Rocz Panstw Zakl Hig 2022;73(1):65-77
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0199

ORIGINAL ARTICLE

CHEMICAL COMPOSITION, ANTIOXIDANTS AND ANTIMICROBIAL ACTIVITIES OF MOROCCAN SPECIES OF *PSIDIUM GUAJAVA* EXTRACTS

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ABSTRACT

Background. During the recent years, appropriate attention has been paid to the oxidative stress which damages the body's cells, proteins, and DNA. Therefore, the need of antioxidants becomes a therapeutic and preventive priority. In addition, microbial infections also constitute a public health problem.

Objective. To find efficient, reliable and safe alternatives sources to synthetic antioxidants, antibiotics and antifungals drugs.

Materials and methods. Extract and essential oil of *Psidium guajava* were screened for their antioxidant and antimicrobial activities against gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), gram negative bacteria (*Citrobacter freundii, Escherichia coli* and *Pseudomonas sp*) and fungi (*Candida albicans,Candida tropicalis* and *Cryptococcus neoformans*), as well as to determine the functional groups of phytochemicals present in the essential oil by Fourier transform infrared spectroscopy (FTIR).

Results. The results indicate that *P. guajava* leaves extract demonstrated very high antioxidant activity and *P.guajava* essential oil showed the highest polyphenols content. The antioxidant capacity showed a significant negative linear correlation to total polyphenolic content (TPC) with *Pearson's* correlation coefficients. P. guajava essential oil shows high antibacterial and antifungal activity against all the studied bacteria and fungi.The FTIR analysis of *P. guajava* essential oil showed the presence of several functional groups (ethers, esters, ketones, terpenes, alkanes, aldehydes, aromatic hydrocarbons, alcohols, and phenols). The relationship between the chemical composition and antimicrobial activity of *P.guajava* essential oil suggests that the attribution of its antimicrobial activity to a particular compound or a synergistic effects between its different constituents remains difficult.

Conclusions. The present study demonstrated that *Psidium guajava* is a valuable source of active compounds with anti-oxidant and antimicrobial activities. This finding suggests the new use of the fruits and the leaves extracts of this plant in the treatment of bacterial and fungal infections, as well as for the extraction of new antioxidants. Therefore, it is necessary to be carried out in another study to identify the active(s) compound(s) in *P.guajava* essential oil with respect to their mechanisms and synergistic actions.

Key words: *antibacterial activity, antifungal activity, polyphenols, medicinal plants, essential oil*

INTRODUCTION

In recent years, there has been an increased interest in the exploitation of medicinal plants in the pharmaceutical, medicinal and agri-food industries for the search of new antibiotics and new antioxidant, this is mainly due to the fact that the medicinal products derived from these plants have been found to be safe for human health and have no side effects compared to chemical synthetic drugs [1].

Psidium guajava, commonly known as guava and belonging to the *Myrtaceae* family,native to Mexico and extends throughout the South America, European, Asia and Africa, has been reported to have several chemical and biological activities. An aqueous extract of guava leaves demonstrated antibacterial activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* [2] and antifungal activity against *Candida albicans* [3] and effects of the guajaverine from guava leaves on growth inhibition

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of *Streptococcus mutans*, a pathogen for dental caries, has been described. It's verified that Flavonoids such as quercetin have expressed significant antioxidant and antibacterial activity [2, 4]. In addition, the lycopene has been found to reduce the risk of cancer through these antioxidant effects.

The leaves of guava contain an essential oil rich in flavonoids, cineol, tannins, resin, eugenol, chlorophyll, malic acid, cellulose and a number of other active compounds [5]. Guava fruits have been reported to have antioxidant activity, contain vitamin C, iron calcium and phosphorus, β-caryophyllene, limonene, antioxidant compound (polyphenols, flavonoids, proanthocyanidins, triterpenes and other constituents), antioxidant dietary fiber [6, 7, 8, 9].

In the present study, the extracts and the essential oil from leaves and fruits of *Psidium guajava* were screened for antioxidant, antibacterial and antifungal activities against gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), gram negative bacteria (*Citrobacter freundii, Escherichia coli*and *Pseudomonas sp*) and fungi (*Candida albicans, Candida tropicalis* and *Cryptococcus neoformans*) as well as to determine the functional groups of the phytochemicals present in *Psidium guajava* essential oil.

MATERIAL AND METHODS

Plant material

The studied *Psidium guajava* (local name Guava) was collected in the region of El Jadida. This cultivated plant was not treated by pesticides. The leaves and the fruits were collected from the surrounding areas, identified, and authenticated by a taxonomist. The leaves and fruits were thoroughly rinsed using water treated and shade-dried over during 2-4 weeks at room temperature. The leaves oriented to the extraction of the essential oil are preserved in the whole state, the leaves and the dried fruits oriented to the preparation of the dichloromethane/ethanol extract have been crushed separately to obtain fine powder.

