ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Anti-Microbial, Anti-Inflammatory and Anti-Oxidant Properties of Guava (*Psidium guajava*) Leaf Extract.

Diana G, Sirak K, Robel B, Betiel S, Adiam, Weini T, Tseghai H, Bahreselam S, and Eyalarasan K*.

Department of Chemical Engineering, Mai-Nefhi college of Engineering and Technology, Eritrea.

ABSTRACT

In Eritrea, traditional healthcare practice has been an integral part of the culture and playing important role in majority of households till date because of their high success rate in curing different types of diseases.Guava leaves are part of such traditional more common medicinal in villages. Guava (Psidium guajava L.) leaves have been used to manage several diseases such asdiarrhea, cough and other infectious diseases. In our work, antimicrobial, anti-inflammatory and antioxidant activity of guava leaves were estimated using different category of microbes. 95%, 84% and 100% of equivalent antimicrobial activities compared to amoxicillin as reference standard were shown by gram positive, gram negative and fungal strain from agar-well diffusion method respectively.Qualitative and quantitative Phytochemical analysis (UV-Spectrometry and FTIR spectrometry) proved that guava leaf extract was rich in wide range of tannins (3g/l), phenol (1.75g/l) and flavonoids (1.20g/l) and tracer amount of triterpenes (terpenoids), glycosides and alkaloids. These constituents are the main reason for the medically valuable activities. FTIR spectrum of guava leaf extract shows a strong peak band for the polyphenol groups which indicates the presence of the pharmacological activities.

Keywords: Antimicrobial, phytochemical, polyphenol.

https://doi.org/10.33887/rjpbcs/2023.14.3.23

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Infectious diseases have been the most dangerous and dominant killer among all other diseases. Despite many more active research has been going on in this area to find antimicrobial agents, natural therapies and natural medicines have been also working out as curing agents. Bioactive antimicrobial compounds extracted from plants has been attracting attention all over the world.

Psidium guajava L. (guava)is plentiful and guava tree plantation is one of the most important plant in Eritrea. Guava leaf extract from boiled water has been used as oral dosage in many part of Eritrea for curing diarrhea, cough, diabetes and as natural mouthwash agent till date. It was generally proved and justified that guava leaf has pharmacological bioactive compounds [1] as well as nutritive compounds. In our work, we mainly investigated to determine the specific contents in guava leaves which reason for such beneficial health effects and we have gone through their detailed mechanism of action. Solvent extraction was our preferred choice for separating the bioactive compounds and optimizing the solvents usage also as added objective.

MATERIALS AND METHODS

Guava leaf Extraction from three different solvents

Dried guava leaves wereplaced into a blender to be grounded into coarse powder which was stored in safe manner. Solvents used are methanol, ethanol and propanol separately for preparing three different extracts. The solvent extract was prepared by mixing 2%[w/v] which was kept for 1 week in dark zone with mild stirring. After 1 week, the extract was filtered using Whattman no-01 filter paper and the filtrate was taken into steam sterilized test tubes.

Figure 1: Guava leaves extract preparation

Zone of inhibition by Agar-well diffusion method

This method is mainly used to measure the antimicrobial activity for the three different categories of microbes. Nutrient agar was prepared and sterilized as per standard procedure mentioned in Manika Das et.al., [1]. The antimicrobial activity of the solvent extract of guava leaves, was estimated against three microbial strains: *Bacillus subtilis* (Gram positive bacterial strain), *Pseudomonas* (Gram negative bacterial strain), *Saccharomyces cerevisiae* (Yeast, fungal strain).For each microbial strain, four petri plates were used, and the usage is mentioned in Table-01. Stock cultures of those microbes were directly streaked and inoculated into the central part of petri-dish containing nutrient agar and incubated for 24 hours at 35˚C. All such procedures were followed in the laboratory laminar flow hood with radiation sterilized environment. For each microbial strain, Petri plates are filled at concentrations of 0.25% [w/v], 0.5% [w/v] and 1% [w/v] respectively. Agar-wells were created to accommodate 0.5ml and 1ml of the extract with the different concentrations as mentioned earlier and microbial growths as well as the zone of inhibition was observed after 24 hours. A ruler was used to measure the inhibition zones in millimeters. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments as triplicates average values.

