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Phytochemical and antimicrobial activities of leaf extract of Guava (*Psidium guajava* L.)

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Abstract

Medicines and products that are produced from natural sources have been gaining a lot of attention. Various fruits and their extracts have been found to exhibit antimicrobial activity. Guava (*Psidium guajava* L.) is believed to have active components that help in treating various diseases and has been used in folk medicine. In this study, the phytochemical analysis of the aqueous and methanolic extracts of guava leaves was carried out which showed the presence of flavonoids, saponins, phenols, glycosides and terpenoids. Minimum Inhibitory Concentration (MIC) of the leaf extracts was checked towards Gram-positive and Gram-negative bacterial isolates and was found to lie in the range of 30 to 60 mg/ml. The leaf extracts were used for determining their antimicrobial activity on *S. aureus* and *E. coli* using agar well diffusion assay method. Future experiments have to be conducted to evaluate the effects of guava leaves on other bacterial isolates.

Keywords: Guava leaves, *Psidium guajava* L., antimicrobial activity, phytochemical analysis, MIC.

1. Introduction

For the treatment of various diseases like pneumonia, diarrhoea, ulcers, bronchitis, colds and diseases of the respiratory tract, the World Health Organization (WHO) has catalogued more than 20,000 plant species with medicinal properties (Gonçalves *et al.*, 2008) [6]. This suggests that plants can be used to inhibit the growth of pathogens since they manifest compounds with relatively high levels of antimicrobial action (Kim and Fung, 2004) [9]. The study of traditional medicines is an integral part of the culture in many parts of the world like for example Indian Ayurveda is among the many other enduring folk medicines which are still practiced. These systems try to improve the quality of life, promote health with therapies based on the use of natural materials. Several approaches are now being carried out to discover new bioactive compounds as plants have been widely used as herbal medicines (Das and Goswami, 2019) [5].

Guava (*Psidium guajava* L.) is an evergreen tree with leaves up to 2-6 inches in length and 1-2 inches in width (Fig. 1). The leaves of the tree are dull green in colour, when crushed give aroma and appear stiff but coriaceous with pronounced veins. The guava leaves help in fighting against pathogens, maintaining blood glucose level and even aiding in weight loss due to the bioactive components present in them (Biswas *et al.*, 2013) [3].



Fig 1: Guava fruit

Scientific classification: (Maurya *et al.*, 2018)^[11]

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: Psidium L.

Species: Psidium guajava L.

Binomial name: Psidium guajava L.

Many parts of the guava tree have been used in traditional medicine to treat conditions like gastroenteritis, malaria, vomiting, diarrhoea, dysentery, ulcers, toothache, cough, sore throat, wounds, inflamed gums, etc (Abdelrahim *et al.*, 2002; Lutterodt *et al.*, 1992)^[1, 10]. The leaves of guava contain an essential oil rich in cineol, tannins, resin, uquenol, malic acid, fat, terpenoids, cellulose, chlorophyll, mineral salts and several other fixed substances (Biswas *et al.*, 2013)^[3]. One of the many methods in which plants are used in traditional remedies is to extract and consume essential plant oils (Gonçalves *et al.*, 2008)^[6].

The efficient use of different parts of guava like leaves, seeds and peels may reduce the risk of their disposal as pollutants since they are treated as wastes by the food processing industries and eventually discarded. Since all the parts of the plant have been used for different purposes like hepatoprotection, antioxidant, antimicrobial, anti-inflammatory, anti-spasmodic, anti-cancer, anti-hyperglycemic, analgesic, anti-stomachache, anti-diarrhoea, etc and shown to have positive effects on health it should be researched more extensively (Barbalho *et al.*, 2012)^[2]. The present study was carried out with an aim to evaluate the effectiveness of the Psidium guajava leaf extracts made using aqueous solvent (distilled water) and organic solvent (methanol) in inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus*.

2. Materials and methods

The media components like Nutrient agar and Nutrient broth used for this study were procured from Himedia, India. The chemicals used were of analytical grade. All the chemicals and media were made in distilled water.

Preparation of guava leaf powder

The leaf samples were collected from guava trees growing in different towns like Kalyan and Ambarnath. Random fresh leaf samples were collected into plastic bags. The collected leaf samples were thoroughly washed and sun-dried for 24 hours. It was then grounded into a fine powder using a mixer grinder. The powder was kept in a clean container and stored in a cool, dry place until further use (Biswas *et al.*, 2013)^[3].