Plant extraction

The dried and powdered leaves and fruits (100 g) was macerated separately for 48 hours at room temperature in a mixture of two solvents, a polar solvent (Ethanol) and a non-polar solvent (Dichloromethane) with a proportion of 50%:50%. The mixture was filterd using Whatman filter. The filtrat was concentrated under low pressure at 40°C using a Rotary evaporator until the total elimination of the solvent and the dried crude extract is obtainedstored in a freezer at 4°C until further tests.Essential oils has been extracted by hydrodistillation technique using Clevenger apparatus. The dried aerial parts of *P.Guajava* (300 g) were hydrodistilled using a Clevenger-type apparatus to extract the essential oils during 4 h. The distilled essential oils has been recovered, filtered and stored at +4°C.

Test microorganisms

Five bacteria species and three fungi from Collection of the Pasteur Institute in Paris (CIP) and from American Type Culture Collection (ATCC) were used (Table 1).

Antimicrobial efficacy testing

The antimicrobial activity of different *P.guajava* extracts was studied using the disc diffusion method. The inoculums of bacteria and fungi were prepared from colonies in phase of exponential growth from the culture from 18 to 24 hours old on *Mueller-Hinton* agar for bacteria and *Sabouraud* agar for fungi.

The evaluation of the antibacterial and antifungal activity of all extracts were validated by the measure of the diameters of the zones of inhibition appearing around the disks in comparison with the standard antibiotics (Ampicillin 30 µg) or the standard antifungal (Econazole 30µg). Every test was realized in triplicate mean inhibition zone was computed.

Antioxidant activity testing

The antioxidant activity of *P.guajava* extracts was determined by a DPPH (diphenyl-1-picrylhydrazyl) assay. The percentage of DPPH inhibition was calculated using the following formula:

Table 1.Bacteria and yeasts used for antimicrobial activity testing

	Microorganisms	Gram	Reference	Origin
Bacteria	*Citrobacter freundii*	Gram-	ATCC8090	American Type Culture Collection
	Escherichia coli		CIP54127	Collection of the Pasteur Institute, Paris
	Pseudomonas sp		ATCC10145	American Type Culture Collection
	Enterococcus faecalis	Gram+	ATCC19433	American Type Culture Collection
	Staphylococcus aureus		CIP 209	Collection of the Pasteur Institute, Paris
Yeasts	Candida albicans		CIP 48.72	Collection of the Pasteur Institute, Paris
	Candida tropicalis R2		CIP1275.81	Collection of the Pasteur Institute, Paris
	Cryptococcus neoformans		CIP960	Collection of the Pasteur Institute, Paris

$$I\% = ((Ac-As)/Ac) \times 100$$

Where:
I%: percentage of DPPH inhibition; Ac: the negative control's absorbance; As: the sample's absorbance tested. The standard of the reaction is the butylatedhydroxytoluene (BHT).

All the tests were made in triplicates and the results were expressed as a mean of the three assays. Ethanolic solution of extract was prepared at concentrations from 0 to 5000 µg/ml. DPPH (0.04 g/l) was added to 0.5 ml of each solution. The negative control was prepared by adding 0.5 ml of methanol to 1.5 ml of the DPPH methanolic solution. Discolorations were measured by the spectrophotometer at 517 nm after incubation of the mixture for 30 min at room temperature in the dark. The absorbance of the positive control (BHT) was measured in the same conditions as well as the extracts. The percentage of DPPH inhibition (I%) was calculated and the IC_{50} values for all the samples were determined using «Origin®Pro8» software.

Polyphenols' content

The method is adapted by *Singleton* and *Rossi* (in 1965) with the reactive of *Folin-Ciocalteu* [10]. Briefly, 2.5 mL of*Folin-Ciocalteu* reagent (diluted10 times) was added to 0.5 mL of aqueous extract (diluted 200times). Sodium carbonate (Na_2CO_3) (75g/L) was added (what favours an alkaline environment to activate the redox reaction). The mixture was incubated in a water bath at a temperature of 50°C during 5 min. Then, the absorbance was measured at 760 nm by a spectrophotometer UV-3100 PC VWR.

The total polyphenols content was calculated from the calibration curve established with a solution of gallic acid (calibration range 0 - 80 µg/ml). The negative control of the reaction was a polyphenol content free. The determination was done in triplicates. The results were expressed by milligram of gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g dw).

FTIR analysis of P. guajava essential oil

To study the chemical composition of *P.guajava* essential oil, the essential oil was scanned in the wavelength range of 4000 - 400 cm^{-1} with a resolution of 2 cm^{-1} using an FTIR spectrometer of type JASCO 4000, equipped with a detector (TGS) and a ceramic source, separated by an optical system using a *Michelson* interferometer. The room was kept at a controlled ambient temperature (25 °C) and relative humidity (30%).

Precisely weighed, essential oil (2 µL) were coated on the KBr tablets to form thin liquid films for infrared spectrometry analysis.The background air spectrum, water vapor and CO_2 interference were subtracted from these spectra. After baseline correction and smoothing were performed using the OMNIC8.0 software, the spectrum data were imported in Unscrambler 9.7 software to standardize the normal variations. the characteristic peaks and their functional groups were detected. FTIR peak values were recorded. Each analysis was repeated three times for spectrum confirmation.