ISSN: 0975-8585

Table 1: Table for Agar-Petri plates for each microbial strain used for agar-well diffusion method

Petri-plate	Purpose of Agar-petri plate	Contents
1	Control plate	Microbial inoculation without adding of GLE and Am-STD
2	Reference standard	Microbial inoculation with addition of Am-STD
3	Test plate with 0.5ml GLE	Microbial inoculation with different concentrations of GLE
4	Test plate with 1ml GLE	Microbial inoculation with Different concentrations of GLE

GLE-guava leaf extract and Am-STD: Amoxicillin standard

Qualitative analysis of the phytochemical content of guava leaf extract

Agar diffusion method was well succeeded for ethanol extract only compared to methanol and propanol. Therefore, the bioactive chemical constituents in the guava leave extract obtained from ethanol were subjected to qualitative analysis using the standard procedure as described. [4] and [1]. It was also later justified that the qualitative analysis of ethanol extract shown much better phytochemical constituents than the other two solvents.

Test for Glycoside: Extract was mixed with 2 mL of glacial acetic acid containing 2 drops of 2% $FeCl_3$. The mixture was poured into another tube containing 2 mL of concentrated sulfuric acid. A brown ring at the interphase indicates the presence of glycosides as mentioned in Bipul et al [2].

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.

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.

Test for phenols and tannins: 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.

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.

Test for Terpenoids (Triterpenes). Extract was mixed with 2 mL of chloroform. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase was formed to show positive results for the presence of terpenoids.

Quantitative analysis: Due to the presence of required contents of medicinal were present mainly in ethanol extract, quantitative analysis was done only for ethanol extract using uv-spectrophotometry and FTIR spectrophotometry. The results of all microbial and chemical analysis were expressed as mean ± standard deviation of triplicate analyses for each experiment.

Phenol content: GLE obtained from Ethanol solvent extract is subjected to Sagbo IJ et.al., method as described. 5ml of GLE and Folin-Ciocalteo [FC] reagent at a dilution of 10% using distilled water were mixed.4ml of Anhydrous sodium carbonate at a concentration of 75%, mixed to the Folin mixture. A blank was prepared and the resulting mixtures were shaken vigorously and incubated at 40°C for 30 minutes. The resulting optical densities were measured at 765 nm using a UV-spectrophotometer. The absorbance against mg of gallic acid was measured and reported [4].

Tannin content: Doss A et.al., method was used in measuring Tannin content of guava leaf extract. Standard Tannic acid solution was prepared by mixing 0.01% [w/v]. This solution was pipetted in 100ml flasks containing 75 ml distilled water in serial different amounts. To each mixture, 5 ml of Folin reagent was added, along with 10ml of 35% [w/v] sodium carbonate solution. The mixture was shaken well and incubated for 30 minutes at 40°C. The absorbance at 760nm against mg of tannic acid was usedas

standard. The sample was prepared by replacing standard tannic acid by GLE and the mixture was incubated at 40˚C for 30 minutes against blank and the absorbance was measured [5].

Flavonoid content: Ordonez AA et.al., method was used. 0.5 mL of the GLE was mixed with equal amount of 2% ethanol prepared aluminum chloride.The mixture was incubated for 1hr and the absorbance was read at 420 nm. Development of yellow color indicated the presence of flavonoid which was calculated as mg/g of quercetin equivalent [6].