Preparation of methanolic and aqueous (distilled water) extract

Two different leaf extracts were made, one in distilled water using hot water extraction method and the other in methanol. The methanolic and aqueous extracts were prepared in sterile 150 ml conical flask by mixing 10 grams of the leaf powder in 100 ml of methanol and boiling distilled water, respectively, making the final concentration of the extracts to 100 mg/ml. The flasks were then kept in a shaker at 250C for 7 hours at 100 rpm. The aqueous extract was filtered through a muslin cloth and transferred into sterile test tubes. In the case of the methanolic extract, the

extract was filtered and then subjected to evaporation by keeping the flask in boiling water bath to evaporate the entire methanol. The dried residue was scraped and diluted with distilled water. Both the extracts were stored at 4°C until further use (Biswas *et al.*, 2013)^[3].

Qualitative analysis of the phytochemical content in the leaf extract

Phytochemical tests were done for the screening of bioactive compounds in the guava leaves with the help of the extracts *viz.* Flavonoids (Lead Acetate Test), Saponins (Foam Test), Tannins (Ferric Chloride Test), Glycosides, Terpenoids (Salkowski Test), Phenols (Ferric Chloride Test), were carried out to check the phytochemical constituents present in the leaf extracts (Biswas *et al.*, 2013)^[3].

Test organisms

The MIC and antimicrobial activity of the leaf extracts were tested against *Escherichia coli* and *Staphylococcus aureus*. These bacterial cultures were procured from the Department of Biotechnology, B. K. Birla College, Kalyan. For regular use, the organisms were cultured and maintained on sterile Nutrient agar slants and stored in a refrigerator (Harley and Prescott, 2002)^[7].

Minimum inhibitory concentration

The minimum concentration of any substance that inhibits the growth of the organism is known as the Minimum Inhibitory Concentration (MIC). The MIC of the extracts was determined for the bacterial isolates *viz.* *E. coli* and *S. aureus*. The concentration range of the leaf extracts (Methanol and Distilled water) were made in the range of 10 mg/ml to 100 mg/ml using Sterile plain Nutrient broth as the diluent. 0.1 ml of 24 hours old culture suspension (*E. coli* and *S. aureus*) was inoculated in the tubes respectively. A positive control (Sterile plain nutrient broth inoculated with test organism) and negative control (Sterile plain nutrient broth) were also kept. All the tubes were incubated at 37°C for 24 hours (Puntawong *et al.*, 2012)^[15]. The lowest concentration that completely inhibited bacterial growth after 24 hours of incubation was determined as the MIC value of the extract (Nayak *et al.*, 2019)^[12].

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed using the well-diffusion/ agar cup method. In this method, the growth response is measured by the zone of inhibition of the test culture in response to different concentrations of the extract. The Sterile molten nutrient agar was seeded with 1ml of the 24 hours old test culture suspensions and the plates are prepared. Wells were made in the solidified media using a cork borer having an internal diameter of 6mm. The methanolic extract (50 mg/ml for *S. aureus* and 30 mg/ml for *E. coli*) and distilled water extract (30 mg/ml for *S. aureus* and 20 mg/ml for *E. coli*) were added to the wells. These values were based on the MIC results obtained. Gentamicin (50 mg/ml) was used as a control. The plates were kept in a refrigerator for 10 minutes for pre-diffusion after which they were incubated at 37°C for 24 hours. The added extract diffuses away from the well in a decreasing concentration gradient so that the susceptibility by the seeded organism can be seen. The zone of inhibitions was measured after the incubation period (Khan *et al.*, 2019).

3. Results and discussion

Preparation of extracts

Guava leaves collected were powdered and suspended in solvents *viz.* methanol and distilled water (Fig. 2) and subjected to shaker conditions at 250C for 7 hours at 100rpm. The solutions obtained after the incubation period were filtered and the filtrates obtained were used as an extract. Effect of plant material depends on its origin, extraction technique, time, the temperature of extraction, solvent concentration, variations, and polarity, quantity and secondary metabolite composition of an extract (Ncube *et al.*, 2006)^[13].