Statistical analysis

All the assays were performed in triplicate and the *Pearson's* correlation coefficient (r) statistics was used. The coefficient of determination (R2) between antioxidant activity and total polyphenolic content (TPC) was carried out using the regression analysis by Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Antioxidant activity

The results obtained are represented in Figure 1.

The antioxidant activity of the extracts and the essential oils of *P. guajava* expressed by IC_{50} (concentration of the extract necessary to reduce 50% of the radical DPPH) is for: *P. guajava* leaves 102 µg/ml, *P. guajava* fruits 966.77 µg/ml, *P. guajava* essential oil 2366.29 µg/ml, and standard antioxidant (BHT) 79.81 µg/ml. The results of the antioxidant characteristics of the different extracts of *P. guajava*, estimated by the DPPH scavenging activity gave the following classification: *P. guajava* leaves >*P. guajava* fruits > *P. guajava* essential oil.

In recent years, appropriate attention has been directed to natural antioxidants. Antioxidant-based drug formulations are used as therapeutic or preventive against several infections and diseases; they synthesize a wide range of secondary metabolic molecules that have antioxidant activities with therapeutic power. Phenolic compounds such as flavonoids, phenolic acids, coumarins, stilbenes and tannins are considered to be the most abundant plant antioxidants [11]. Polyphenols and any reducing compounds, even non-electroactive species, will contribute for the overall antioxidant power. Therefore, reducing sugars, polysaccharides, vitamin C may influence the results of antioxidant activity in plant material [12].

The reduction power is generally due to the existence of one or more hydroxyl functions carried by the benzene ring that exert an antioxidant action by donating a hydrogen atom to break the free radicals chain reaction or to prevent the formation of peroxide [13].

The analytical principle of DPPH radical scavenging assays is based on the conversion of former radical (DPPH°) to the reduced form(DPPH-H), which is observed by the discoloration effect (transition

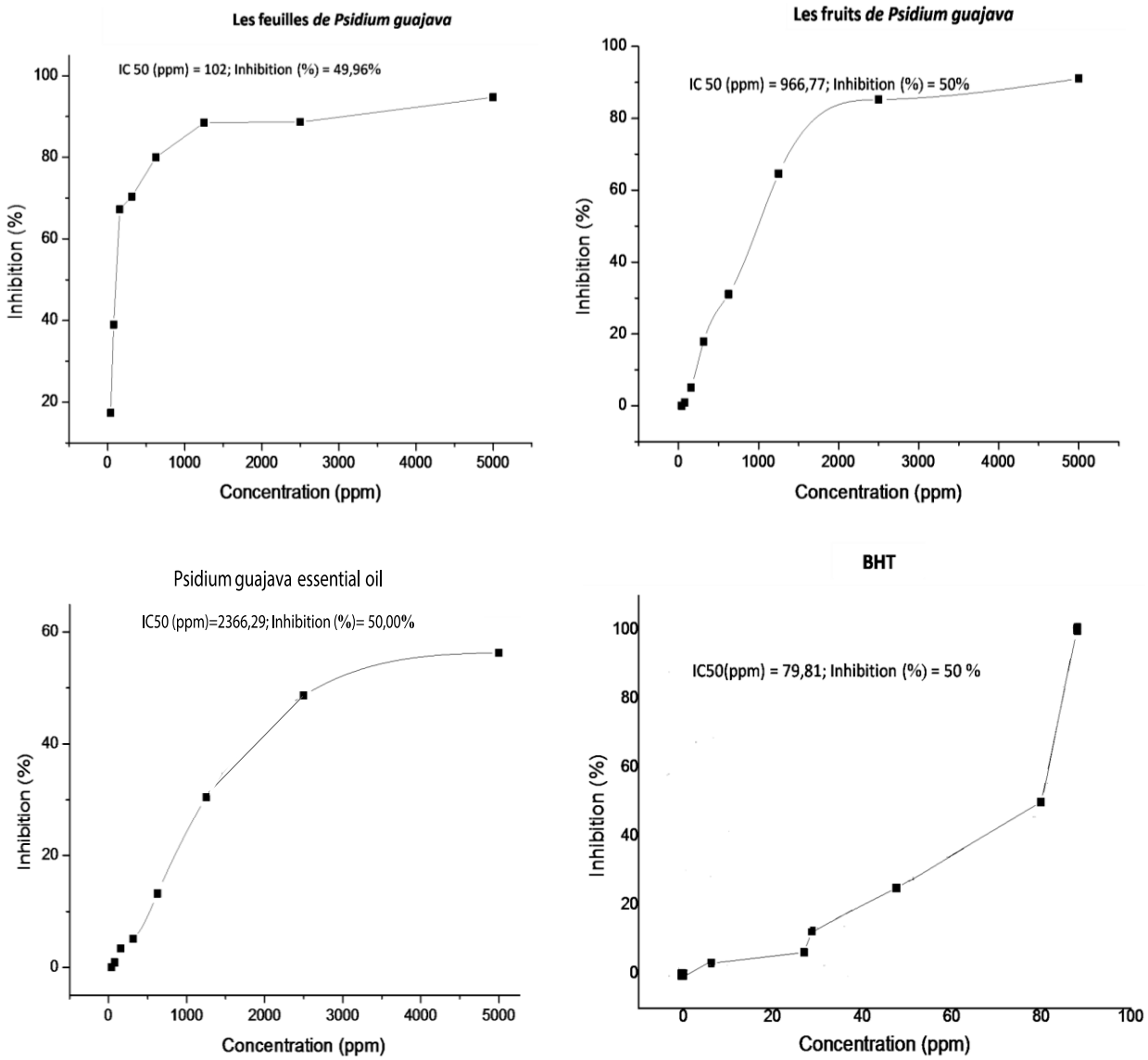

Figure 1. Inhibition percentage of different plant extracts based on extract concentration

from violet to yellow) measured spectrometrically at wavelength of 517 nm [14]. Thus, the lower is the IC_{50} value, stronger is the antioxidant activity.

