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Original Research Article

Antifungal and Antibacterial Property of Guava *(Psidium guajava)* Leaf Extract: Role of Phytochemicals

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ABSTRACT

The traditional medical practice is an integral part of the culture and the interpretation of health by indigenous populations in most of the world. Guava (Psidium guajava L.) leaves have traditionally been used to manage several diseases such as rheumatism, diarrhea, diabetes mellitus, and cough. In this present investigation antifungal and antibacterial property of guava leaves were estimated using Bacillus subtilis (Gram positive bacterial strain), Escherichia coli (Gram negative bacterial strain), Saccharomyces cerevisiae (Yeast, fungal strain) and Aspergillus niger (Mould, fungal strain) strains. The growth of gram positive bacteria and fungal strains were inhibited strongly, whereas gram negative bacterial strain displayed less sensitivity against the antimicrobial (antifungal and antibacterial) property of guava leaf extract. Zone inhibition assay also confirmed the result. Phytochemical analysis (qualitative and quantitative) revealed that guava leaf extract was rich in wide range of poly phenols. It was found that guava leaves are rich in phenols, flavonoids and tannins whereas components like alkaloids, flavonoids, saponins and triterpenes are present in comparatively lesser amounts. As polyphenols have strong antimicrobial property, it can be concluded that rich source of phenols, flavonoids and tannins are the probable cause of anti microbial property of guava leaves.

Keywords: Antifungal, antibacterial, guava leaf, phytochemical, polyphenol

1. INTRODUCTION

Study of traditional medical practice, is an integral part of the culture and the interpretation of health by indigenous populations in many parts of the world. [1] For example, Indian Ayurveda and traditional Chinese medicine are among the most enduring folk medicines still practiced. These systems try to promote health and improve the quality of life, with therapies based on the use of indigenous drugs of natural origin. [2] Given that plants have been widely used as herbal medicines, several approaches are now being carried out to discover new bioactive compounds. [3]

Psidium guajava L., popularly known as guava, is a small tree belonging to the myrtle family (Myrtaceae). [4] Native to tropical areas from southern Mexico to northern South America, guava trees have been grown by many other countries having tropical and subtropical climates, thus allowing production around the world. [5] Traditionally, preparations of the leaves have been used in folk medicine in several countries, mainly as anti-diarrheal remedy. [6] Moreover, other several uses have been described elsewhere on all continents, with the exception of Europe. [6-8] Depending upon the illness, the application of the remedy is either oral or topical. The

consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders, such as rheumatism, diarrhea, diabetes mellitus, and cough in India, China, Pakistan, and Bangladesh, [6-9] while in Southeast Asia the decoction is used as gargle for mouth ulcers. [6,8,9] For skin and wound applications, poultice is externally used in Mexico, Brazil, Philippines, and Nigeria. [6-9] In addition, chewing stick is used for oral care in Nigeria. [9]

Currently, there is increasing interest in studying of plants regarding their chemical components of bioactive compounds, their effects on several diseases, and their use for human health as functional foods and/or nutraceuticals. [10] In recent years, guava leaves tea and some complementary guava products are available in several shops in Japan as well as on the Internet, [11] because guava leaf phenolic compounds have been claimed to be food for specified health use (FOSHU), since they have beneficial health effects related to the modulation of blood–sugar level. [12] In this present investigation we tried to find out the antifungal and antibacterial property of guava leave and the probable cause behind it.

2. METHODOLOGY

2.1. Preparation of guava leaf powder:

Fresh guava leaves were collected and air dried for 10 days. The dried leaves were then crushed and churned in a blender to form a coarse powder. The powder was collected in an air tight container and stored in a cool, dry place, away from sunlight.

2.2. Preparation of methanol extract of guava leaf powder:

The methanol extract was prepared by mixing 20 grams of guava leaf powder with 100 ml of methanol, which was kept for 3 days in a cool, dark place along with occasional stirring. After 3 days, the extract was filtered into sterilised test tubes.

2.3. Preparation of nutrient agar (500ml):

Weighed amounts of peptone (2.5 gm), glucose (5 gm), and beef extract (0.75 gm) and agar (10gm) were dissolved in distilled water (500 ml), in a conical flask. The mixture was sterilised in an autoclave.