Fig 2: Methanolic (left) and distilled water (right) extract

Qualitative analysis of the phytochemical content in the leaf extract

The phytochemical analysis was carried out on a qualitative basis to determine the presence of chemical constituents in guava leaf extracts. The presence of bioactive compounds was observed in both methanol and distilled water extracts. The methanol extract showed the presence of phenols, flavonoids, terpenoids, glycosides and saponins whereas distilled water extract showed the presence of all the phytochemicals except glycosides as shown in Fig. 3, Table. 1. These matched with the results of the phytochemical analysis of guava leaf extract carried out by Das and Goswami (2019)^[5]. They showed that phenols, flavonoids and tannins are present in large amounts whereas components like triterpenes, alkaloids and saponins are present in comparatively lesser amounts in guava leaf extract (Das and Goswami., 2019)^[5]. Flavonoids are active compounds showing antibacterial properties. Terpenoids have also been found to be potential antibacterial agents against inhibiting bacteria inspite being mostly used for their aromatic properties (Biswas *et al.*, 2013)^[3]. Phenols inhibit ergosterol which is a component of fungal cell membrane and glucosamine, a growth indicator present only in the fungal cells, indicating the presence of anti-fungal activity. Tannins which are water-soluble compounds, act as an antimicrobial agent with the help of different mechanisms like deprivation of substratum, inhibition of oxidative phosphorylation, extracellular enzyme inhibition, etc. Hence it can be concluded that the most probable cause of the antimicrobial activity of guava leaves extract is the wide range of polyphenolic compounds (Das and Goswami., 2019)^[5].



Fig 3: Phytochemical testings of plant extract

Table 1: Phytochemical analysis of Psidium guajava extracts

S. No.	Extracts	Saponins	Flavonoids	Terpenoids	Glycosides	Phenols
1.	Methanol	+	+	+	+	+
2.	Distilled water	+	+	+	-	+

Key: +: presence of the constituent, -: absence of the constituent

Minimum Inhibition Concentration

Minimum Inhibition Concentration is the lowest concentration of a compound at which the test micro-organism does not demonstrate any visible growth. Two

different extracts (Aqueous and Methanolic) were investigated to determine their Minimum inhibition Concentrations against test micro-organisms *viz.* Escherichia coli and Staphylococcus aureus. The methanolic extract

showed MIC in the range of 30-40 mg/ml and 50-60 mg/ml against *E. coli* and *S. aureus* respectively. While distilled water extract showed the result in the range of 20-30 mg/ml and 30-40 mg/ml against *E. coli* and *S. aureus* respectively as shown in Table. 2. Numerous studies have demonstrated that the guava leaf extract could inhibit the growth of *E. coli* with different MIC values. This can be attributed to the

presence of different active compounds present in them. Tannins might be the active compound in guava leaves that can be used to control bacterial resistance to antibiotics. The different factors like cropping areas of the herbs, the season, extract, and extraction methods may affect the MIC value (Chukiatsiri *et al.*, 2019)^[4].

Table 2: Minimum Inhibition Concentration

S. No.	Test organisms	Methanol (mg/ml)	Distilled water (mg/ml)
1.	<i>Escherichia coli</i>	30-40	20-30
2.	<i>Staphylococcus aureus</i>	50-60	30-40

Antimicrobial Susceptibility Test

The antimicrobial activity of the Guava leaves was studied using Agar well diffusion method against two organisms *viz.* Gram-positive organism (*Staphylococcus aureus*) and Gram-negative organism (*Escherichia coli*). The result indicated that both the methanolic and aqueous extracts prepared from the leaves of *Psidium guajava* showed inhibitory activity, indicating the susceptibility of both the micro-organisms towards the extracts. The methanolic extract with a concentration range of (50 mg/ml) and distilled water extract with a concentration range of (30 mg/ml) showed a zone of inhibition of 16 mm in diameter, while a zone of inhibition of 20 mm was observed with 50 mg/ml of Gentamicin concentration (Antibiotic used as a control) against *S. aureus* as shown in Fig. 4, Table. 3. Whereas the methanolic extract with a concentration range of (30 mg/ml) and distilled water extract with a concentration range of (20 mg/ml) showed a zone of inhibition of 17 mm, while a zone of inhibition of 21 mm was observed with 50 mg/ml of Gentamicin concentration against *E. coli* as shown in Fig. 5, Table. 4. The size of the

molecules (active components) of the solvent extracts may affect the inhibition zone values (Power, 1995)^[14]. The results in this study indicate that the inhibitory activity of both the extracts was comparatively greater than that of Gentamicin. The methanolic extracts of *P. guajava* leaves showed significant antibacterial activity against *S. aureus* and *E. coli*. Bacterial cells can be killed by the rupture of cell walls and membrane and by the irregular disruption of the intracellular matrix when treated with plants extracts (Kim and Fung., 2004)^[9]. The mesh-like peptidoglycan layer of the Gram-positive bacteria is more accessible to the extracts by permeation. Whereas the resistance of the Gram-negative bacteria could be attributed to its cell wall structure as they are mostly resistant to plant-origin extracts. An effective permeability barrier of the Gram-negative bacteria is comprised of a thin lipopolysaccharide membrane, which restricts any penetration. But the *P. guajava* leaf extracts contain active components which show inhibitory effect against Gram-negative bacterium by penetrating its cell wall (Biswas