P. *guajava* extracts, evaluated in this study, demonstrated variability in antioxidant characteristics. This is the first report for antioxidant capacity of *P. guajava* in Morocco, since is recently introduced in Morocco, it's native to the Caribbean and Central America. Thee extract of *P. guajava* leaves extract demonstrated very high antioxidant activity (IC_{50} =102 µg/ml) very close to that of BHT (IC_{50} =79.81 µg/ml), despite that this extract contains a low content of polyphenol (2.52±1.12 mg GAE/g dw). These results are in accord with high antioxidant activity (IC_{50} =100 µg/ml) reported previously for the aqueous extract of *P. guajava* leaves in South Korea [15]. This high antioxidant activity may be due to blockage of the chain reaction of linoleic acid [16] or free radical scavenging activity by quercetin, quercetin-3-o-glucopyranoside murine [4] and ferulic acid [17] or other antioxidants such as phenolic compounds like flavonoids, phenolic acids or carotenoids. However, the effectiveness of flavonoids as effective antioxidants depends on several factors such as environmental factors, which can even alter their effectiveness as antioxidants.

The dichloromethane/ethanolic extract of *P. guajava* fruits exhibited low to moderate activity by the DPPH, (IC_{50}=966.77 µg/ml), compared to that of BHT (IC50= 79.81 µg/ml) [18]. Several previous studies have obtained similar IC_{50} values[19,20]. In other study, *Ademiluyi* et al.[19] have shown that even if the IC_{50} value obtained was 92 0µg/ml, but this value has been interpreted as ted as signifying a high antioxidant activity due to the richness of this fruit in polyphenols [21], as shown by the results obtained with the total content of polyphenols of 18.09 ± 3.4 mg GAE/g dw, which is confirmed by the presence of Kaempferol, Quercetin, Schottenol ferulate and Esculin in *P.guajava* fruits extract [22].

Concerning *P.guajava* essential oil, the results obtained in our study indicate that it showed low antioxidant activity with a IC50 value of 2366.29 µg/ml, even if the polyphenols content is 45.67±2.88 mg GAE/g dw. Indeed, this result agrees favorably with previous reports suggests weak antioxidant activity of *P.guajava* essential oil (IC50 values between 18.52 - 33.72 mg/ml), this result can be explained by the absence of compounds as flavonoids, one of the main responsible compounds for the antioxidant activity of medicinal plants [23,24,25].

Polyphenols content

Phenolic compounds, such as catechins, quercetin, caffeic acid, chlorogenic acid, rutin, naringin and gallic acid, are the most important in the plant constituents known for their antioxidant power [26]. The total phenolic content (PC) data is presented in Table 2. Among all the tested extracts, the highest PC was observed in *P.guajava* essential oil 195.67±2.88 mg GAE/g dw and was the least in dichloromethane/ ethanol leaf extract 2.52±1.12 mg GAE/g dw.

Table 2. Total polyphenols contents in extracts from *P.guajava* (mg GAE/g dw)

Extracts	Total phenolic mg GAE/g dw
P. guajava leaves	2.52 ± 1.12
P.guajava fruits	18.09 ± 3.41
P.guajava essential oil	195.67±2.88

The comparison contents of total phenolic compounds in the three extracts of *P.guajava*, indicates the following order: *P.guajava* essential oil > *P.guajava* fruits extract > *P.guajava* leaves extract. This finding is in agreement with reported data from studies carried out by *Mahomoodally* et al., which showed similar result (209.16 ± 6.15 mg GAE/g) [27]. As regarding to *P.guajava* fruits extract its previously confirmed that its polyphenol content remains significant compared to other studies[18]. For *P.guajava* leaves extract with low polyphenolic content not exceeding 2.52 ± 1.12 mg GAE/g dw, even if this value is higher than that found in other study that is interpreted to be very rich in polyphenols [28]. Therefore, we can conclude that this extract is being rich in phenolic compounds as gallic acid, quercetin, protocatechuic acid, chlorogenic acid, caffeic acid, kaempferol and ferulic acid [28].

Correlation between phenolic compounds and antioxidant activity

The phenolic compounds were supposed to play an important role in the antioxidant activity. To reveal the correlation between total polyphenols content (TPC) and antioxidant activity (estimated by $1/IC_{50}$) is in Figure 2. This correlation showed a low determination coefficient ($R^2 = 0.371$, ($Y= -0.029x + 5.901$)). *Pearson's* correlation coefficient was applied to evaluate the relationship between antioxidant activity and total polyphenolic contents.

