



REVIEW ARTICLE

Harnessing *Psidium guajava* (Guava) Leaf Phytochemicals: *In Vitro* Antimicrobial Activity and Implications for Alternative Therapies

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ABSTRACT

Psidium guajava, well known for its antimicrobial potential is alternative and effective substitutes for conventional antibiotics which is responsible for emergence of antimicrobial resistance (AMR). *P. guajava* (guava) leaves are used as traditional medicine in treating various disorders. It is also an excellent source of bioactive substances such as flavonoids, tannins, phenolic compounds, and terpenoids. In the present paper, the evolution of in vitro antimicrobial activity of *P. guajava* leaf extracts against important pathogenic microorganisms has been studied. The available literature shows that *P. guajava* leaves possess different amounts of antibacterial activity against both Gram-negative and Gram-positive bacteria. Influencing antimicrobial efficacy are extraction solvent, concentration, phytochemical composition, and bacterial susceptibility. *P. guajava* leaves can thus be a natural source of antimicrobial elements, offering an alternative or additional option to antibiotics. Nonetheless, more research will need to be performed to optimize the extraction methodology, as well as to determine the usefulness of guava in antimicrobial therapy.

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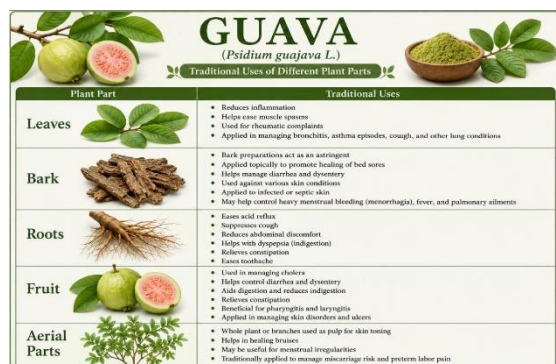
1. Introduction

Antimicrobial resistance has become a significant global health issue, caused by the number of factors, including the ease of availability and improper use of antimicrobial medications. While the introduction of novel antibiotics is a significant strategy in the fight against AMR, emergence of resistance in newly developed antimicrobial agents continues to pose challenges. In this regard, there has been a lot of interest in medicinal plants and their bioactive components as possible replacements for traditional antimicrobials. Natural medicinal plants may have some benefits, such as cheaper prices and fewer side effects compared to some synthetic antimicrobials [1]. Traditional medicine has had a major role, especially in indigenous and local communities. *P. guajava* has been traditionally used for treating a range of ailments, including diabetes, obesity, cancer and hypertension. [2].

Apart from that, various components of the plant have been used to treat acute and inflammatory diseases like gastroenteritis, injuries, burns, and gingival inflammation. The antimicrobial action of the plant is commonly due to the presence of bioactive phytochemicals. These chemicals can have different effects, such as changing the permeability of their membranes, damaging the bacteria's cell walls and membranes, and disturbing their internal components, which results in the death of bacteria [3]. The guava tree, scientifically known as *Psidium guajava*, is a tropical tree that is grown all over the world. It belongs to the Myrtaceae family, which contains about 133 genera and almost 3,800 species [4]. The various properties of *P. guajava* leaves, both biological and therapeutic, can be associated with the different bioactive components contained in them, which include quercetin and other

flavonoids, as well as phenolic acids like caffeic, ferulic, and gallic acids [5-6].

Fig 1. Traditional use of *Psidium guajava* plant extract



Plant Part	Traditional Uses
Leaves	- Reduces inflammation - Helps ease muscle spasms - Used for rheumatic complaints - Applied in managing bronchitis, asthma episodes, cough, and other lung conditions
Bark	- Bark preparations act as an astringent - Applied topically to promote healing of bed sores - Helps manage diarrhea and dysentery - Used against various skin conditions - Applied to infected or septic skin - May help control heavy menstrual bleeding (menorrhagia), fever, and pulmonary ailments
Roots	- Eases acid reflux - Suppresses cough - Reduces abdominal discomfort - Helps with dyspepsia (indigestion) - Relieves constipation - Eases toothache
Fruit	- Used in managing cholera - Helps control diarrhea and dysentery - Aids digestion and reduces indigestion - Relieves constipation - Beneficial for pharyngitis and laryngitis - Applied in managing skin disorders and sores
Aerial Parts	- Whole plant or branches used as pulp for skin toning - Helps in treating bruises - May be useful for menstrual irregularities - Traditionally applied to manage miscarriage risk and preterm labor pain

2. Phytochemicals

The leaves of *Psidium guajava* L. have a great number of antioxidant phytochemicals that take part in the pharmacological and therapeutic properties of the plant. The primary antioxidant components include polyphenols such as gallic acid, chlorogenic acid, ellagic acid, caffeic acid and ferulic acid; flavonoids such as myricetin, catechin, kaempferol, quercetin, rutin, epicatechin and terpenoids including limonene and β -caryophyllene. Other notable bioactive substances are anthocyanins including cyanidin-3-O-glucoside, tannins and vitamin C. These compounds show antioxidative and radical scavenging properties and could be responsible for the anti-inflammatory and anticancer effects of *P. guajava* leaves [7].

