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Original Article

Evaluation of Antimicrobial and Antioxidant Activities of *Psidium guajava* L growing in Libya

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ABSTRACT

The present study focuses on the phytochemical screening, antimicrobial and antioxidant activities of the leaves extracts of Guava (*Psidium guava Linn),* belonging to the Myrtaceae family. The plant samples were collected from Tajura, eastern part of Tripoli in Libya. Aqueous and methanol extracts of dried and ground plant materials were prepared. Qualitative phytochemical screening of the extracts showed positive tannins, flavonoids, alkaloids, saponins, terpenes in varying proportions while alkaloids were the only exclusion in the methanolic extract. The strong positive result of flavonoids in both plant extracts motivated us to perform an individual extraction and test for flavonoids. The water, methanol crude extracts as well as flavonoids extract were effective against both Gram-positive and Gram-negative bacteria: *Buscillis subtils, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*, giving a range of 12-25 mm inhibition zone diameter. According to the minimum inhibitory concentration MIC results, flavonoids extract was the most effective against bacteria (with MICs of 2.5-5 mg/ml), followed by methanolic extracts (with MIC of 5.5-11 mg/ml). The water was found to be the least effective with MIC (12-15 mg/ml). Therefore, more investigation for antimicrobial properties of flavonoids extracts was carried out using bioautography method to specify the biologically active separated spots (against *B. sub*) on thin layer chromatograms from flavonoids extracts. Thin Layer-Bioautographic application showed the presence of five biologically active spots (S1-S5) with Rf values of 0.46-0.8. These results could be a preliminary orientation concerning how many possible bioactive plant components need separation and identification.

Total phenols were also determined in methanolic and aqueous extracts of *Psidium guajava L.* in order to assess their contribution to the antioxidant activity; both extracts (aqueous and methanolic) showed reasonably high content of polyphenols, 66.21 and 64.97 mg/g, respectively. Results generally suggest that Guava leaves are not only reliable natural sources of antimicrobials but also potential sources of phenolic antioxidants and hence could be nominated for future intensive studies.

Key words: medicinal plants, *Psidium guajava,* Phytochemical, Antimicrobial, Antioxidant phenolics, flavonoids

Introduction

Psidium guajava is an important food crop and medicinal plant in tropical and subtropical Countries, widely used like food and in folk medicine around the world. This study aims at exploring the chemical constituents, pharmacological, and clinical uses of this plant. Different pharmacological experiments in a number of *in vitro* and (Martha P´erez Guti´errez *et al.,* 2008) *in vivo* models (Martha P´erez Guti´errez *et al.,* 2008; Aadesariya *et al.,* 2019) have been carried out to identify the medicinally important phyto-constituents, a number of metabolites in good yield and some of which have been shown to possess useful biological activities (Martha P´erez Guti´errez *et al.,* 2008; Khan Muluh *et al.,* 2019). Belonging mainly to phenolic, flavonoid, carotenoid, terpenoid and triterpene, extracts and metabolites of this plant, particularly those from leaves and fruits possess useful pharmacological activities.

Psidium guajava, which is considered a native to Mexico (Martha P´erez Guti´errez *et al.,* 2008) extends throughout the South America, European, Africa and Asia. Based on archaeological evidence.

It has been used widely and known in Peru since pre-Columbian times. It grows in all the tropical and subtropical areas of the world, adapts to different climatic conditions but prefers dry Climates (Stone, 1970).

The phytochemicals during this plant justifies its traditional medicinal uses (El-Shahaby *et al.,* 2019). The *guajava* (*Psidium guajava*) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. Many parts of the plant are utilized in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, pharyngitis, inflamed gums, and a variety of other conditions (Abdelrahim *et al.,* 2002; Jaiarj *et al.,* 1999; Lutterodt, 1992). Plants are potential sources of antimicrobial compounds and that is why different researchers have examined the antimicrobial activities of medicinal plants utilized in traditional or alternative healthcare systems (Asemave and Anure, 2019).