RESULTS AND DISCUSSION

Estimation of antimicrobial activity of guava leaf extract

The antimicrobial activity of the three solvents extract of guava leaves was assessed against three microbial strains: *Bacillus subtilis* (Gram positive bacterial strain), Pseudomonas (Gram negative bacterial strain), *Saccharomyces cerevisiae* (Yeast; fungal strain). After one day of incubation, the microbial growth was observed for bacterial as well the fungal strains with their zone of inhibition. Here methanol and propanol were relatively showing less diameter of zone of inhibition compared to ethanol extract. Therefore, Ethanol extract of guava leaves were taken into account for our further study and the methanol and propanol extract were omitted from our observation of agar-well diffusion method. Direct streaking of stock culture of particular microbes was done on nutrient agar. Table-02 shows clearly that yeast and bacillus subtilis growth were strongly inhibited whereas the growth of pseudomonas also approached slightly lesser as 84% equivalent inhibition. The petri-plates were prepared as mentioned in table-01. It was clearly noted the GLE obtained from ethanol extract shown effective zone of inhibition as crude extract itself and if it was subjected to further downstream processing such as crystallization, equivalent antimicrobial effect for different microbes except gram negative pseudomonas may be shown.

Table 2: Estimation of antimicrobial activity of ethanol extracted guava leaf extract comparing with the reference standard Amoxicillin [Am-STD]

Microbes used for test	Microbial zone of inhibition of 1 % [w/v] solution		
	Category	Am-STD [1ml]	GLE [1ml]
Bacillus subtilis	Gram +ve	100%	95.00%
Pseudomonas	Gram –ve	100%	84.00%
Saccharomyces cerevisiae	Yeast	100%	100%

Table 3: Zone of inhibition of bacterial and fungal strains against different concentration of guava leaf extract [GLE] and Amoxicillin standard [Am-STD]

Microbial strain	Zone of Inhibition [cm]					
	0.25% [w/v] GLE	0.25% [w/v] Am-STD	0.5% [w/v] GLE	0.5% [w/v] Am- STD	1% [w/v] GLE	1% [w/v] Am-STD
Bacillus subtilis	**2.90**	3.12	**3.10**	3.19	**4.40**	4.50
Pseudomonas	**1.85**	2.21	**2.00**	2.37	**3.25**	3.88
Saccharomyces cerevisiae	**3.25**	3.35	**3.85**	4.00	**4.50**	4.5

It is more evident that the infectious diseases caused by gram positive bacterial strains such as Bacillus Subtillis, E.Coli etc., can be well treated by guava leaf extract and the same can also be expected with fungal strains such as yeast, mold Aspergillus Niger etc. Gram negative bacterial strain such as Pseudomonas can also be controlled by almost 84%. Pseudomonas shows less sensitiveness to this guava leaf extract and the sensitiveness can be increased by increasing the downstream processing such as crystallizing and keeping in well-sterile storage systems.

ISSN: 0975-8585


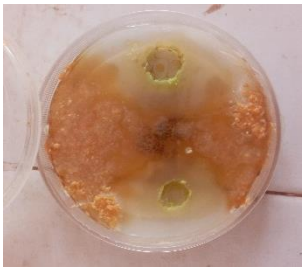


Figure 2 **Figure 3** **Figure 4**

Figure 2, 3 and 4 represents zone of inhibition from ethanol extracted guava leaves for yeast, pseudomonas and *bacillus subtillis* respectively.

Optimizing solvent extraction based on Qualitative analysis

Qualitative analysis of the phytochemical content of guava leaf extract using the three different solvents were shown in Table-04. It was observed that the essential phytochemicals such as phenols, tannins, flavonoids and glycosides were relatively present more amount in ethanol extract of GLE than methanol extract whereas saponins were absent in both extracts. Propanol extract shows only the presence of glycosides and all the other essential phytochemicals were not present. It was decided that ethanol extract of guava leaf is much better than the extract obtained from methanol and proponal, Therefore the ethanol extract was taken for further study mainly quantitative analysis.

Table 4: **Result of qualitative Analysis of Guava Leaf Extract**

Extracts	Glycosides	Alkaloids	Flavonoids	Phenols and Tannins	Saponins	Terpenoids
Ethanol	++	+	++	++	-	+
Methanol	-	+	+	+	-	+
Propanol	-	-	-	-	-	-

++: Strongly observed +: Tracer observed -: Not present





Figure 5: Results of qualitative tests: Phenol, Flavonoids, Tannins, triterpenes, Anthroquinones

Quantitative analysis of GLE

UV-Spectrophotometry: Phytochemical bioactive compounds were observed by UV-spectrophotometer and the results were tabulated in table:05 along with the methods used.