Flavonoids including catechin, kaempferol, rutin, myricetin and quercetin, have secondary antioxidant functions since they are able to mitigate oxidative damage and prevent the process of oxidation. These compounds exhibit antioxidant properties plus antibacterial, anticancer, antispasmodic and antihyperglycemic activities. Therefore, the presence of these bioactive compounds could be responsible for the many applications of *P. guajava* leaves in traditional and pharmaceutical practices. *P. guajava* leaves extract have been traditionally applied in the treatment of diseases connected with the gastrointestinal tract including diarrhea, dysenteries, stomachache, gastroenteritis, indigestion, as well as hyperglycemia, hypertension, rheumatism, fever inflammation, respiratory problems, and ulcer disorders (Table-1) [8-9].

P. guajava leaves contain many bioactive substances which include flavonoids, triterpenoids, heteroterpenes, tannins, sesquiterpenes, meroterpenoids, benzophenone and glycosides [10]. Flavonoids found in *P. guajava* leaves are thought to be responsible for its antidiarrheal property and contribute to the traditional use of guava in treating gastrointestinal disorders. Moreover, tannins present in *P. guajava* leaves are thought to support digestive health by improving blood circulation [11]. Polyphenols and

carotenoids found in *P. guajava* help the leaves support its antioxidant activity as well. The other studies have shown that the leaves of *P. guajava* exhibit several pharmacological activities including antidiarrheal, antioxidant, antitumor, hypotensive, hypoglycemic and antibacterial properties [12]. Flavonoids have several biochemical and pharmacological effects like anti-inflammatory, hepatoprotective, anti-cancer, antiviral, anti-allergic, antioxidant, an anti-thrombotic properties. Tannins found in *P. guajava* leaves have also shown immense pharmacological potential; they were traditionally used for hemostatic, anti-hemorrhoid, as well as anti-diarrhea purposes [13]. Saponins from *P. guajava* leaves have anti-inflammatory and antioxidant properties, and their role in lowering cholesterol should also be taken into consideration. *P. guajava* leaves have been found to contain terpenoids that are believed to have abilities to help heal wounds and thus have serious therapeutic potential [14].

Recently, a number of bioactive compounds have been found in the aqueous extract of *P. guajava*. These are benzene-1,2-diol, 20-O-methyl guanosine, 5-bromo-8-(5-nitrosalicylideneamino) quinoline hydrochloride, guavanoic acid, protocatechuic acid, oleanolic acid, and 2 α -hydroxyursolic acid, and they are said to be responsible for the antioxidant and antidiabetic properties of this extract [15]. Likewise, the ethanolic leaf extract of *P. guajava* contains a wide range of phenolic and flavonoid compounds, including ellagic acid, hyperin, guajaverin, reynoutrin, genistin, rutin, avicularin, quercitrin, kaempferol, and quercetin, as well as triterpenoids maslinic acid, corosolic acid, ursolic acid, and oleanolic acid [16].

Table-1. Major Phytochemicals of *Psidium guajava* (Guava) Leaves

No	Phytochemical / class	Mode of action	Potential health benefits	Ref
1	Quercetin Flavonol	Affect microbial membranes and enzymes.	Antimicrobial, antioxidant, and antidiabetic potential.	[37]
2	Kaempferol Flavonol	Modulates inflammatory signaling; reported antibacterial activity.	Antioxidant, antimicrobial, and antidiabetic potential.	[37]
3	Guajaverin Flavonoid glycoside	Quercetin glycoside; may affect microbial growth and enzymes.	Antimicrobial, antioxidant and antidiabetic potential.	[38]

