Staphylococcus aureus* was most inhibited. The present study has shown that *P. guajava* leaf extract has excellent antibacterial activity against the drug resistant strains of both gram-positive and gram-negative bacteria.

Keywords: *Psidium guajava*, antibacterial activity, disc diffusion method.

Introduction

Antibiotics are important agents in combating bacterial infections. However, now antibiotics have become less effective because of the emergence of drug-resistant bacteria. It is imperative to investigate newer drugs which are active against drug resistant bacteria. Drugs derived from plants play a significant role in the prevention and treatment of human diseases. The bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents[¹]. In many developing countries, traditional herbal medicine is one of the primary healthcare systems[²,³]. The phytochemicals of many medicinal plants may form rich source of antimicrobial agents with a different mechanism of action[⁴,⁵]. Thus, many studies have been undertaken to find the effectiveness of plant extracts on bacteria from different parts of the world[⁶]. Many works have been done on ethnomedicinal plants of India[⁷].
Plants are rich source of phytochemicals such as flavonoids, glycosides, tannins, terpenoids, alkaloids, etc., which have been found to have antimicrobial properties\(^\text{[8, 9]}\).

*Psidium guajava* L., Myrtaceae, is used widely in traditional medicine for the treatment of diarrhoea, dysentery, gastroenteritis, stomach aches, and indigestion\(^\text{[10]}\). It is used for diarrhoea and dysentery in many countries\(^\text{[11]}\). The plant is a rich source of flavonoids like quercetin and their natural derivatives like avicularin, guaijaverin, isoquercetin, hyperin, quercitrin, quercetin 3-O-gentiobioside and quercetin 4’-glucuronide\(^\text{[12-17]}\). Quercetin has several pharmacologic actions like antioxidant properties, anti-inflammatory activity, antibacterial, antiviral and antitumor activities\(^\text{[18-21]}\). The present study aimed at studying the antibacterial activity of the ethanolic extract of leaves of *P. guajava* against the standard strains of bacterium and the antibiotics resistant clinical strains of bacterium.

**Materials and Methods**

**Collection of plants**
The fresh and apparently healthy leaves of the plant *P. guajava* were collected in and around Chennai and were identified in department of botany, Chennai.

**Preparation of plant extract**
The extraction of the *P. guajava* leaves was carried out following the standard protocol\(^\text{[14]}\). The plant materials were washed and shade dried. Then they are powdered in a grinder. The powder (50.0 g) of the plant material was extracted by 900 ml of hydroalcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) and concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The extracts were then stored in sterile bottles until further use. The dry weight of the plant extracts was obtained is measured in mg/ml.

**Test organisms**
The standard strains of *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), and *Pseudomonas aeruginosa* (MTCC 424) are obtained from Institute of Microbial Technology, Chandigarh, India. The clinical isolated obtained pus, throat swab and urine were also included in the study.

**Antibacterial study by disc diffusion assay**
The screening of the extracts for antibacterial activities was conducted by the disc diffusion method. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) with a concentration of 0.1 g/mL. Paper discs (6 mm in diameter) were impregnated with 30 μL of plant extracts and placed on Mueller Hinton agar plates and blood agar plates, which were inoculated with test organisms according to the standard protocol described by the National Committee of Clinical Laboratory Standards\(^\text{[15]}\).

The plates were incubated at 37 °C and the diameters of the inhibition zones were measured after 18 h. Filter paper discs containing DMSO without any test compounds served as a control and no inhibition was observed. Tetracycline (15 mg/L, 30 μL) was used as a reference standard. Each assay was performed in triplicate and repeated three times.

**Results**
The results of antibacterial activity for standard strains are given in the Table 1. From the table it is evident that the extract at various concentrations have shown antibacterial activity to the standard test organisms. Similarly, the antibacterial activity for multidrug resistant clinical strains are given in the Table 2. The table clearly shows that the extract at various concentrations have antibacterial activity to the organisms.

All the bacterial strains under study were inhibited. *Staphylococcus aureus* was most inhibited with zone diameter of 28±0.09, while *Pseudomonas aeruginosa* was the least inhibited (24.4±0.33). The zone diameter for MTCC strains
varied from 24.4±0.33 to 28±0.09. For E.coli the inhibitory diameter was 25.8±0.42. All the resistant bacterial isolates from clinical samples were also inhibited by the plant extract. The diameter of zone of inhibition ranged between 23.8±0.81 to 27±0.65. In the case of resistant strains also, *Staphylococcus aureus* was most inhibited, while *Pseudomonas aeruginosa* was the least inhibited. Standard strain and penicillin resistant strain of *Strep. pyogenes* was also inhibited by the ethanolic leaf extract.

Table 1: Antibacterial activity of ethanolic extract of leaves of *P. guajava* against standard strains of gram-positive and gram-negative organisms

| Organism           | Average zone diameter ± SD in mm | 5µg/mL | 25µg/mL | 50µg/mL | 100 µg/mL | Penicillin |
|--------------------|----------------------------------|--------|---------|---------|-----------|------------|
| *Staphylococcus aureus* | 10.3±0.25                       | 18.8±0.55 | 28±0.09 | 16.5±0.26 | 27±0.56   |
| *S. pyogens*         | 7.5 ±0.06                        | 17.8±0.28 | 26.1±0.83 | 18.7±0.34 | -         |
| *E. coli*            | 6.1±0.12                         | 18.4±0.57 | 25.8±0.42 | 17.5±0.16 | -         |
| *Pseudomonas aeruginosa* | 8.1±0.62                       | 14.9±0.39 | 24.4±0.33 | 17.6±0.31 | -         |