The antioxidant capacity showed a significant negative linear correlation to TPC with *Pearson's* correlation coefficients of r= -0.59. For example, the leaves extract of *P.guajava*, which had the lowest polyphenols content (2.52±1.12 mg GAE/g dw), showed the highest antioxidant activity (102 ppm). Indeed, several studies have demonstrated that there is no correlation between antioxidant activity and total polyphenols content [29]. This suggests that the relationship between polyphenols and antioxidant requires an explanation. Firstly, the free radical scavenging activity is not only affected by polyphenols concentrations, but also by the structure of the polyphenol compounds in the extract. Indeed, for

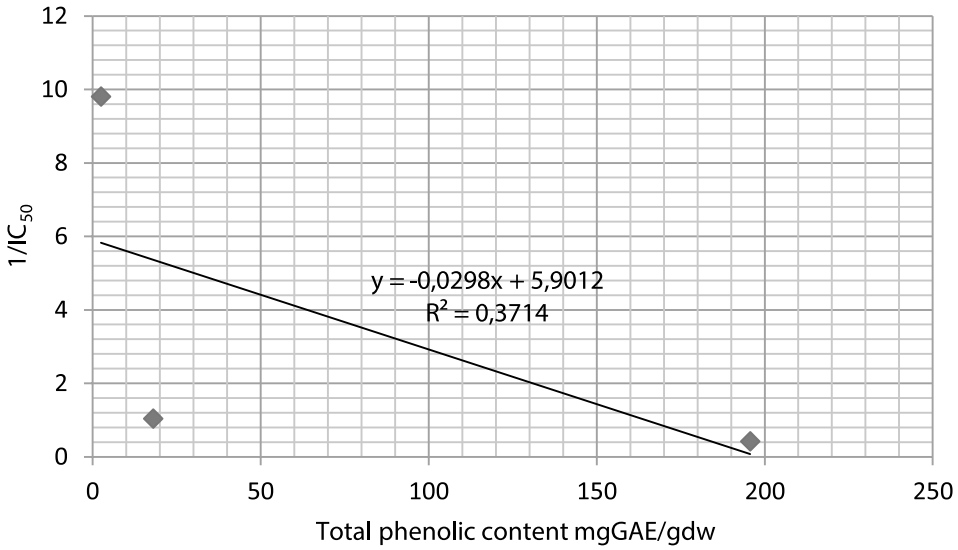

Figure 2. Correlation between total phenolic compounds and antioxidant activity ($1/IC_{50}$)

polyphenols that act *via* reactive species scavenger's pathway, their activity is affected by the positions and the numbers of phenolic hydroxyl groups in the structure of aromatic ring in phenols [30].

Also, the degree of stability conferred on the flavonoid phenoxyl radicals is the most effective radical scavengers, participant in electron delocalization [31]. The glycosylation of phenolic compounds can also decrease antioxidant activity. Moreover, the DPPH using in this study does not consider the effect of polyphenols others than free radical scavenging activity, via pathway of lipoxygenase inhibition or via reducing agents for metmyoglobin which requires others analysis method [32]. Others finding indicate that no correlations confirm that phenolic compounds are not the only contributor to the antioxidant activities of the medicinal plant extracts, several others non-phenolic antioxidants as nitrogen compounds, alkaloids, carotenoids, ascorbic acid, vitamin E and β-carotene may be responsible for the antioxidant activity [33, 34, 35]. Moreover, the antioxidant activity is the result of a synergetic effect between phenolic antioxidants and non-phenolic antioxidants [36].

Antibacterial activity

The antibacterial activity screening results presented in Table 3 show that *P.guajava* essential oil (PgEO) shows high antibacterial activity against all the studied bacteria, with maximum activity against *Enterococcus faecalis* and minimum activity against *Escherichia coli*. *P.guajava* leaves extract also shows antibacterial activity against all bacteria tested, but with an inhibition zone of average diameters, ranging from 8 to 11 mm. *P.guajava* fruit extract exhibit moderate antibacterial activity against *E. coli, Pseudomonas sp, E. faecalis* and *S. aureus*, with an inhibition zone of medium diameters between 8 mm and 12 mm.

The sensitivity of the bacteria according to their Gram to the *P.guajava* extracts studied shows that the antibacterial action of the three studied extracts of *P.guajava* is more pronounced on Gram positive bacteria compared to Gram negative bacteria, which are the most resistant. Indeed, Gam negative bacteria recorded lower inhibition diameters (between 8 mm and 20 mm) compared to Gram-positive bacteria, which showed higher inhibition zones reaching 24 mm (Figure 3).