4	Avicularin Flavonoid glycoside	Radical-scavenging activity; may modulate oxidative and inflammatory processes. Scavenges reactive oxygen species and protects cellular components from oxidative damage.	Antioxidant, anti-inflammatory and antimicrobial potential.	[38]			
5	Rutin Flavonol glycoside	Potent radical scavenger; modulates oxidative, inflammatory and metabolic pathways.	Antioxidant, anti-inflammatory, antidiabetic and antimicrobial potential.	[38]			
6	Myricetin Flavonol	Modulates inflammatory signaling and oxidative stress; experimental antimicrobial effects are reported. Scavenges radicals and can interact with microbial membranes and proteins. Quercetin O-glycoside with antioxidant activity; can modulate enzymes and oxidative processes.	Antioxidant, anti-inflammatory and antimicrobial potential.	[38]			
7	Apigenin Flavone		Antioxidant, anti-inflammatory and antimicrobial potential.	[38]			
8	Catechin Flavan-3-ol		Antioxidant, antimicrobial and metabolic-health potential.	[38]			
9	Quercitrin Flavonol glycoside		Antioxidant, antimicrobial and anti-inflammatory potential.	[37]			
10	Gallic acid Phenolic acid		Electron/hydrogen donation neutralizes reactive oxygen species; can inhibit microbial enzymes and damage cellular structures. Strong antioxidant and antiproliferative activity; can interact with proteins and microbial targets. Antioxidant and enzyme-modulating effects; can affect microbial membrane integrity and metabolism. Scavenges radicals and modulates oxidative/inflammatory pathways; can inhibit microbial growth. Lipophilic membrane interaction; also activates CB2-related signaling involved in inflammatory regulation.	[38]			
11	Ellagic acid Polyphenol		Antioxidant, antimicrobial and anti-inflammatory potential.	[38]			
12	Chlorogenic acid Phenolic acid		Antioxidant, antimicrobial and antidiabetic potential.	[38]			
13	Caffeic acid Phenolic acid		Antioxidant, antimicrobial and anti-inflammatory potential.	[38]			
14	β -Caryophyllene Sesquiterpene		Anti-inflammatory, antimicrobial and antioxidant potential.	[39]			

15	Linalool Monoterpene alcohol	Disrupts microbial membrane integrity and membrane potential and can interfere with cellular energy metabolism.	Antimicrobial, antioxidant and anti-inflammatory potential.	[40]
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a. Proximate Composition

P. guajava leaves (GLs) are a significant hub of both macro- and micronutrients as well as many biological active compounds. According to estimates, *P. guajava* leaves contain 3.64% ash, 82.47% moisture, 0.62% fat, 12.74% carbohydrates and 18.53% protein. In addition, the *P. guajava* leaves comprise approximately 103 mg ascorbic acid and 1,717 mg gallic acid equivalents (GAE)/g of total phenolic compounds [17].

b. Vitamins and Minerals

P. guajava leaves are an excellent source of many important minerals and vitamins, such as sodium, potassium, sulfur, magnesium, calcium, iron, manganese, boron, and vitamin B and C. The reason is due to its high levels of magnesium, sodium, sulfur, boron, and manganese, which enhance the nutritive value of the *P. guajava* leaves. This shows its potential to function as a source of micronutrient for human nutrition and animal feeds and may assist in mitigating the risk of having an insufficiency of any micronutrients [18].

3. Biologically Active Properties of Guava Leaf Extracts

The extracts from *P. guajava* leaves have several active compounds that exhibit different biological and pharmacological properties. The active compounds have the antioxidant, anti-inflammatory, antimicrobial, hypoglycemic, anticancer, and other therapeutic properties, emphasizing the medicinal importance of *P. guajava* leaves.

a) Anti-diarrheal activity

The practice of employing the *P. guajava* leaves as well as many traditional treatments has been known as one of the ways of dealing with childhood diarrhea and gastrointestinal diseases. The guava buds and leaves have bioactive flavonoids in their composition, like quercetin and quercetin-3-arabioside, which are able to provide an analgesic-like effect due to their capacity to reduce neurotransmitter secretion and intestinal contraction, thus affecting the tone of muscle tissue. In addition, Asiatic acid extracted from *P. guajava* leaves was shown to possess a dose-dependent spasmolytic effect in the concentration range of 10–500 µg/mL meaning that the constituents of *P.*

guajava leaves can play the role in relieving intestinal spasms [19].

b) Antihypertensive Activity

Having normal blood pressure is important for decreasing the chances of developing cardiovascular complications like stroke and coronary artery disease. There are multiple epidemiological studies indicating that guava's plant chemical components may provide cardioprotective effects by regulating the increased blood pressure levels and enhancing cardiovascular well-being [20].