Table 2: Antibacterial activity of ethanolic extract of leaves of *P. guajava* against multidrug resistant clinical strains of gram-positive and gram-negative organisms

| Organism           | Average zone diameter ± SD in mm | 5µg/mL | 25µg/mL | 50µg/mL | 100 µg/mL | Penicillin |
|--------------------|----------------------------------|--------|---------|---------|-----------|------------|
| *Staphylococcus aureus* | 9.3±0.25                       | 17.5±0.24 | 27±0.65 | 25.2±0.65 | 27±0.56   |
| *S. pyogens*         | 6.2 ±0.06                        | 17.9±0.55 | 24.2±0.48 | 27.5±0.54 | -         |
| *E. coli*            | 5.1±0.12                         | 18.8±0.65 | 25.4±0.59 | 26.9±0.65 | -         |
| *Pseudomonas aeruginosa* | 4.1±0.62                       | 12.7±0.38 | 23.8±0.81 | 28.9±0.42 | -         |

Discussion

It has been identified that the activity of *P. Guajava* is probably due to their ability to complex with extracellular and soluble proteins, and to complex with bacterial cell walls. More lipophilic flavonoids may disrupt the microbial membranes[22]. The flavonoids present in guava leaves were identified to be responsible for the antimicrobial activity[23]. The *P. Guajava* has many tannins that have received a great deal of attention in recent years, since it has been found to cure and prevent a variety of illness[23].

Conclusion

The present study has shown that *P. guajava* leaf extract has excellent antibacterial activity against the drug resistant strains of both gram-positive and gram-negative bacteria.

Acknowledgements

Author is thankful to the central research laboratory, Tagore Medical College and Hospital for extending help in the completion of this research study.

References

1. Towers GH, Lopez A, Hudson JB. Antiviral and antimicrobial activities of medicinal plants. J Ethnopharmacol. 2001; 77:189–96.
2. Farnsworth NR. Ethno pharmacology and future drug development: The North American experience. J Ethnopharmacol. 1993;38:145–52.
3. Houghton PJ. The role of plants in traditional medicine and current therapy. J Alter Complement Med. 1995;1:131–43.
4. Runyoro D, Matee M, Olipa N, Joseph C, Mbwambo H. Screening of Tanzanian medicinal plants for anti-Candida activity. BMC Complement Altern Med. 2006;6:11.
5. Shahidi BH. Evaluation of antimicrobial properties of Iranian medicinal plants against Micrococcus luteus, Serratia marcescens, Klebsiella pneumonia and Bordetella bronchoseptica. Asian J Plant Sci. 2004;3:82–6.
6. Reddy PS, Jamil K, Madhusudhan P. Antibacterial activity of isolates from Piper longum and Taxus baccata. Pharmaceutical Biol. 2001;39:236–8.
7. Maheshwari JK, Singh KK, Saha S. Economic Botany Information Service, NBRI. Lucknow: 1986. Ethno botany of tribes of Mirzapur District, Uttar Pradesh.
8. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol. 2000;32:S81–118.
9. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564–82.
10. Gutiérrez RM, Mitchell S, Solis RV: Psidium guajava: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2008, 117:1-27.
11. Dham SK. Treatment of diarrheal diseases. In Diarrhoeal diseases: Current status, research trends and field studies Edited by: Raghunath D, Nayak R. New Delhi: Tata McGraw-Hill Publishing Company Limited; 2003:245-254.
12. Abdel Wahab SM, Hifawy MS, El Gohary HM, Isak M. Study of carbohydrates, lipids, protein, flavonoids, vitamin C and biological activity of Psidium guajava L growing in Egypt. Egypt J Biomed Sci. 2004;16:35–52.
13. El Khadem H, Mohamed YS. Constituents of the leaves of Psidium guajava L: Part II: Quercetin, avicularin, and guaijaverin. J Chem Soc. 1958;32:3320–3.
14. Arima H, Danno G. Isolation of antimicrobial compounds from guava (Psidium guajava L.) and their structural elucidation. Biosci Biotechnol Biochem. 2002;66:1727–30.
15. Seshadri T, Vasishta K. Polyphenols of the leaves of Psidium guava; quercetin, guaijaverin, leucocyanidin, amritoside. Phytochemistry. 1965;4:989–92.
16. Kandil FE, El-Sayed NH, Micheal HN, Ishak MS, Mabry TJ. Flavonoids from Psidium guajava. Asian J Chem. 1997; 9:871–2.
17. Murray MT, Pizzorno JE. Flavonoids-Quercetin, citrus flavonoids, and HERs (hydroxylethylrutosides) London: Harcourt Brace and company Ltd; 1999. Text book of Natural Medicine 2nd ed; pp. 745–50.
18. Mucsi I, Pragai BM. Inhibition of virus multiplication and alteration of cyclic AMP level in cell cultures by flavonoids. Experientia. 1985;41:930–1.
19. Kaul T, Middleton E, Ogra P. Antiviral effects of flavonoids on human viruses. J Med Virol. 1985;15:71–9.
20. Farkas L, Gabor M, kallay F, Wagner HI. New York: Elsevier; 1982. Flavonoids and Bioflavonoids; pp. 443–50.
21. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564–82.
22. Rattanachaikunsopon P, Phumphachorn P. Contents and antibacterial activity of flavonoids extracted from leaves of Psidium guajava. J Med Plants Res. 2010;4:393–6.
23. Gutierrez RM, Mitchell S, Solis RV. Psidium guajava: A review of its traditional, phytochemistry and pharmacology. J Ethnopharmacol. 2008;117:1–27.