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SHORT COMMUNICATION

Bioactive Potential of Guava Leaves against Bacterial Pathogens

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ABSTRACT

Objectives : The study was conducted to analyze antimicrobial activities of leaf extract of Guava against Gram positive and Gram negative bacteria.

Methods: The leaf samples of Guava were collected from different parts of Lalitpur district and were processed to analyze the antimicrobial property of different solvent extract of guava leaf by using Agar well diffusion method.

Results: The results of the study indicated that three out of three of the crude solvent extract prepared from the leaves of guava, Methanol, Ethanol and Distilled water showed inhibitory activity against bacteria. Only Gram-positive bacteria, *Staphylococcus aureus* were susceptible to the all the extract, while *Escherichia coli* did not show any inhibitory action. Among the four sample extract of guava leaf from different solvent, the distilled water showed highest zone of inhibition by 25mm, followed by methanol extract with 20mm zone of inhibition and ethanol extract by 19mm zone of inhibition. At 10mg/50µL, the distilled water extract showed more antimicrobial activity with mean zones of inhibition 23.66mm than Methanol and Ethanol extracts with mean zone of inhibition 18mm and 16.33mm respectively.

Conclusion: Leaf extract of Guava showed the antibacterial activity against *Staphylococcus aureus*.

Keywords: Guava leaves, Medicinal properties, Agar well diffusion method, solvents extract, Antimicrobial activity

INTRODUCTION

From the ancient times different plant based herbal medicine are used to cure the diseases. Medicinal plants are used for the ailment of several microbial and non-microbial originated diseases due to their valuable effects in health care (Akroum, et al., 2010). The affordable, reliability, and low toxicity of medicinal plants in therapeutic use has made them popular and acceptable by all religion for implementation in medicinal health care all over the world. According to WHO about 80% of the developing world's population rely on traditional herbal medicine for their primary health care (WHO, 1993). The wide spread antibiotic resistance observed is now posing a serious health concern with medical scholars warning a return of pre-antibiotic era (Davies, et al., 2010). Due to increase in drug resistance the use of alternative therapies and natural remedies has rapidly increased in present context.The bioactive compounds are type of chemical found in small amounts in plants and promotes good health. They are being studied in the prevention of cancer, heart, disease etc. Example are: Flavonoids, tannins, terpenoids, glycoside. Among all plant organs, leaves are the largest accumulators of bioactive compounds, such as secondary metabolites. Several recent studies reported phytochemical profiles and biological activities of leaf extracts of various cultivated plants (Kumar, et al., (2021). Hence, although plant leaves are considered as agricultural waste, they are a rich source of high-value nutra-pharmaceutical compounds.

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The guava (*Psidium guajava* L.) tree, belonging to the Myrtaceae family, is a very unique and traditional plant which is grown due to its diverse medicinal and nutritive properties. Guava has been grown and utilized as an important fruit in tropical areas like India, Nepal, Indonesia, Pakistan, Bangladesh, and South America etc. Different parts of the guava tree, i.e., roots, leaves, bark, stem, and fruits, have been employed for treating stomachache, diabetes, diarrhea, and other health ailments in many countries. Guava leaves, along with the pulp and seeds, are used to treat certain respiratory and gastrointestinal disorders, and to increase platelets in patients suffering from dengue fever (Mateus, et al., 2020).

METHODS

Study duration and sample sites

The study was carried from October 2021 to December 2021 at the Microbiology Laboratory, Department of Microbiology, D.A.V. College. The total of four Guava leaves samples were collected from different part of Lalitpur district.

Preparation of Guava leaves extract

The leaf samples of Guava were collected from different areas of Lalitpur district. The leaves were first washed thoroughly with tap water to remove dirt and later washed with distill water. The samples were sun dried 2-3 days, after the leaves were dried it was grind in the grinder to make a powder of leaves and preserved at room temperature (RT) for future use. The three solvent were taken which Alcohol, Methanol and Distill water. The leaf powder was added to each of solvents to make a 20% concentration i.e. 20gm of leaf extract in a 100ml of solvents. Then it was kept for 3 days in a room temperature and filtered by Whattman No.1 filter paper. The extract was then centrifuged at 4000rpm for 10 min. The supernatant was collected and stored at 4oC until further use. Standard reference is missing. Add references in each method section

Phytochemical Analysis

Chemical test for the screening and identification of bioactive chemical constituents in the guava leaf were carried out with the extract using the standard procedure as described by Chapman and Hall (1973). In a test tube 2ml solvent extract was taken and shaken vigorously, the formation of stable foam was taken as an indication for the presence of saponins. In another test tube 1ml solvent extract was taken and added 2% of FeCl3. The black coloration indicated the presence of phenols and tannins. Similarly, 1ml extract was mixed with chloroform and added 2ml of concentrated sulfuric acid and was shaken gently. A reddish brown coloration of the interphase was formed to show presence of terpenoids. Same amount of extract was taken in another test tube and mixed with magnesium ribbon fragments. The concentrated hydrochloric acid was added dropwise. Orange red coloration indicated the presence of flavonoids. Similarly, 1ml extract was taken and mixed with 2ml glacial acetic acid containing 2 drops of 2% FeCl3. The mixture was poured into another tube containing 2ml of concentrated sulfuric acid. A brown ring at interphase indicated the presence of glycosides reference ????.