In Libyan folk medicine, decoction of *guajava* leaves is used to relieve cough and cold as it helps to get rid of mucus. It is also believed to disinfect the respiratory tract, throat and lungs.

Guava has exhibited remarkable antimicrobial activity against microorganisms such as Bacillus, E. coli, Staphylococcus, Pseudomonas, Clostridium, Shigella, Salmonella and yeast such as Candida species (Fugaban, 2016).

When one refers to plants of medicinal value, one often lists their active ingredients, which might include alkaloids, flavonoids or glycosides , essential oils , tannins (Dahanukar *et al.,* 2000) and some other unusual substances (Naili *et al.,* 2010). Alkaloids are group of nitrogen-containing organic compounds; many of which are poisonous, whilst others are very useful as codeine and morphine, which alleviate pain. Alkaloids (McDevitt *et al.,* 1996) and essential oils are most often effective on skin and mucous membranes as expectorants, while yellow colored flavonoids are found in many plants and can be used to treat

atherosclerosis and hypertension . Though further studies could also be used for the advancement of conventional medicines and introducing novel active compounds from the therapeutic plants, they will produce a completely unique substance to treat several incurable illnesses. (Solomon *et al.,* 2018).
This study accordingly investigates the phytochemical, antibacterial and antioxidant properties of *Psidium guajava* leaf extracts.

Materials and methods

Plant materials

The plant materials (leaves) of *Psidium guava* (family: Myrtaceae) that were utilized in this study were collected from Tajoura eastern part of Tripoli in Libya. The herbarium specimens were authenticated by examining the morphological and anatomical features in the Botany Department, Faculty of Science Tripoli University, Tripoli, Libya.

Chemicals and reagents

All chemicals used during this work were products of Aldrich and Sigma Chemical Companies of reliable commercial grade, hence utilized without further purification. Folin-Ciocalteu Reagent (FCR) was always freshly prepared following the procedure described in literature (Varley, 1976); Silica gel 60 F254 thin chromatographic plates were purchased from Merck (Germany).

Bacterial strains

For assaying the antibacterial potential of the plant extracts, a narrow spectrum of antibiotics-sensitive clinically isolated bacterial species (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella typhi*) was used. These organisms were generously provided by the Laboratory of Clinical Microbiology, Tripoli Medical Center, Tripoli, (Libya). Bacterial strains were routinely grown and preserved on Nutrient Broth or Mueller Hinton (NB, Difco). Medium (2.0% agar was added whenever needed).

Extraction of Psidium guava leaves

The fresh parts of plant leaves were washed enough with tap water then twice with distilled water and open air-dried for a week in shadowed place (26 ± 2.0 ºC). They were then crushed into powder with an electric blender. Then two portions (20 g each) of the freshly prepared samples were individually boiled with 250 ml distilled water (Aqueous extract) or soaked for 48h in 200 ml of 80º/º methanol (Methanolic extract). Both samples were macerated at room temperature with continuous stirring for further 24 h. Then all crude extracts were filtered through Whatman filter paper (No. 1) and kept at 4 °C until tested.

Phytochemical tests

The fresh aqueous and methanolic crude extracts were qualitatively screened (Harborne, 1983) for the following constituents: flavonoids, coumarines, hydrolysable tannins, alkaloids, terpenes, anthraquinones and saponins. The qualitative results of the methods have been rated from (+ve) for faint to (+++ve) for dense turbidity (see Table 1).

Table 1. Phytochemical analysis on solvent fractions of *Psidium Guava*

Phytochemical compounds	Water extract	Methanol extract
Alkaloids	+ ve	- ve
Saponins	+ ve	++ve
Anthraquinones	- ve	- ve
Tannins	+ ve	+ ++ve
Terpenes	++ve	++ve
Flavonoids	++ve	+ ++ve
Coumarines	- ve	- ve

Extraction of flavonoids

The strong positive result of flavonoids in both plant extracts motivated us to perform an individual extraction and testing for flavonoids as described in literature (Harborne, 1983). The plant tissues were hydrolyzed with HCl (2 M) for 30–40 min at 80 °C using water bath, and then cooled to room temperature, the acidic solution was extracted twice with methanol and the combined extracts were evaporated to dryness. The crude extract was re-dissolved in the least volume of methanol for flavonoids testing; then bioassays and thin layer chromatography were conducted.

