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Review Article

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ANTIMICROBIAL ACTIVITY OF LEAVES OF *PSIDIUM GUAJAVA* LINN – AN OVERVIEW

Dr. Deena Mendez* and Dr. Mamatha Kunder

Assistant Professor, Department of Biochemistry, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education & Research, Tamaka, Kolar, Karnataka, India, 563103.

***Corresponding Author: Dr. Deena Mendez**
Assistant Professor, Department of Biochemistry, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education & Research, Tamaka, Kolar, Karnataka, India, 563103.

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ABSTRACT

In recent years, plant extracts have gained popularity in treatment of several diseases and a lot of attention is paid on the production of medicine from natural sources. *Psidium guajava* (Guava) has a history of being used for medicinal purposes. The potential of the leaf extract of *Psidium guajava* in treatment of bacterial infections has been attributed to the bioactive compositions of the leaves which are rich in tannins, flavonoids and essential oils. This review article compiles the results of the antimicrobial activity of the guava leaves so that further research can be focused on identification and purification of bioactive compounds from the leaves which can be an efficacious treatment against bacterial infections as guava leaf extract appears to be a suitable candidate in the search for a natural antimicrobial agent.

KEYWORDS: *Psidium guajava*, Antimicrobial activity, Phytochemicals.

INTRODUCTION

Plants have been used for medicinal purposes for several hundreds of years. *Psidium guajava* is one such plant. *Psidium guajava* Linn popularly known as guava is a small tree belonging to Myrtaceae family.[1] It is a native to tropical countries and hence is grown widely in countries having tropical and subtropical climate.[2] The leaves of guava plant are essentially rich in phytochemicals such as cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, cellulose, chlorophyll, mineral salts and a number of other fixed substances.[3, 4] These phytochemicals are non-nutritive chemicals produced by plants for their own protection but have been found to protect humans against diseases and all of them work in a different manner.[5] The plant is known for its antimicrobial, anti–inflammatory, hypoglycemic, anti-diarrheal and anti-oxidant properties which can be attributed to the presence of these phytochemicals.[1]

AIM

The aim of this review article was to examine the beneficial effects of guava leaves and summarize its antimicrobial activities invitro.

Many of the studies for determining antimicrobial activity of the leaves involve preparation of crude extracts of the air dried, coarsely powdered leaf material. The extracts are then dissolved in suitable solvents such as water, methanol, ethanol and acetone. The techniques for medicinal plant extraction includes maceration, percolation, Soxhlet extraction and aqueous- alcoholic extraction by fermentation. Maceration is a crude form of extraction where the solvents diffuse into solid plant material and solubilize compounds with a similar polarity.[6] Antimicrobial activity is usually determined by using Agar well diffusion method or Disc diffusion methods.[7,8,9,10]

Aqueous and organic extracts of the leaves have been shown to have anti-bacterial as well as antifungal activity. However, the antifungal activity appeared to be less when compared to the inhibitory effect on that of bacteria.[8, 11, 12] Anti-bacterial activity of the leaf extracts have been shown to have inhibitory action against both gram positive and gram negative bacteria.[8,11,12,13,14] When treated with these plant extracts, bacterial cells may be killed by the rupture of cell walls and membranes and by irregular disruption of intracellular matrix.[15,16]

The inhibitory effects against *Staphylococcus aureus* strains have been documented in several studies.[17,18,19] and good antimicrobial activity against nine different strains of *Staphylococcus aureus* were shown.[20] There have been contrasting reports on the type of solvent which can bring about maximum inhibition. In one study, Sanches *et al.*, found that the ethanol extract of the leaves was more active than the aqueous extract against *Staphylococcus aureus*.[19] Methanolic extract of the leaves have been shown to have an inhibitory effect on the growth of different strains of bacteria such as