The essential oil of *P. guajava* (PgEO), indicated strong antibacterial activity against *E.coli, C. freudii, S.aureus* and *E. faecalis* with an inhibition zone ranging from 11.67±2.08 mm to 24±3.61mm. *Hanif* et al. concluded that PgEO has moderate antibacterial potential against *E.coli* (15.0±0.8 mm), *S. aureus*

Table 3. Antibacterial activity of *P.guajava* extracts from leaves, fruits and essential oil (EO)

Extracts	Inhibition zone diameter (mm)				
	Gram negative bacteria			Gram positive bacteria	
	Escherichia coli	Pseudomonas sp	Citrobacter freundii	Enterococcus faecalis	Staphylococcus aureus
P. guajava (leaves)	8±1.00	11±2.00	8±1.00	11±1.73	11±2.65
P.guajava (fruits)	9±1.73	12±0.58	-	9±2.00	8±0.00
P.guajava (EO)	11.67±1.5	19±1.7	20±0.00	24±0.58	14.33±1.7
Ampicillin 30 µg	27	25	25	29	24

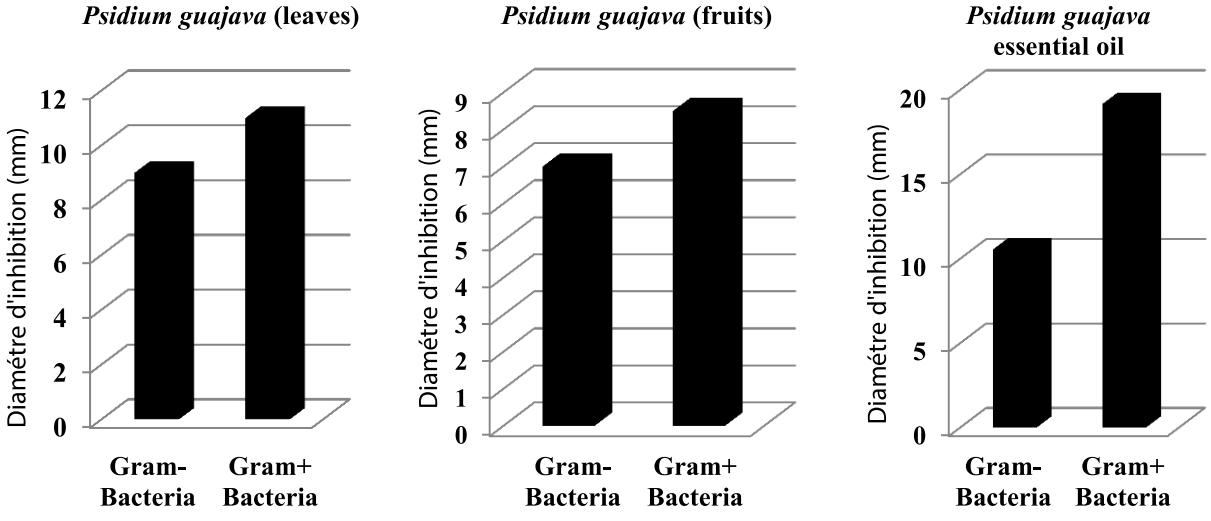

Figure 3. Sensitivity of the bacteria according to their Gram to *P.guajava* extracts

(9.0±0.5mm), and *S. pyrogenes* (11.0±0.6 mm) [37]. *Weli* et al. obtained smaller inhibition diameters, not exceeding 13 mm and concluded that PgEO was characterized by significant antibacterial activity[38].

Concerning to dichloroethanoic extract of *P.guajava* leaves, it should be noted that the results obtained are very similar to those found by *Biswas* et al. [48] when the ethanolic extract showed an inhibition zone of 6.11 mm and 11 mm against *S. aureus* and *B. cereus*, respectively. Indeed, several other studies confirm the antibacterial effect of *P. guajava* extracts, methanolic and ethanolic extracts showed an inhibitory activity against Gram-negative bacteria, known by their resistance as *E. coli* and *Pseudomonas* Sp, and also against Gram positive bacteria like *S. aureus* [39].

Antibacterial activity of *P.guajava* fruits extract was found to be less pronounced than previous extracts (inhibition zone 8-12 mm). Another evaluation of ethanolic extract found very close results, with inhibition zones ranging from 7 to 13 mm [40].

Antifungal activity

The screening of the antifungal activity of *P.guajava* extracts (Table 4) indicates that *P.guajava* fruits extract showed a moderate antifungal activity against all yeasts tested, with an inhibition diameter between 11 mm and 12 mm compared to the standard antifungal used Econazole 30 µg which showed an inhibition diameter between 20 mm and 22 mm. The results obtained for this extract are very similar to those found by *Malaviya* et al., for the alcoholic extract and the aqueous extract of *P. guajava* fruits extract against *Candida albicans* [47] or those obtained by *Panedy* et al., for the methanolic extract, ethanolic and ethyl acetate extract against *Microsporum canis*, *Tripchopythonrubrum*, *Aspergillus niger* and *Candida albicans* [48].

P.guajava essential oil is active against all tested fungi with inhibition diameter between 9 cm and 16 cm. The maximum activity was observed against *Candida albicans* (d=16 mm). Close to that of Econazole, it showed the strongest antifungal activity against *Candida tropicalis* (d=14 cm)known by strong resistance to antifungal drugs.In agreement with numerous studies, which confirmed the antifungal power of *P.guajava* essential oil against *Candida* strains and phytopathogenic fungi (*Curvularia lunata* and *Fusarium chlamydosporum*) [49]. This activity can come to the action of secondary metabolites such as phenolic compounds like ellagic [50]. This antifungal activity obtained in our study remains important given the pathogenicity of the tested yeasts, since they are responsible for several infections and diseases [51,52].