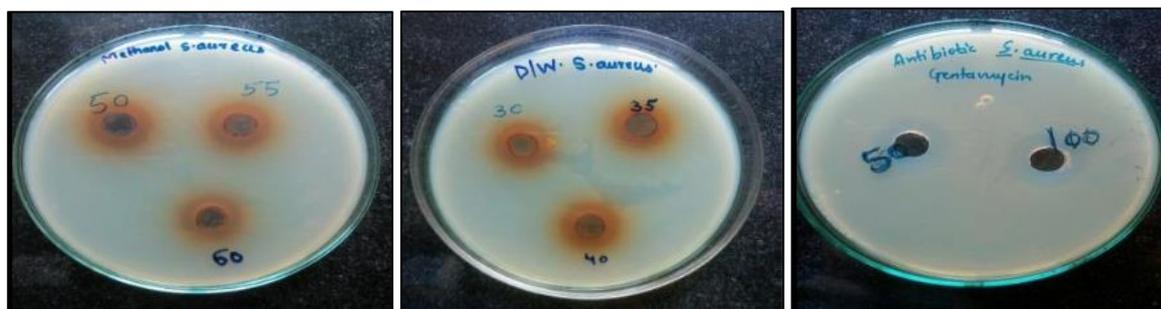


Fig 4: Antimicrobial activity of methanolic, distilled water extract, gentamicin against *S. aureus*



Fig 5: Antimicrobial activity of methanolic, distilled water extract, gentamicin against *E. coli*

Table 3: Inhibitory action of methanolic, d/w extract and gentamicin on *S. aureus*

Test organism	Zone of inhibition(mm)		
	Methanolic Extract (50 mg/ml)	Distilled water Extract (30 mg/ml)	Gentamicin (50 mg/ml)
<i>S. aureus</i>	16	16	20

Table 4: Inhibitory action of methanolic, d/w extract and gentamicin on *E. coli*

Test organism	Zone of inhibition(mm)		
	Methanolic Extract (30 mg/ml)	Distilled water Extract (20 mg/ml)	Gentamicin (50 mg/ml)
<i>S. aureus</i>	17	17	21

4. Conclusion

This study was undertaken with an aim to determine the phytochemical constituents and the antimicrobial activity of guava leaves. The phytochemical analysis showed that *P. guajava* leaves were rich in a wide range of bioactive compounds like phenols, flavonoids, terpenoids, glycosides and saponins. Research indicates these bioactive compounds to be the active antimicrobial agents in guava leaves. From the results of MIC, it can be suggested that the methanolic and aqueous extract produced inhibitory activity with varying degrees against both the Gram-positive and Gram-negative strains. The MIC of the aqueous extract was lower than the MIC of the methanolic extract for both *S. aureus* and *E. coli*. This indicates that the aqueous extract was more effective than the methanolic extract at inhibiting the growth of the micro-organisms. The antimicrobial activity was checked, and it was seen that the growth of *S. aureus* and *E. coli* was inhibited by the methanolic and aqueous extracts with visible zones of inhibition using the well diffusion/agar cup method. Gentamicin which is a common antibiotic used for treating various bacterial infections was taken as a control and it showed lesser inhibition against the test organisms as compared to the extracts. Various chronic degenerative diseases have reached epidemic proportions in many countries, increasing the socio-economic burden for the public health system (Barbalho *et al.*, 2012) [2]. More efforts are being taken to find alternate antimicrobial agents due to the rapid development of resistance to existing agents. Several studies have suggested that products isolated from plant species are preferable compared to synthetic products. Given this, in the search for a natural antimicrobial agent to fight against human pathogens, *P. guajava* leaves could prove to be a good candidate. However, further studies need to be carried out to determine the mechanism of *P. guajava* on other bacterial isolates.

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