Escherichea coli, *Pseudomonas aeruginosa*, *Proteus spp* and *Shigilla Sp.*[21] Both the aqueous and organic extract of the leaves were found to be active against *Staphylococcus aureus* and *Proteus Spp*[22] while other studies showed that water extracts more active compounds than methanol.[23] Again, contradicting these are studies reporting that methanol leaf extracts brought about a greater degree of inhibition than water.[18, 24] Gitika et al., in their study showed that the methanol and ethanol extracts of the leaves had significant inhibition against gram positive *M.lutens*, *B.subtilis* and *Streptococcus sp* as well as gram negative bacteria *E.coli*, *Pseudomonas aeruginosa* and *S.typhimurium* and their study also showed that the aqueous extracts were not as effective as those from alcoholic solvents.[25] The reason for these contrasting results may be because the chemistry involved in boiling, soaking and rubbing these plant extracts is quite complex. It thus does not seem likely that active compounds present in these water extracts are the same as those present in methanol/ethanol extracts. However, it should be remembered that in traditional medicine, plant extracts are prepared with water for decoctions or infusions and plasters. Moreover, besides the fact that different solvents are being used, there will be variation in extraction methods such as length of extraction period, solvent to sample ratio, concentration of the solvent, the quantity of the extracts used and the temperature at which extraction was done and this can lead to variations in the results obtained. Thus, the type of plant extract used can result in the type of microbial inhibition. Besides, the secondary metabolism of plants is quite complex and leads to a large variety of metabolic products. So, a high degree of variability can be expected.

Contrasting results have also been obtained with some studies which claim that the leaf extracts are virtually inactive against gram negative bacteria such as *E. coli* and *Pseudomonas aeruginosa*.[22] It was suggested that Gram negative bacteria may be more resistant to the leaf extract due to its cell wall structure.[26] Gram negative bacteria have an effective permeability barrier made up of a thin lipopolysaccharide membrane which probably restricts the penetration of the plant extract while Gram positive bacteria have a mesh like peptidoglycan layer which is more permeable to the extracts.[27,28] The results that gram negative bacteria are not inhibited by the leaf extracts has been opposed by several other studies which showed significant inhibitory activity against *Salmonella sp*, *Pseudomonas aeruginosa* and *E.coli.*[24,25,29,30,31,32]

Guava leaves have been used in the treatment of diarrhea and have been shown to have significant inhibitory activity against *E.coli*, *Vibrio cholera*,*V. parahemolyticus*, *Aeromonas hydrophila* and *Salmonella spp.*[33,34,35] Crude aqueous mixtures and methanol extract from leaf of *Psidium guajava* have shown strong anti-bacterial activity against multidrug resistant *V. cholera*.[35] and it reduced production of cholera toxin by binding to gangliosidemonosialic acid.[36]

Guava leaves are traditionally used for wound healing as well as for treatment of ulcers on the skin.[37,38,39] At the wound site, bacteria release proteolytic enzymes to promote their invasive power. Bacterial Elastase is one such enzyme which is an important virulence factor in several types of infection. The invasive effect of elastase by *Pseudomonas aeruginosa* has been well documented and this enzyme is commonly found in chronic ulcers.[40,41] The host secretes Human Neutrophil Elastase (HNE) as the first line of defense against invasive microorganisms. HNE is also found to be elevated in wounds which are slow in healing.[42] High levels of proteases at a wound site impair healing as they degrade the extracellular matrix.[42] So, application of these leaves can facilitate the healing of wounds as they inhibit these enzymes.

Psidiumguajava leaf extract (5gm/kg) caused reduced occurrence of cough which was induced by capsaicesi aerosol by 54% within 10 minutes and since *Staphylococcus aureus* and *β-streptococcus* is inhibited by aqueous and chloroform extract of leaves it can be given for cough.[43] The methanolic leaf extract showed good inhibition of influenza viruses including Oseltamivir resistant strains.[8,44]

Though, there is a wide variation in the results due to the degree of variability in the extraction methods, what really matters is the powerful inhibitory effect of guava leaves on many pathogenic bacteria.

Phytochemicals present in Guava leaves: The antimicrobial activity of the guava leaves as mentioned above is related to the phytochemicals present which are extracted in various solvents both aqueous and organic. As different regions have different climatic conditions ranging from soil, rainfall availability, temperature and humidity this can result in plants having different bioactive compounds though they are of the same species.[45]

Tannins are polyphenolic compounds and exhibit anti-microbial activity by binding to proline rich protein thus inhibiting protein synthesis.[19, 46, 47, 48] The effect may be further compounded due to the ability of tannins to inactivate microbial adhesions, enzymes and cells that transport proteins.[49] The anti- microbial effect could also be related to the concentration of gallic acid and catechen present.[13,50] as well as butulinic acid and lupeol.[51]