Table 4. Antifungal activity screening of *P.guajava* extracts

Extracts	Inhibition zone diameter (mm)		
	Candida albicans	Candida tropicalis	Cryptococcus neoformans
Psidium guajava (fruits)	12±1.00	11±2,.64	11±0.5
Psidium guajava (leaves)	12±1.5	-	10±1.16
*Psidium guajava (*EO*)*	16±1.73	14±1.04	9±0.76
Econazole 30 µg	20	21	22

On the other hand, the fact that Gram negative bacteria are more resistant than Gram positive bacteria is confirmed by previous results showing greater antibacterial activity of herbal extracts against Grampositive bacteria compared to Gram negative bacteria [41]. This observation can be explained by the difference in bacterial membrane structure between Gram positive bacteria and Gram negative bacteria, the efflux pump system of Gram-negative bacteria that can serve as a mediator for such a difference and also the periplasmatic space of Gram-negative bacteria that can contain enzymes capable of breaking down foreign molecules introduced into the bacterial cell from the outside [42]. This bacterial resistance is caused by the impermeability of the lipopolysaccharide membrane of the bacterium, in the presence of active compounds of *P.guajava*, especially tannins, which have the effect of limiting the multiplication of *S.aureus* by inhibiting the phosphorylation of bacteria to form its cell wall during bacterial multiplication [43]. Also, some bioactive compounds such as: saponins, flavonoids, tannins, alkaloids, phenols and phytosterols, effective against several strains of pathogenic bacteria, may have a protein degradation effect against bacterial proteins [44, 45, 46].

The leaf extract of *P.guajava* is active against *C. albicans* (d=12 mm) and *C. neoformans* (d=10 mm), but inactive against *C. tropicalis*, which is considered a resistant yeast.As shown by several previous researches, which confirmed the antifungal effect of this plant against *C. albicans* and also against *C. krusei*, *C. glabrata* and *M. canis* [53]. In this fact, *P. guajava* essential oil, the only strongly active extract against *Candida tropicalis*, which seems very interesting for the development of anti-*Candida tropicalis* bio-antifungals.

FTIR analysis of *P. guajava* essential oil

The infrared spectrum of *P. guajava* essential oil (Figure 4) shows the following bands: the very broad absorption band observed around 3422 cm^{-1} may be due to the presence of bonded O–H stretching of acids, with another very strong absorption band appearing in the region 1065 cm^{-1} due to C–O stretching vibration. The combination of these two bands indicates the presence of alcohols as linalool, cadinol, santalol, pogostol, muurolol, viridiflorol, spathulenol, cubebool, guaiol, nerolidol [38, 54, 55] and phenols such as durohydroquinone, chavibetol, thymol and 2,5-diethylphenol [55].

Two other bands found at 1454 cm^{-1} and 3076 cm^{-1} associated with C=C and =C-H of aromatic hydrocarbons such as calacorene, calamene, eugenol acetate, phenylethyl butyrate, o-cymene, benzyl benzoate and safrole [55, 56].

In the region between 1705 $cm^{-1}$and 1725 cm^{-1}, A medium intense absorption bandexisted at 1712 cm-1 associated with C=O ketones groupement, which significant the presence of ketones in the essential oil especially the tagetone [57]**.**

The very low absorption band appearing in the region 1634 cm^{-1} is due to C=C stretching vibration of the alkenes group, confirmed by another absorption band of =C-H groupement beyond 3000 cm^{-1}, shows the presence ofterpenes already confirmed in the essential oil of *Psidium guajava*, this includes thuyere, myrcene, limonene, ocimene, copaene, aryphyllene, humuene, amorphene, seychellene, viridiflorene, aromadendene, bisabolene, caryophyllene [38, 54, 55].

The relationship between the chemical composition and biological or chemical activities of *P. guajava* essential oil is confirmed by several researches. Indeed, antimicrobial activities of 1,8-cineole has been demonstrated against *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. faecalis* and *C. albicans* [59].On the other hand, the most abundant esters in the essential oils as bornyl acetate, geranyl acetate, *α*-terpenyl acetate, iso-bornylformateare responsible to the antibacterial effect [60, 61, 62, 63]. However, sabinyl acetate and vinyl crotonate showed a low to moderate antimicrobial activity [64, 65, 66]. Tagetone presented antifungal activities *in vitro* against *Candida lipolityca, Candida parapsilosis, Trichosporon asahii* and *Sphaceloma ampelinum* [67].