Table 5: Quantitative analysis of phytochemicals of Guava leaf extract:

Phytochemicals	Concentration [g/l]	Medicinal Activitities	Analytical Method used
Tannin	3.00	Antimicrobial and Anti-inflammatory	Doss A et.al [5]
Phenol	1.75	Antimicrobial and Antioxidants	Sagbo IJ et.al.[4]
Flavonoids	1.20	Antimicrobial	Ordonez AA et.al.[6]
Terpenoids	0.25	Anti-Inflammatory	Ansari MA.et.al [10]
Alkaloids	1.40	Antioxidants	Ansari MA.et.al [10]
Glycosides	1.58	Antioxidants	Ansari MA.et.al [10]

Table-05 shows that the antimicrobial, anti-inflammatory and antioxidant activities of guava leaves were obtained from these three phytochemicals majorly. The functional groups present in the guava leaf extract also were tested using FTIR spectroscopy and the results were reported as follows.

FTIR Spectrum for guava leaves:

FTIR Spectrum of guava leaf extract shows a strong peak in wave number of 1050 to 1100 $(cm)^{-1}$ range corresponds to the hydroxyl [O-H] stretching vibrations which indicate the presence of polyphenolic groups and tannin containing hydroxylated polyphenol groups which are the main reason for the pharmacological activities. The peak obtained at 1400 to 1500 $(cm)^{-1}$ indicates the presence of phenolic secondary compounds characterized by C_6-C_3-C_6 structure, justifies the presence of flavonoids.

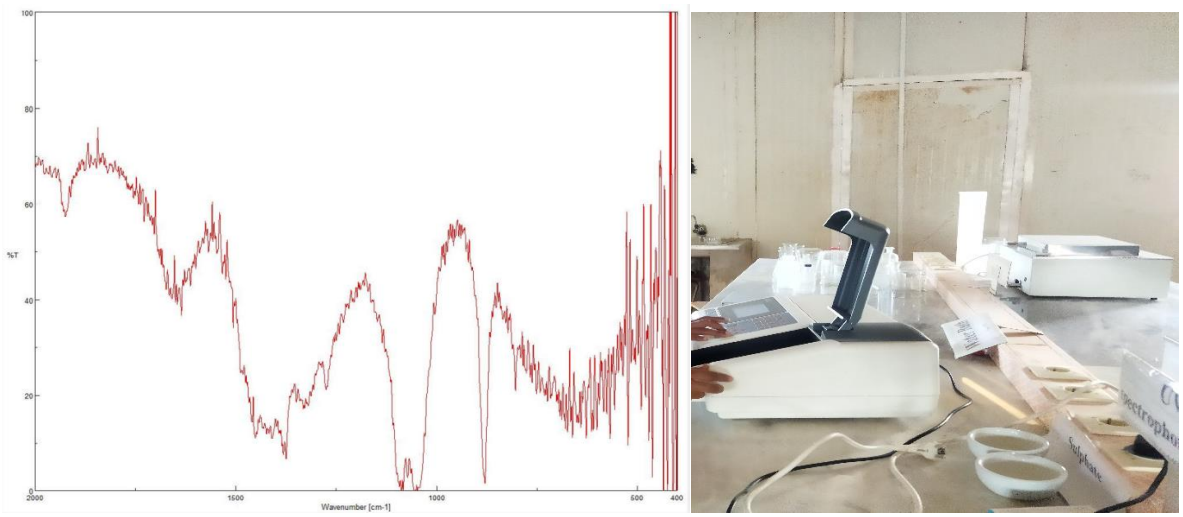

Figure 6: FTIR Spectrum of Ethanol extract and UV-spectrometry

DISCUSSION

Agar well diffusion method

It was observed at first that the extract obtained from ethanol shown relatively better zone of inhibition compared to methanol in agar well diffusion method. Propanol extract shown zero zone of inhibition. Therefore, the detailed antibiotic activity test was undergone for ethanol extract and Table-03 and 04 summarizes that fungal, gram positive and gram negative strains indicated 100%, 95% and 84% of equivalent antimicrobial property compared to the reference standard. The cell walls of Gram negative bacteria are very stronger and consist of outer lipopolysaccharide membrane, a peptidoglycan cell wall

and an inner cytoplasmic membrane which act as a strong barrier towards some phytochemicals to the cells. [7]and [1]. Intrusion of bioactive compounds are prevented by such strong outer cell membrane makes gram negative microbes more resilient to die than other microbes.