Antimicrobial susceptibility assay

The agar well diffusion method was performed to analyze the antimicrobial activity of guava leaf extract of different solvent on *S. aureus* and *E. coli.* All these test organisms were obtained from the Microbiology Laboratory of D.A.V. College, Lalitpur. The inoculums of test organisms were prepared by inoculating 3-4 bacterial colonies to 5ml Nutrient broth and incubated at 370C for 4 hour, after which it was adjusted to the 0.5 McFarland standard solution. The agar plates were inoculated with test organism using sterile cotton swab and was allowed to dry for 5 minutes. With the help of sterile 6 mm cork borer, a well was made on all agar plate. Then 50 microliters of each respective sample extract and neat (negative control) in separate wells respectively. It was allowed to rest for 15 minutes for diffusion of enzymes in media and incubated at 370C for 24 hours. After the observation was made for clear zone around the extract well and zone of inhibition was measured and noted.

RESULTS

Phytochemical Analysis

The results showed the presence of active compounds in guava leaf sample in three different solvents extracts. As the table shows, the methanol and ethanol extracts indicate the presence of tannins, Phenols, flavonoids, terpenoids, and glycosides, but absence of saponins. Distilled water is the only that showed the presence of all the phytochemicals in all the four sample of guava leaves.

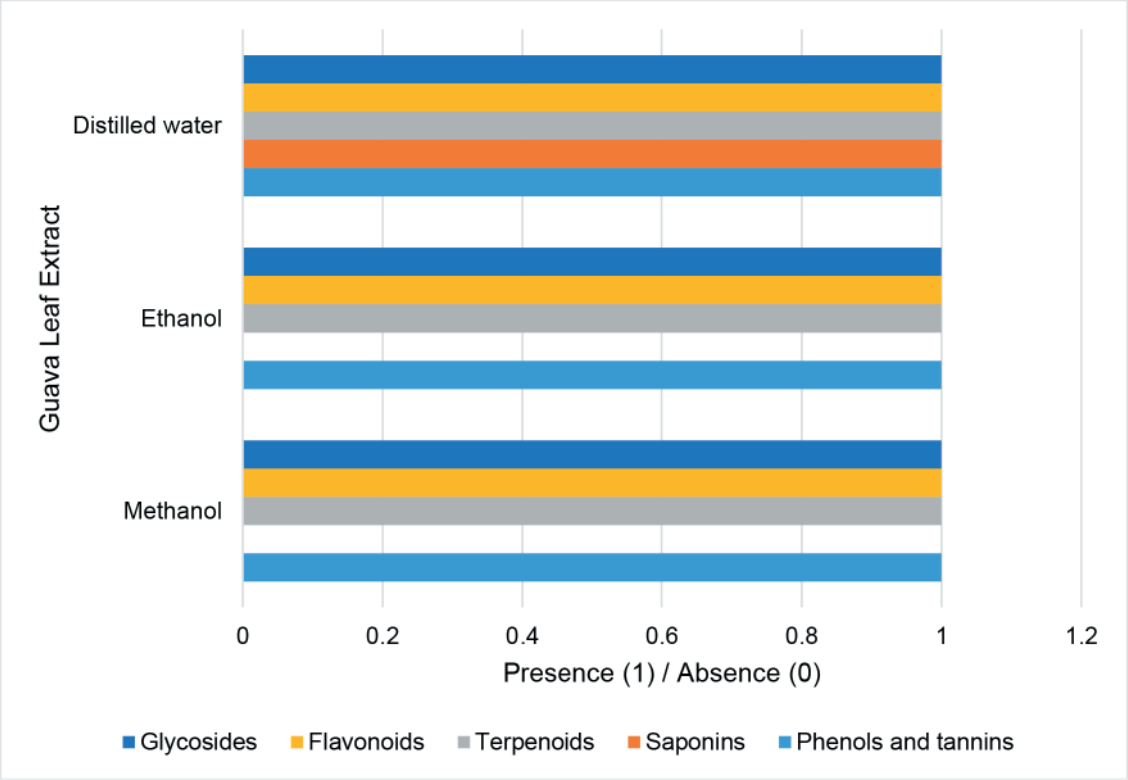

Figure 1: Phytochemical constituents of guava leaf extracts

Antibacterial activities of guava leaf extracts against Gram positive and Gram negative bacteria

All of the solvent extract showed highly effective against Gram-positive bacteria (*Staphylococcus aureus)* but the Gram-negative bacteria (*E. coli*), did not show any zone of inhibition against all three solvent extract.