2.4. Estimation of antimicrobial activity of guava leaf extract

The antimicrobial activity of the methanol extract of guava leaves, was estimated against four microbial strains: *Bacillus subtilis* (Gram positive bacterial strain) [MTCC No: 441], *Escherichia coli* [MTCC No: 1696](Gram negative bacterial strain), *Saccharomyces cerevisiae* [MTCC No: 3090](Yeast, fungal strain) and *Aspergillus niger* [MTCC No: 10180] (Mould, fungal strain).

2.4.1. Examination of microbial (fungus and bacteria) growth in the presence and absence of Guava leaf extract

For each microbial strain, four petri plates were used, of which one was used as ‘Control’ plate, while the rest were used as ‘Test’ plates containing different amount of Guava leaf extract as for example 0.5 ml, 1ml and 2ml, respectively along with nutrient agar. The petri plates were streaked with the above-mentioned microbial strains and were incubated at 37ºC. Microbial growths were observed after 24 hours.

2.4.2. Estimation of zone inhibition using Well Diffusion method

Before preparing the petri plates, liquid cultures of the microbial strains were prepared by inoculating lactose broth with previously prepared sub cultures and incubating it for 24 hours at 37C to observe the turbidity. [13] The nutrient agar containing petri plates were inoculated with these liquid cultures using spread plating method.

For each microbial strain, one petri plate was kept aside as ‘Control’ plate while another was used as ‘Test’ plate which contained Guava leaf extract in three wells, at concentrations of 50 µl, 100 µl and 150 µl, respectively. The petri plates were incubated at 37ºC and zone of inhibition was observed after 24 hours.

2.5. Qualitative analysis of the phytochemical content of guava leaf extract

Chemical tests for the screening and identification of bioactive chemical constituents in the guava were carried out with the extracts using the standard procedure as described. [14]

2.5.1. Test for alkaloids: The leaf extracts of guava were dissolved and filtered in dilute hydrochloric acid. Wagner's reagent was prepared by mixing 6 gm of potassium iodide and 2 gm of iodine in 100 ml of distilled water, and then added to the previously obtained filtrate. The presence of alkaloids would be confirmed with the appearance of reddish-brown precipitate.

2.5.2. Test for anthraquinones: 0.2 ml of guava leaf extracts were taken, to which 2 ml of chloroform was added. The mixture was shaken and filtered, and was mixed with 10% ammonia solution. The presence of anthraquinones would be confirmed with the presence of bright pink precipitate.

2.5.3. Test for flavonoids: 0.5 ml of guava leaf extracts were taken, to which few drops of 10% sodium hydroxide was added. The appearance of bright yellow colour, which will disappear with the addition of dilute acid, would confirm the presence of flavonoids.

2.5.4. Test for phenols: 0.5 ml of guava leaf extracts were taken, to which few drops of 10% ferric chloride solution was added. The presence of phenol would be confirmed with the appearance of bluish black colour.

2.5.5. Test for saponins: The guava leaf extract was taken in a test tube and shaken vigorously. Formation of a stable foam would confirm the presence of saponins.

2.5.6. Test for tannins: 0.5 ml of guava leaf extracts were taken, to which 10% sodium chloride solution containing 1% gelatin solution was added. The presence of tannins would be confirmed with the appearance of white precipitate.

2.5.7. Test for triterpenes: to guava leaf extracts, chloroform was added and filtered. To it, few drops of concentrated sulphuric acid was added, shaken and kept aside. The presence of triterpenes was would be confirmed with the appearance of golden yellow colour.

2.6. Quantitative analysis of phenol content of guava leaf extract:

The total phenolic content of guava leaf extract was determined using a spectrophotometer and Folin Ciocalteu's reagent. The Folin reagent was first prepared at a dilution of 10% using distilled water, and to it, 5 ml of the guava leaf extract was mixed. Thereafter, anhydrous sodium carbonate was prepared at a concentration of 75%, and 4ml of it was added to the Folin mixture, hence producing a blue coloured solution. A blank was prepared and the resulting mixtures were shaken vigorously and incubated at 40C for 30 minutes. The resulting optical densities were measured at 765 nm using a spectrophotometer. The absorbance against mg of gallic acid was measured and the graph obtained was used to determine the phenol content of guava leaf extract. [14]

2.7. Quantitative analysis of tannin content of guava leaf extract

Tannin content of guava leaf extract was determined spectrophotometrically using Folin Ciocalteu's reagent. Standard Tannic acid solution was prepared by mixing 100 mg Tannic acid in 1000 ml water. This solution was pipetted in 100ml flasks containing 75 ml distilled water in amounts of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml. To each mixture, 5 ml of Folin reagent was added, along with 10ml was sodium carbonate solution (prepared by dissolving 35 gm anhydrous sodium carbonate in 100ml water). The mixtures was shaken well and incubated for 30 minutes at 4^0C. The absorbance against mg of tannic acid was plotted and the graph obtained was used to determine the concentration of tannin in guava leaf extract. [15]

The sample was prepared by pipetting 1 ml of guava leaf extract in a flask containing 80 ml water. To it, 5 ml of Folin reagent was added along with 75% sodium carbonate solution. The volume was made up and the mixture was incubated at 4^0C for 30 minutes against blank and the absorbance was measured.