Quantitative and Quantitative phytochemical analysis

Table-03 shows the summarized phytochemical screening of chemical constituents of guava extracts under study on qualitative basis. The results revealed the presence of active compounds in the three different extracts. As the table shows, the ethanol extracts indicate the stronger presence of tannins, phenols, flavonoids and tracer presence of terpenoids as well as glycosides. Saponins were absent in all extracts which is also not an essential constituent. Solvent propanol failed to show none of the phytochemicals whereas solvent methanol succeeded to show them but may not be in appreciable amount. Therefore, it was concluded that the ethanol extract is the best among all other extracts and it was considered for further quantitative analysis [2].

The analysis of the plant extracts revealed the presence of phytochemicals which are known to exhibit medical and physiological activities. It was also justified that polyphenols show anti-fungal activity by inhibiting fungal cell membrane [ergo sterol] [8]. Inhibition of important macromolecular synthesis in microbes are some of the underlying antimicrobial mechanisms of action of polyphenolic compounds present in the phytochemicals [9].

Tannins are water soluble polyphenolic compounds that affects protein synthesis and exhibit antibacterial activity. [2] Tannin act as antimicrobial agent by the mechanism of extracellular enzyme inhibition, deprivation of substratum and inhibition of oxidative phosphorylation [10]. Flavonoids are hydroxylated polyphenolic compounds well known natural inhibitor of microbial infections by forming complexes with cellular structural proteins and bacterial cell walls. Terpenoids are full of aromatic qualities have also been found to be potential agents against inhibiting bacteria. It was also well observed that the phenolic and polyphenolic compounds are proven agents as antimicrobial, anti-inflammatory and antioxidant. FTIR and UV spectrophotometry provided clear-cut presence of functional groups and compounds reason for such pharmacological activities.

CONCLUSION

The results indicate that ethanol is better solvent than methanol and propanol for the extraction of essential phytochemicals of guava leaves. The present work also demonstrates the Guava leaves extracted using ethanol possess strong medicinal property even though the gram negative bacteria [Pseudomonas] showed slightly less sensitivity towards the antimicrobial property of guava leaves. Phytochemical analysis showed that Guava leaves are rich in wide range of polyphenolic compounds such as Phenol, flavonoids and tannins are the main reason for antimicrobial, anti-inflammatory and antioxidant properties. This study paves a way to further investigate other pharmacological properties of guava in future. Potency against gram negative bacteria may be improved by adding other adjuvants or by enriching concentrations of extract. On the basis of the present finding, P. guajava leaves possess the capabilities of being excellent natural antimicrobial agent against infections caused by gram positive bacterial and fungal strains.

ACKNOWLEDGEMENT

This work was majorly supported by Department of Chemical Engineering, Mai-Nefhi college of Engineering and Technology [MCOETEC], Eritrea and Azel Pharmaceuticals share company.

REFERENCES

[1] Manika Das, Subhagata Goswami, Antifungal and antibacterial property of Guava (psidium Guavaja): role of phytochemicals, IJHSR, Vol-09, issue-02, 2019

[2] Bipul Biswas, Kimberly Rogers, Fredrick McLaughlin, Dwayne Daniels, and Anand Yadav, "Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative and Gram-Positive Bacteria published in Hindawi, 2013.

[3] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 2001; 109: 69–75.

[4] 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.
[5] 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.
[6] Ordonez AA, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of Sechium edule (jacq). swart extracts. Food Chem 2006; 97: 452-8.
[7] 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.
[8] Coppo E, Marchese A. Antibacterial Activity of Polyphenols. Current Pharmaceutical Biotechnolog.2014; 15 (4): 380 – 392.
[9] 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.
[10] 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.