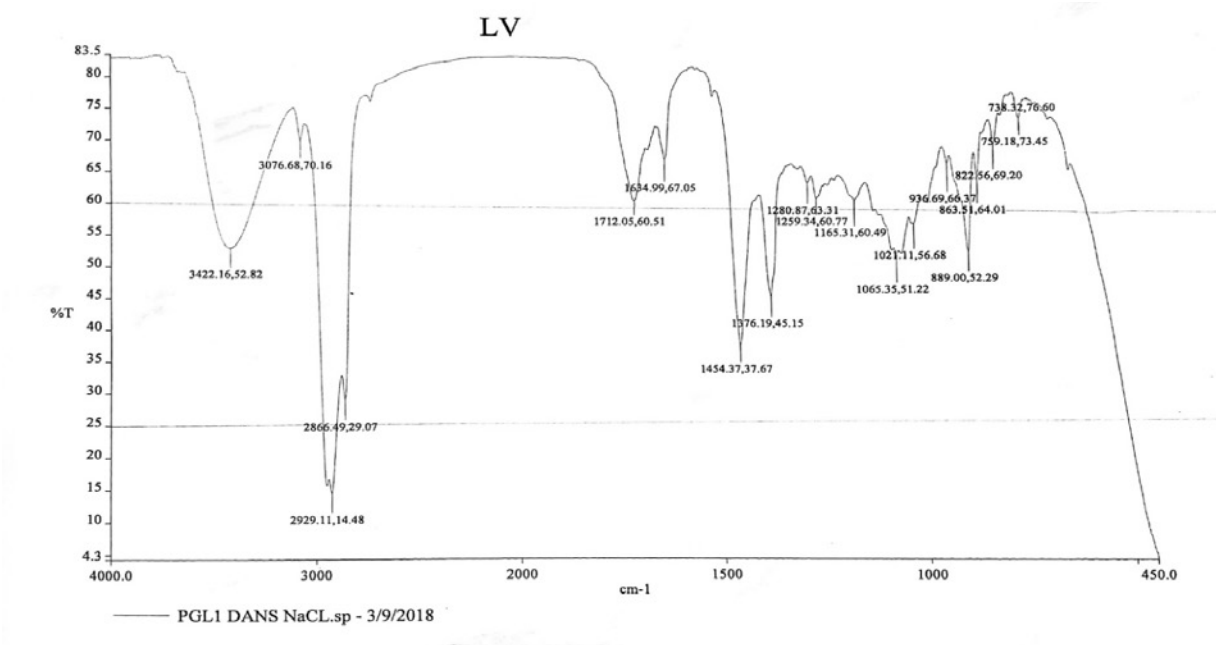

Figure 4. Infrared spectrum of *P.guajava* essential oil

Around the 3000 cm^{-1} region, there are two intense bands of 2929 cm^{-1} and 2866 cm^{-1}, which can be associated with the O=C-H aldehydes grouping, especially the nerol, citronellal, methanal phenylated, benzaldehyde and farnesol [38, 55, 56, 57] and also with the C-H alkanes, such as heptadecane, pristane, octadecane, phytane, phenosane and octacosan[58].

Three bands exist at 1021 cm^{-1}, 1259 cm-1 and 1280 cm^{-1}, possibly related to the C-O ether grouping, such as 1,8-cineol and the C-C esters grouping, including citronellyl acetate, bornyl acetate, geranyl butyrate, terphenyl acetate, dihydrocarveol acetate, isopulyl acetate, isobornylformate, sabinyl acetate and vinyl crotonate [54, 55]**.**

As regarding the terpenes in .*guajava* essential oil, previous studies have shown that β-Myrcene, β-Caryophyllene, α-Humulene, Germacrene, D-Limonene, β-ocimene and viridiflorene had a positive relationship with the antimicrobial activity

[68, 69, 70, 71]. The must alkanes that have shown excellent antimicrobial activity are heneicosane, tetracosane, heptadecane and eicosane [72, 73]. In addition, aldehydes such as cis-citral, farnesol and citronellal, found in essential oil are effective against several bacteria and fungi [63,74,75]. Moreover, others finding confirmed the high antimicrobial activity of aromatic hydrocarbon, especially Eugenol acetate, calamenene, phenylethyl butyrate, o-cymene and safrole showed higher antibacterial and antifungal activities [23, 63, 76, 77, 78, 79], these compounds may act alone or in combination with other compounds as *β*-caryophyllene, thioamide drugs, citral and carvacrol by a synergistic interactions [23, 63, 76, 77]. For alcohols and phenols, they reported the fungicidal and the bacterial effects of linalool, τ-cadinol, *cis*-α-santalol, pogostol muurolol, viridiflorol, spathulenol, trans-nerolidol, cubebol, terpineol against bacteria and fongi such as *E. faecalis, S. aureus*, *E. coli, E. faecalis, C.neoformans* and *candida sp* [80, 81, 82, 83]. Finally, durohydroquinone, chavibetol and thymol are the main phenols in *P. guajava* essential oil that have shown high antibacterial and antifungal properties [84, 85, 86].

CONCLUSIONS

The present study demonstrated that *Psidium guajava* is a valuable source of active compounds with antioxidant and antimicrobial activities. This finding suggests the new use of the fruits and the leaves extracts of this plant in the treatment of bacterial and fungal infections, as well as for the extraction of new antioxidants. Therefore, it is necessary to be carried out in another study to identify the active (s) compound(s) in *P.guajava* essential oil with respect to their mechanisms and synergistic actions.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Sara Moujabbir and Abdelghani Aboukhalaf for technical assistance.

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Received: 15.10.2021
Accepted: 05.02.2022
Published online first: 10.02.2022