Flavonoids are hydroxylated polyphenolic compounds with properties like free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action as well as antimicrobial activity.[49,52] This property could be attributed to their ability to form complexes with extracellular soluble proteins and

bacterial cell walls.[53] Flavonoids such as guaijavarin and quercetin are anti-inflammatory and antimicrobial.[54,55] Two new flavanoid glycosides, morin-3-O-alpha lyxopyranoside and morin-3-O-alpha arabopyranoside which have been identified were effective against *Salmonella enteritides* and against *Bacillus cereus* respectively.[35,55] Guaijaverin showed anti-plaque effect by inhibiting growth of *Streptococcus mutans*.[56] The guava leaf extract has trypanocidal properties which may be due to iron chelating activity of flavonoids and tannins present.[52] The leaves of guava are rich not only in quercetin but also quercetin glycosides as well as polyphenols such as β-sitosterol, Uvaol, Oleanolic acid, Urasolic acid and triterpenoid-guajanoic acid.[32,57]

The essential oils from *Psidium guajava* have been analyzed and identified by GC-MS. The major components were carophyllene, copaene, azulene and eucalyptol.[58] Other volatile oils obtained by hydrodistillation of the leaves and identified by GC-MS are α-pinene, 1,8-cinole and β-bisabolol.[59] There is much variation in the essential oil compositions which depends on the particular chemotype present and this may also have implication in its traditional uses and biological activity.[60] Goncalves *et al*., showed the antimicrobial effect of essential oils obtained from the leaves against *Staphylococcus aureus* and *Salmonella sp* could be the source of a potential new antimicrobial compound.[61] The mechanism by which they can inhibit microorganisms is by penetration of the lipid bilayer of the cell membrane making it more permeable and leading to a leakage of the cell contents.[62,63]

Saponins which are glycosides and terpenoids are also potential agents against bacteria.[19] Saponin from the leaves was found to interfere with the envelop subunit gp41 from the critical 6-HB structure, thus inhibiting the entry of HIV into target cells.[64] Several triterpinoids isolated from leaves of *Psidium guajava* have been identified by NMR spectroscopy as guajavanoic acid, obtusiningoreishic acid, guavanoic acid, guavacoumaric acid along with 2α-hydroxyurosolic acid, jacomaric acid, isoneriucoumaric acid, asiatic acid, β-sitosterol, guajavolide, guavenoic acids along with a pentacyclic terpenoid psidiumoic acid.[58,65]

In a detailed study on the chemical examination of the leaves by spectroscopic analysis, it was found that specific compounds such as quercetin and 6β-20β-dihydroxy-12-ursen-28-oic acid and the methanolic, chloroformic and ethyl acetate fraction were found to bring about the inhibition of elastase enzyme and thus promotes wound healing.[15,66]

A study by this author had aimed to identify a specific protease inhibitor of proteinous nature in the leaves but failed in its attempt as an aqueous extract of the leaves after removal of all phenolic compounds did not exhibit any recordable inhibitory activity on elastase showing there was no specific inhibitor of protein nature.[24] The study also showed that aqueous and methanolic extract of the leaves were found to have an inhibitory effect on elastase enzyme of *Pseudomonas aeruginosa* as well as HNE. However, inhibition brought about was due probably to phenolic compounds such as tannins as well as flavonoids and triterpernoids and not due to a specific protease inhibitor. The practice of using these leaves for wound healing however can be justified as there was inhibitory effect on elastase released by both *Pseudomonas aeruginosa* and HNE.

CONCLUSION

This review article indicates that the leaves of the guava plant possess compounds which can suppress bacterial growth when extracted with a suitable solvent. This article gives a scientific insight to further investigate the pharmacological properties of guava leaves and its possible application as an adjunct to conventional therapy. This review article has made an attempt to shed light on the therapeutic potential of the guava leaf and its beneficial antimicrobial effects. The extensive literature survey has shown that the leaves which have a proven history of treating microbial infections has to be further investigated to utilize its full potential. On the basis of this article, the leaves of *Psidium guajava* appears to be an ideal candidate in the search for a natural antimicrobial agent as prolonged treatment by allopathic drugs has led to drug resistance and this is most frequently seen during antimicrobial therapy.

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