2.7. Quantitative analysis of flavonoid content of guava leaf extract

The flavonoid content was determined according to the method described by Ordonez et al. [16] Briefly, 0.5 mL of the extract was mixed with 0.5 mL of 2% aluminum chloride (prepared in ethanol).The mixture was incubated for 1 h at room temperature, after which the absorbance was read at 420 nm. Development of yellow color indicated the presence of flavonoid. Total flavonoid content was calculated as mg/g of quercetin equivalent using the calibration curve.

The results of all microbial and chemical analysis were expressed as mean ± standard deviation of triplicate analyses.

3. RESULTS & DISCUSSION

3.1. Estimation of antimicrobial activity (antifungal and anti bacterial) of guava leaf extract

The antimicrobial activity of methanol extract of guava leaves was assessed against four microbial strains: *Bacillus subtilis* (Gram positive bacterial strain), *Escherichia coli* (Gram negative bacterial strain), *Saccharomyces cerevisiae* (Yeast; fungal strain) and *Aspergillus niger* (Mould; fungal strain).

3.1.1. Examination of microbial (fungus and bacteria) growth in the presence and absence of Guava leaf extract

After 24 hours of incubation, the microbial growth was observed for bacterial as well the fungal strains **(Table: 1)**

Table 1: Estimation of antimicrobial activity of guava leaf extract using pour plating technique

Microbial Strain	Microbial growth observed			
		Amount of Guava leaf extract		
	Control	0.5 ml	1 ml	2 ml
Bacillus subtilis	+++	+	-	-
Escherichia coli	+++	++	++	-
Saccharomyces cerevisiae	+++	+	-	-
Aspergillus niger	+++	+	-	-

++ : High Growth, +: Low growth, - : No growth

The petri plates streaked with gram positive strain and fungal strain plenty of growth was observed in the 'Control' plate. In the petri plate containing 0.5 ml of guava leaf extract, less microbial growth was observed. No microbial growth was observed in the petri plates containing 1 ml and 2 ml of guava leaf extract, respectively. In the petri plates streaked with gram negative bacterial strains (*Escherichia coli*), abundant growth was observed in the 'Control' petri plate, as well in the petri plates containing 0.5 ml and 1 ml guava leaf extract, respectively. Negligible growth was observed in the petri plate containing 2 ml guava leaf extract. The result of this study indicated that, gram negative bacterial strain *Escherichia coli*, was less sensitive towards the antimicrobial activity of guava leaf extract.

3.1.2. Estimation of zone inhibition by Well Diffusion method

After 24 hours of incubation, the Zones of Inhibition were measured for the bacterial and fungal strains **(Table: 2).** Results showed that zone of inhibition were much less in case of gram –ve bacteria compared to gm +ve bacteria and fungal strain.

Table 2: Zone of inhibition of bacterial and fungal strains against different concentration of guava leaf extract

Microbial strain	Zone of Inhibition (cm)		
	50 µl Guava leaf extract (mm)	100 µl Guava leaf extract (mm)	150 µl Guava leaf extract (mm)
Bacillus subtilis	14 ± 3.2	16 ± 4.3	20 ± 4.2
Escherichia coli	08 ± 2.6	10 ± 2.5	13 ± 3.6
Saccharomyces cerevisiae	16 ± 4.3	18 ± 3.3	24 ± 5.2
Aspergillus niger	15 ± 4.2	19 ± 4.4	21 ± 5.4

Inhibition zones are the mean including borer (5 mm) diameter ± standard deviation

3.2. Qualitative analysis of the phytochemical content of guava leaf extract

Phytochemical analysis of guava leaf extract showed that phenols, flavonoids and tannins are present in significantly large amounts whereas components like alkaloids, saponins and triterpenes are present in comparatively lesser amounts in guava leaf extract **(Table: 3).**