Antibacterial and minimum inhibitory concentration (mic) assessment of plant extracts

The antimicrobial activity of the crude and flavonoid extracts was determined by the "hole-plate diffusion" method (Daud *et al.,* 2005). Each test organism was maintained on nutrient agar slant and was recovered for testing by growth in nutrient broth (Biolab, Difco) for 14 h at 37 °C before streaking. Cultures were routinely adjusted to a suspension of 1×10^6 to 2×10^6CFU/ml using pre-made calibration curve representing viable cell count (X $\times10^6$) against OD660 nm (Y). 150 µl aliquots of the extract were placed into wells of 8 mm diameter. The plates were kept for 1 h at 4 °C for allowing better diffusion of the extract into the agar. Subsequently, plates were incubated at 37 °C for 18 h. Tetracycline (30 µg each) was used as positive control while sterile water or 70% methanol was used as negative control. Diameters of inhibition zones (DIZ) were measured in mm and the results were recorded as the mean of triplicate experiments. The MIC was carried out using agar two-fold serial dilution assay (Irith *et al.,* 2008). The concentrations (mg/ml) of extracts were prepared. Reference antibiotics and the solvents were also assayed. The MIC was recorded as the lowest concentration of the sample that inhibits visible growth giving clear zones of inhibition after 24 h incubation. For bioautographic assay (Nostro *et al.,* 2000). 10 µl of each extract (90 mg/ml) was applied onto 5 × 20 cm Silica gel 60 PF254 glass plate. Best separation was accomplished using methanol, acetic acid, and benzene (2:1:8) for both extracts (Figure 1). The developed TLC plates were dried under a continuous stream of air for 3 h till complete removal of the solvent. The dried chromatograms were overlaid with nutrient agar medium seeded with B. subtilis (106–107 cfu/ml) and then incubated for overnight at 37 ºC (see Figure 1). For comparative purposes, additional chromatogram was run alongside under same conditions and sprayed for visualization of flavonoids (Krebs *et al.,* 1969).

Determination of total phenolics content

The content of total phenolics in plant samples was determined using the Folin-Ciocalteu method (Singleton *et al.,* 1999). Absorbance was measured at 765 nm after 1 h. Gallic acid was used as a standard. 2.9.1.

Reducing power

The antioxidant activity was investigated using the ferric reducing antioxidant power (FRAP) assay for plant extracts according to the method of (Oyaizu, 1986). Plant extract of 1, 10, 50 or 100 mg in 1 ml of ethanol was mixed with 2.5 ml of phosphate buffer (2 M, *p*H 6.6) and 2.5 ml of potassium ferricyanide (10 g/L) then the mixture was incubated at 500 C for 20 min. Next, 1.5 ml of trichloroacetic acid (100 g/L) was added to the mixture, which was centrifuged at 3000 rpm for 10 min. Finally, 0.5 ml of the supernatant solution was mixed with 1.0 ml of distilled water and 0.5 ml of $FeCl_3$ (1.0 g/L) and the absorbance was measured at 665 nm.
Increased absorbance of the reaction mixture indicates the increasing of reducing power of plant ingredients.