c) Anti-inflammatory Activity

Research has shown that *P. guajava* leaf extracts possess anti-inflammatory properties. Studies indicated that ethyl acetate extracts of *P. guajava* leaves can decrease inflammatory and immune processes and exhibit antiviral effects. Such effects may be associated with the expression of heme oxygenase-1, as well as with inflammatory signaling pathways, thus indicating possible applications in the treatment of inflammatory skin diseases. The effect of ethanolic guava leaf extracts shows an inhibition of nitric oxide production and a decrease in prostaglandin E₂ formation, contributing to their anti-inflammatory activity. The flavonoids and benzophenone derivatives present in *P. guajava* leaves may play an important role in anti-inflammatory activities since they can influence nitric oxide and histamine production [21].

d) Antimicrobial activity of *P. guajava* leaf

The antibacterial properties of aqueous and alcoholic extract methods, prepared from *P. guajava* leaves and roots, were tested on *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Proteus spp.*, *Shigella spp.*, and *Escherichia coli*. These are a group of harmful microorganisms linked with intestinal disease in humans, where the extracts showed antimicrobial action in the laboratory using agar disk diffusion method. The results have reported inhibitory activity against the tested microorganisms, confirming the use of *P. guajava* for treatment of infectious intestinal diseases [22].

Several studies have investigated the antimicrobial properties of *P. guajava* leaves based on ethanolic extraction methods, fractionation, and further purification of flavonoids from the leaves. Researchers used the agar diffusion technique to check whether the fractions obtained and the isolated compounds exhibited any antimicrobial property against bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as *Candida albicans* [23].

The utilization of plant-derived bioactive compounds for their antimicrobial properties can have several mechanisms of action, such as inhibition of bacterial cell wall synthesis, production of reactive oxygen species (ROS), interference with adenosine triphosphate production, inhibition of DNA

replication and transcription and neutralization of bacterial toxins [24].

Also, the leaves of *Psidium guajava* have significant antimicrobial properties due to the high concentration of essential oils, anti-inflammatory properties, and both organic and inorganic antioxidants [21]. The flavonoid quercetin plays a major role in the pharmacological effects of *P. guajava* leaves. In addition, the triterpene compounds betulinic acid and lupeol might prevent the growth and reproduction of bacterial and fungal pathogens in an unfavorable environment [9]. While another research results indicated that the methanol extract of *P. guajava* leaves was effective against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. Paper disk diffusion technique was employed to measure the inhibitory effect of *P. guajava* leaf extract on bacterial strains, and this finding was supported by the turbidometric test [25].

Methanolic extracts obtained from the leaves and bark of *P. guajava* showcased significant inhibitory effects on the strains of *Bacillus* and *Salmonella* species [26].

The compounds present in *P. guajava* leaves might have a bacteriostatic and fungistatic action since they can stop the growth and proliferation of the microorganisms. It has also been demonstrated that the compounds derived from the *P. guajava* leaves might have antiviral potential concerning some viruses like the influenza virus. The antiviral effect might have a direct relation with the ability of the bioactive compounds present in guava extracts to impact the viral proteins, thus inhibiting viruses from being replicated and surviving [27]. Moreover, the essential oil of the guava has been shown to have antimicrobial properties towards such pathogenic bacteria as *Salmonella* species and *Staphylococcus aureus* which proves that *P. guajava* might become a natural source of anti-microbial agents [28].

According to researcher, *P. guajava* leaves extract is beneficial for reducing blood glucose, as well as stimulating insulin secretion in plasma [29]. Moreover, extracts from *P. guajava* leaves and bark have proven their ability to increase glucose transportation in muscle cells, as well as they have proved their ability to inhibit α -amylase, and thus have potential antidiabetic properties. *P. guajava* leaves have been traditionally used among those cultures due to their antiseptic and medicinal properties when it comes to the treatment of hypertension, pain, fever, and respiratory problems [30]. Additionally, *P. guajava* leaves have been traditionally used for treating kidney inflammation in instances of gastroenteritis and in case of diarrhea in children related to various infections. Their healing properties might be explained by their high content of dietary fibers and bioactive phytochemicals that are believed to help in maintaining digestive system health. In addition to that, the leaves are known to be effective enough to fight against a variety of pathogenic microorganisms, which is connected with large amounts of Vitamin C and

numerous phytochemicals contained in the leaves themselves [31].