Table 3: Qualitative Analysis of Guava Leaf extract

Qualitative Analysis of Guava Leaf extract	
Compound tested	**Result**
Alkaloids	+
Anthroquinones	-
Flavonoids	++
Phenols	++
Saponins	+
Tannins	++
Triterpenes	+

++: Strongly Detected, +: Detected; −: Not Detected

3.3. Quantitative analysis of the phytochemical content of guava leaf extract

Since qualitative analysis of guava leaf showed that it is very rich in phenol, tannin and flavonoid, quantitative analysis (spectrophotometrically) were performed for phenol, tannin and flavonoid. **(Table: 4)**

Table 4: Quantitative analysis phenol, tannin and flavanoid. of Guava leaf extract

Compound analysed	Concentration (mg/ml)
Tannin	3.07 ± 0.6
Phenol	2.62 ± 0.7
Flavanoids	0.9 ± 0.3

4. DISCUSSION

Overall result indicated that Gram negative bacteria (*Escherichia coli*) exhibited less sensitivity compared to the Gram positive bacteria (*Bacillus subtilis*) towards the antimicrobial property of Guava leaves, whereas the fungal strains showed strong sensitivity towards the antimicrobial property of guava leaves. Gram negative bacteria are less sensitive to antimicrobial components as compared to Gram positive bacteria, may be due to a difference in their respective cell wall structures.

The cell wall of Gram negative bacteria consist of three distinct layers, which act as an envelope towards certain bioactive components. These include: an outer membrane, which consists of lipopolysaccharide, a peptidoglycan cell wall consisting of partially cross linked peptide chains, and an inner or cytoplasmic membrane. [17]

Gram positive bacteria are generally devoid of this outer membrane as compared to gram negative bacteria. The concerned outer membrane acts as a barrier with limited permeability, which prevents the penetration of certain antibiotic components and drugs into the cell. The presence of this outer membrane is one of the major reasons contributing towards the increased antibiotic resistance of Gram negative bacteria, as compared to their gram positive counterparts. [18]

The value of edible/ medicinal plants to mankind is well established, as numerous discoveries have shown that plants' extracts contain not only minerals and primary metabolites, but also a wide range of secondary metabolites with great therapeutic efficiencies.

Phytochemical analysis of guava leave showed the guava leaves are rich in flavonoids, phenols and tannins whereas components like alkaloids, saponins and triterpenes are present in comparatively lesser amounts in guava leaf extract. It is well known that along with powerful anti-inflammatory, free radical scavenging, antioxidant and anti-carcinogenic properties, polyphenolic compounds contain potent antimicrobial activity. [19] Inhibition of RNA, DNA, and depolarization of cytoplasmic membrane and inhibition of macromolecular synthesis are some of the underlying antimicrobial mechanisms of action of polyphenolic compounds. [20] Evidences also suggested that phenols show anti-fungal activity by inhibiting ergosterol which is the component of fungal cell membrane and glucosamine, a growth indicator present only in the fungal cells of some genera and some other proteins. [21]

Tannins are water-soluble chemical compounds found widely in the plant kingdom, which exhibit strong antimicrobial property. The various mechanism which are

present in tannins to inhibit microbial growth include deprivation of iron via iron chelation, disturbance in the metabolic activities of microbes via inhibition of oxidative phosphorylation, deprivation of essential compounds responsible microbial growth and also, inhibition of enzymes required in the extracellular cytoplasmic membrane. [22] Tannin act as a antimicrobial agent by the mechanism such as: extracellular enzyme inhibition, deprivation of substratum, inhibition of oxidative phosphorylation. [23] From the above discussion it can be concluded that rich source of wide range of poly phenolic compounds (Phenol, flavonoids and tannin) may be the most probable cause of the antimicrobial property of guava leaves.

CONCLUSION

From this investigation it can be concluded that Guava leaves posses strong antimicrobial (antibacterial and antifungal) property although the gram negative bacteria showed less sensitivity towards the antimicrobial property of guava leaves. Phytochemical analysis showed that Guava leaves are rich in wide range of polyphenolic compounds (Phenol, flavonoids and tannin). As the polyphenolic compounds posses the antimicrobial property, it may be the most probable cause of antifungal and antibacterial property of guava leave. Not only that for the antimicrobial property, guava leaves can be used in pharmaceutical industry as well as in food industry as bio preservative. For their detail mechanism of action further investigations are required.