Results

Phytochemicals

Psidium guajava L., popularly known as guava, is a small tree belonging to the myrtle family (Myrtaceae) (Morton, 1987). Native to tropical areas from southern Mexico to northern South America, guava trees have been grown by many other countries having tropical and subtropical climates, thus allowing production around the world (Salazar *et al.,* 2006). Traditionally, preparations of the leaves have been used in folk medicine in several countries, mainly as anti-diarrheal remedy (Gutiérrez *et al.,* 2008). Moreover, other several uses have been described elsewhere on all continents, with the exception of Europe (Gutiérrez *et al.,* 2008; Morais-Braga *et al.,* 2014). Depending upon the illness, the application of the remedy is either oral or topical. The consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders, such as rheumatism, diarrhea, diabetes mellitus, and cough (Das and Goswami, 2019).
In Libya, a decoction of guava leaves is used as a cold remedy and an agent to ease stubborn cough. The phytochemical screening properties of *P. guava* leaves showed that the aqueous Extracts were poor in tannins (+), alkaloids (+ve) and saponins (+ve), moderate in terpenes and flavonoids. All these phytochemicals with the exception of alkaloid were also detected in the methanolic extracts that proved to be richer in tannins and flavonoids (+++ve), whereas anthraquinones and coumarins were absent in both solvents (Table 1).
Since nearly all of the identified components from plants active against microorganism are aromatic or saturated compounds, they are most often obtained through initial ethanolic or methanolic extraction (Nostro *et al.,* 2000). Although water is almost universally the practical solvent used to extract active compounds, the current results revealed higher existence of different ingredients in methanol.
Lots of evidence is indicating that the interaction between various phytochemicals, especially phenolic and flavonoid compounds, is involved in reducing the risk of various degenerative diseases (Ness and Powles, 1997; Riboli and Norat, 2003). Flavonoids are found in many plants and can be used to treat atherosclerosis and hypertension. A prominent property of flavonoids is their antioxidant capacity which could afford some

protection against oxidative stress. The antioxidant activity of flavonoids and other polyphenols content appear to play a major role, due to their ability of scavenging reactive oxygen and nitrogen species (Frei and Higdon, 2003). In this study, the total polyphenols content of the methanolic extract of *P.Guava* leaves was 66.21 mg/g while that of water extract was 64.97 mg/g.

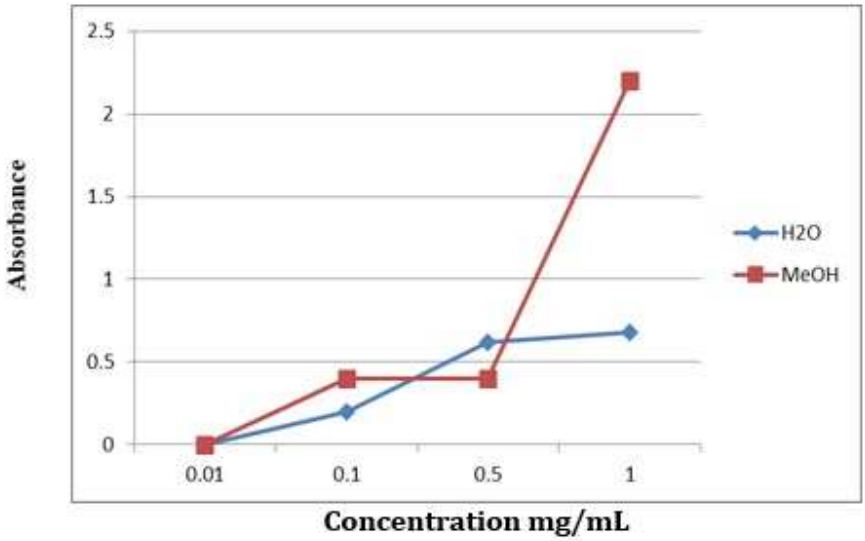

Figure 1. Reducing ability of aqueous and methanolic extracts of *P. guajava leaves*

Figure 1 shows the level of reducing power of the *P. guajava* extracts, as a function of their concentrations. The reducing power of methanol and water extracts were found to be very potent and increased with increasing concentration of reducers (Duh *et al.,* 1999), in which the highest reducing power was obtained at the highest concentration tested (1 mg/ml). The reducing power capacity for water extract was noticeably less than that of recorded for methanolic extracts especially at highest concentration (1 mg/ml).