e) Anti-Cancer/Anti-Tumor Activity

Cancer is a complicated disease due to the uncontrolled and abnormal development of cells leading to tissue damage and tumor formation. A variety of internal and external factors may be responsible for excessive production of reactive oxygen species resulting in oxidative stress and tissue damage. Long-standing oxidative stress may lead to chromosomal changes, DNA cross-linking, nucleic acid degradation, mutations, and cellular membrane damage and promote the process of carcinogenesis [32]. The leaves of *P. guajava* have a variety of phytochemicals with the following active components: flavonoids, glycosides, triterpenoids, tannins, sesquiterpenes, psigaudials, volatile oils, and quinone compounds [33]. These phytochemicals are believed to be responsible for anticancer and antitumor properties of *P. guajava* leaves. In particular, it is proposed that flavonoids and terpenoids exert tumor cell inhibition effects through inhibition of tumor cell proliferation and angiogenesis, modulation of immune reactions, and blockade of cellular signaling pathways and tumor cell migration or adhesion [34].

f) Other Uses of *Psidium guajava* Leaves

P. guajava leaves are well known for their various medicinal properties. Different leaf preparations such as decoctions, infusions, boiled preparations, and fresh leaf extract preparations have been used for treatment of rheumatic pain, dysentery, diabetes, mouth sores, gastroenteritis, and skin infections. The crushed leaf extracts are said to possess antibacterial and wound healing properties, thereby being used externally for cuts, sores, and boils [35]. *P. guajava* leaves have also been used in case of gastro-intestinal disorders and inflammation of gums.

The activity of *P. guajava* leaves might be due to the bioactive components of the leaves that are known to disturb the bacterial cell membrane and cell wall. The compounds derived from plants can interfere with the lipid bilayers via physical bonding and thereby increase their permeability, causing the content of the affected cells to leak out, which can eventually kill the bacteria [3,26]. The effect could thus explain why the extracts of *P. guajava* leaves are effective against some bacteria even though the concentration is relatively high.

4. Factors influence antimicrobial activity

Various studies show that guava leaf extracts inhibit a large group of pathogenic microorganisms and suggest their broad antibacterial activity. Differences in the activity of the extracts may depend on the extraction methods used to concentrate the compounds found in *P. guajava* leaves; the solvent used during extraction can impact the yield of phenolic compounds [36,37].

Extraction methods play a vital part in maintaining the biological effectiveness of *P. guajava* leaves [37]. High temperatures, especially when prolonged boiling is used in the extraction process, can negatively influence some heat-sensitive phytochemicals. Thus, when the method of preparing *P. guajava* leaf extract is chosen, both the type of solvent and the temperature of the extraction process should be thoroughly taken into account. The most commonly used solvents for phenolic extraction are aqueous acetone, ethanol, or their mixtures due to their particular extraction efficiency and polarity. Overall, the previous usage of *P. guajava* leaves in traditional medicine and shown antibacterial properties gives a promise to *P. guajava* leaves as bio stimulants in various health-related situations [36,37].

5. Conclusions and Future Perspectives

P. guajava leaves (GLs) are abundant and easily accessible sources of naturally available bioactive compounds with great nutrition, pharmacology, and medicinal potential. Many studies indicate that *P. guajava* leaf extracts exhibit several biological activities such as antioxidant, antimicrobial, anticancer, hypoglycemic, and anti-inflammatory activity. The high content of proteins, vitamins, minerals, and other nutrients also suggests the use of *P. guajava* leaves as an important nutrition source. The biological effects of *P. guajava* leaves are owing to their active compounds including flavonoids, triterpenoids, sesquiterpenes, glycosides, alkaloids, and saponins among other phenolic compounds. These compounds play a role in

physiological and metabolic regulation and have been researched for their ability to manage chronic diseases, including diabetes, cancer, digestive tract disorder, neurodegeneration, and heart diseases.

Some constituents of biological activity like vitamin E, apigenin, and β -caryophyllene appear to provide antiproliferative activity against colon cancer and other types of cancer cells, while the biological activities of *P. guajava* leaves may indicate the potential for them to serve as a low-cost source of natural products for use in pharmaceuticals, nutraceuticals, and functional foods. There is a specific relevance of their antimicrobial properties in light of growth in antimicrobial resistance, which allows for the possibility of guava properties being employed in the discovery of new or alternative antimicrobial agents. However, by these findings, further studies are necessary to ensure the safety and efficacy of guava leaf products, the mechanism of their action, the proper methods of extraction, and standardized dosages.

Authors contribution

Maham Sheikh: Original draft writeup, Reviewing & Editing, **Muhammad Shafee:** Conceptualization & Reviewing, **Nadeem Rashid, Zoha Usman:** Investigation and reviewing, **Abdul Samad:** Reviewing & Editing, **Sahiba Muhammad Khan:** Data collection.

Conflict of Interest

The authors declare no conflict of interest.

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