Table No: 1 Comparison of antibacterial activities of guava leaves extract on bacteria.

Guava Extract	Organism	Mean Zone of inhibition
Methanol	*Staphylococcus aureus*	18mm
	Escherichia coli	-
Ethanol	*Staphylococcus aureus*	16mm
	Escherichia coli	-
Distilled water	*Staphylococcus aureus*	23.25mm
	Escherichia coli	-

DISCUSSION

About four samples were collected from different parts of Lalitpur district. The bacteria was provided by the Microbiology laboratory of DAV College, this was further re-confirmed by identifying colony characteristics, gram staining and biochemical testing.

The result showed that the methanol and ethanol extract contained phenol and tannins, flavonoids, terpenoids and glycosides but lacked saponins, while distilled water showed the presence of saponin, phenols and tannin, flavonoids, terpenoids and glycosides in all the four samples of guava leaves. This analysis has led the presence of phytochemical that has antimicrobial property. For example, tannins are polyphenolic compounds that bind to proline rich protein that interferes with protein synthesis and has shown antimicrobial property (Ulubelen, 2003). Flavonoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infection to which this aspects has been studied and found to have antimicrobial activity (Cowan, 1999). Terpenoids are mainly used for the aromatic purpose but it has also been found to have potential inhibitory action against bacteria (Tsuchiya, et al., 1996). Saponins which are glycosides have been found to have inhibitory effects on Gram-positive organism (*Staphylococcus aureus).*

Therefore, the phytochemical analysis showed that the methanol, ethanol and distilled water extract have chemical compounds that have been found to possess the antimicrobial activities.

The results of the study indicated that three out of three of the crude solvent extract prepared from the leaves of guava, Methanol, Ethanol and Distilled water showed inhibitory activity against bacteria. Only Gram-positive bacteria, *Staphylococcus aureus* were susceptible to the all the extract, while Gram-negative i.e *Escherichia coli* did not show any inhibitory action. Among the four sample extract of guava leaf from different solvent, the distilled water showed highest zone of inhibition by 25mm, followed by methanol extract with 20mm zone of inhibition and ethanol extract by 19mm zone of inhibition. At 10mg/50µL, the Distilled water extract showed more antimicrobial activity with mean zones of inhibition 23.66 than Methanol and Ethanol with mean zone of inhibition 18 and 16.33 respectively. Distilled water showing more antimicrobial activity might be the reason that it contained all the phytochemical mentioned (table 4) while other solvent lacked saponin. Saponins possess detergent-like properties and might increase the permeability of bacterial cell membranes without destroying them (Jacob, et al., 1991). This may have facilitated the influx of other phytochemical into the cell of bacteria which might be the reason that distilled water containing saponin showed highest zone of inhibition. Sanches, et al. (2005), found that the aqueous extract of guava was effective against *Staphylococcus.* Beside distilled water, methanol showed the second highest zone of inhibition with mean zone of inhibition of 18mm while the ethanol extract showed least of the zone of inhibition with mean zone of inhibition 16.33. Biswas, et al. (2013) conducted similar study where they found out that methanol extract showed highest zone of inhibition followed by ethanol extract. But the distilled water did not show any zone of inhibition throughout the study. In case of bacteria used, gram-positive *Staphylococcus aureus* only showed the zone of inhibition while the gram-negative *Escherichia coli* did not show any zone of inhibition.

The resistance of the Gram-negative bacteria could be due to its cell wall structure. Gram-negative bacteria have an effective permeability barrier, comprised of thin lipopolysaccharide exterior membrane, which could restrict the penetration of the plant extract. It has been reported earlier that Gram-negative bacteria are usually more resistant to the plant antimicrobial property and even show no effect, compared to gram-positive bacteria (Tajkarimi, et al., 2010). Gram-positive bacteria have a mesh like peptidoglycan layer which is more accessible to permeation by the extract (Rameshkumar et al., 2007).

Nascimento, et al. (2000) conducted a study which supports the finding of the present study in which the guava extract was able to have inhibitory effects against *Staphylococcus aureus* and no effect on *Escherichia coli.* Mahfuzul Hoque, et al. (2007) found no effect on antibacterial activity of ethanolic extract of guava against *E. coli;* however, Viera, et al. (2001) found that the guava sprout extracts were effective against inhibiting *E. coli.*

CONCLUSION

The current study demonstrate the antimicrobial activity of guava leaves extract using various solvent. The result showed that the distilled water was better than methanol and ethanol respectively. It also showed that the guava leaves extract has no antimicrobial effect on the gram-negative bacteria (*Escherichia coli).* The observed inhibition of gram-positive bacteria, *Staphylococcus aureus,* suggests that guava possess compounds containing antimicrobial properties that can effectively suppress the gram positive bacterial growth.

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