The co-linearity between the total polyphenols and reducing power activity suggests that polyphenols represent the majority of reducing power or in other words antioxidant activity of leaves of *Psidium guajava.* This indicates the antioxidant potential of *P. guajava. L.*

Antibacterial activity of *P. guava* leaves extract

In this study, crude methanol, flavonoids and water extracts were found to be effective in inhibiting the growth of all the tested bacteria giving a range of 12-25 mm inhibition zone diameter (Table 2). The maximum inhibitory effect of flavonoid extract was observed on *Bacillus subtilis and Esch, erichia coli*. Meanwhile, it was moderate against *Salmonella typhi and Methicillin-resistant Staphylococcus aureus* (MRSA). Crude methanol extract exhibited strong antibacterial effect against *E. coli*, and moderate antibacterial effect against *S. aureus* and *B. subtilis*, with weak effect against *S. typhi*. Crude water extract demonstrated minimum inhibitory effect on all tested bacteria.

According to MIC results (Table 3), flavonoids extract was the most effective one against bacteria (with MICs of 2.5-5 mg/ml), followed by methanolic extracts (with MIC of 5.5-11 mg/ml). The water was found to be the least effective with MIC (12-15 mg/ml).

The antibacterial activity of *P. guajava leaves* extracts can be related to their content of flavonoids and phenols, which have been found as effective antimicrobial substances against a wide array of microorganism's *in vitro* (Srinivasan *et al.,* 2001). Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Tsuchiya *et al.,* 1996).

More investigation on antimicrobial properties of flavonoids extracts was carried out using bioautography method (Nostro *et al.,* 2000) to specify the biologically active separated spots (against *B. sub*) on thin layer chromatograms from flavonoids extracts (Table 4). Thin Layer-Bioautographic application showed the presence of five biologically active spots (S1-S5) with Rf values of 0.46-0.8. These results could be a preliminary orientation concerning how many possible bioactive plant components need separation and identification.

Table 2. *In vitro* antibacterial activities of the extracts of *Psidium guava leaves*

Test bacteria	Aqueous	Methanolic	Flavonoids	Tetracycline (30 µg)
B. subtilis	+ve	+ +ve	+ ++ve	+ +ve
S. aureus	+ve	+ +ve	+ +ve	+ ++ve
E. coli	+ve	+ ++ve	+ ++ve	+ ++ve
S. Typhi	+ve	+ve	+ +ve	+ +ve

DIZ, diameter of inhibition zone (NB.paper disc =5 mm)
+ve (8-12 mm); ++ve (13-17 mm); +++ve (18-25 mm)

Table 3. Minimum Inhibitory Concentration (MIC) of *Psidium Guava* leaves aqueous, methanolic and of flavonoids extracts against *B. subtilis*, S. *aureus, E. col* and *S. Typhii* Patient examination and clinical competences of clinical officer interns

Test	Water crude extract	Methanol crude extract	Flavonoid extract organisms
B. subtilis	14	11	2.5
S. aureus	15	9	5
E. coli	10	5.5	3
S. Typhi	12	10	5

Values represent an average of three determinations

Table 4. Bioautographic profile (with Rf values) of Flavonoid extract of *P.guava L.*

Spot n	S1	S2	S3	S4	S5
R_f	0.46	0.54	0.61	0.68	0.8

All spots separated were active against the most sensitive test bacterium (*Bacillus subtilis*). Best separation was accomplished using, Benzene: Methanol: acetic acid: (8:2:1 v/v)

Conclusions

From the obtained results in this investigation, it can be concluded that Guava leaves possess strong antimicrobial property against the gram-negative and gram-positive organisms. Phytochemical analysis showed that Guava leaves are rich in wide range of polyphenolic compounds (flavonoids and tannins). As the polyphenolic compounds possess the antimicrobial property, it may be the most probable cause of antibacterial property of guava leave. Another prominent property of flavonoids is their antioxidant capacity, which could

afford some protection against oxidative stress. The antioxidant activity of flavonoids and other polyphenols content appear to play a major role, due to their ability of scavenging reactive oxygen and nitrogen species. These results suggest a possible use of *P.guava* L. as a source of natural antioxidant and antimicrobial agents. However, further studies are required to disclose their detail mechanism of action.

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