REFERENCES

1. Anyinam C. Ecology and ethnomedicine: Exploring links between current environmental crisis and indigenous medical practices. Soc. Sci. Med. 1995; 40: 321–329.
2. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: A comparative overview. Evid. Based Complement. Altern. Med. 2005; 2: 465–473.
3. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 2001; 109: 69–75.
4. Morton JF. Fruits of Warm Climates; Creative Resource Systems, Inc.: Winterville, NC, USA, 1987.
5. Salazar DM, Melgarejo P, Martínez R, Martínez, JJ, Hernández F, Burguera M. Phenological stages of the guava tree (Psidium guajava L.). Sci. Hortic. 2006; 108: 157–161.
6. Gutiérrez RMP, Mitchell S, Solis RV. Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 2008; 117: 1–27.
7. Shruthi SD, Roshan A, Sharma S, Sunita S. A review on the medicinal plant Psidium guajava Linn. (Myrtaceae). J. Drug Deliv. Ther. 2013; 3: 162–168.
8. Morais-Braga MFB, Carneiro JNP, Machado AJT, Dos Santos ATL, Sales DL, Lima LF, Figueredo FG, Coutinho HDM. Psidium guajava L., from ethnobiology to scientific evaluation: Elucidating bioactivity against pathogenic microorganisms. J. Ethnopharmacol. 2016; 194: 1140–1152.
9. Sanda KA, Grema HA, Geidam YA, Bukar-Kolo YM. Pharmacological aspects of P. guajava: An update. Int. J. Pharmacol. 2011; 7: 316–324.
10. Bernal J, Mendiola, JA, Ibáñez E, Cifuentes A. Advanced analysis of nutraceuticals. J. Pharm. Biomed. Anal. 2011; 55: 758–774.
11. Matsuda K, Nishimura Y, Kurata N, Iwase M, Yasuhara H. Effects of continuous ingestion of herbal teas on intestinal CYP3A in the rat. J. Pharmacol. Sci. 2007; 103: 214–221.
12. Arai S, Yasuoka A, Abe. K. Functional food science and food for specified health use policy in Japan: State of the art. Curr. Opin. Lipidol. 2008; 19: 69–73.
13. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. Antimicrobial Activities of Leaf Extracts on Two Gram-Negative and Gram-Positive Bacteria. International Journal of Microbiology. 2013; 5 (6): 1 – 7.
14. Sagbo IJ, Afolayan AJ, Bradley G. Antioxidant, antibacterial and phytochemical properties of two medicinal plants against the wound infecting bacteria. Asian Pacific Journal of Tropical Biomedicine. 2017; 7 (9): 817 – 825.

15. Doss A, Dhanabalan R. Antibacterial activity of tannins from the leaves of Solanum trilobatum Linn. Indian Journal of Science and Technology. 2009: 2 (2): 41 – 44.
16. Ordonez AA, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of Sechium edule (jacq). swart extracts. Food Chem 2006; 97: 452-8.
17. Exner M, Bhattacharya S, Christiansen B, Gebel J, Trautmann M.. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? GMS Hygiene and Infection Control. 2017; 12 (5): 1 – 24.
18. Miller SI. Antibiotic Resistance and Regulation of the Gram-Negative Bacterial Outer Membrane Barrier by Host Innate Immune Molecules. American Society for Microbiolog.2016: 7 (5): 1 – 3.
19. Xie Y, Yang W, Tang F, Chen X., Ren L. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. Current Medical Chemistry. 2015; 22 (7): 132 – 149.
20. Ozcelik B, Orhan DD, Ozgen S, Ergun, F. Antimicrobial Activity of Flavonoids against Extended-Spectrum β-Lactamase (ESβL)-Producing Klebsiella pneumoniae. Tropical Journal of Pharmaceutical Research. 2008; 7 (4): 1151 – 1157.
21. Coppo E, Marchese A. Antibacterial Activity of Polyphenols. Current Pharmaceutical Biotechnolog.2014; 15 (4): 380 – 392.
22. Mailoa NM, Mahendradatta M, Laga A, Djide N. Tannin Extract of Guava Leaves (Psidium guajava L.) Variation with Concentration Organic Solvents. International Journal of Scientific and Technology Research. 2013; 2 (9): 106 – 110.
23. Ansari MA, Amiya A, Fatima Z, Hameed, S. Natural Phenolic Compounds: A Potential Antifungal Agent. Microbial Pathogens and Strategies for combating them: Science, Technology and Education. 2013; 7 (8): 1189 – 1195.

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