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Antibacterial Activity Evaluation of *Psidium guajava* L. (Myrtaceae) Crude Extracts Against Selected Bacterial Pathogens

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Abstract. Methanol, ethanol, acetone, ethyl acetate and hot water extracts of different parts (leaves, seeds, fruits and twigs fractions) of *Psidium guajava* L. were investigated for their antibacterial activities against 8 bacterial isolates. Inhibitory effects of *P. guajava* L. extracts have been screened by disc-diffusion method (Zone of inhibition, ZI), activity index (AI) and minimum inhibitory concentration (MIC) determination. Ciprofloxacin antibiotic was used as standard for *P. guajava L*. antimicrobial activity comparison. From the ZI, AI and MIC values, methanolic and hot water extracts of twigs < 1cm diameter were the most potent against all tested micro-organisms by showing the highest ZI and AI value and the lowest MIC values. Whereas, no inhibitory activity was recorded for both seeds and fruits extracts using all tested solvents. These observations make this plant a potential source that can be used in management of bacterial infections. Moreover, methanolic and hot water extracts of twigs < 1cm required further in depth study.

Keywords: *Psidium guajava*, antibacterial activity, minimum inhibitory concentration (MIC)

Introduction

The genus *Psidium* belongs to the family Myrtaceae, which is considered to have originated in tropical South America. Guava crops are grown in tropical and subtropical areas of the world especially Thailand, Pakistan, Egypt, Hawaii, Florida, Palestine, Syria and India, and others. The genus *Psidium* comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits (Mani *et al*., 2011). The most commonly cultivated species of *Psidium* is *P. guajava* L. with common name "Guava".

Psidium guajava L. (Guava) is a perennial fruit tree that offers a great economic potential. Guava is one of the few medicinal plants which has been extensively investigated in terms of pharmacological activity of its phytochemical components (Farhana *et al*., 2017; Gitika1 and Kumar, 2016; Saleh *et al*., 2015; Mailoa *et al*., 2014; Taura *et al*., 2014; Ofodile *et al*., 2013; Biswas *et al*., 2013; Pandey and Shweta, 2012; Esimone *et al*., 2012; Elekwa *et al*., 2009; Nwinyi *et al*., 2008).

Guava is rich in antioxidants compounds, tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins (A, B, C & G), fibre and fatty acids. The biology of these compounds indicated the great potent of their antibacterial activities. Guava fruit is higher in potassium, copper and manganese and about four times the amount of vitamin C as an orange (Hassimotto *et al*., 2005). Guava fruits are also a good source of pectin - a dietary fibre. The wood is hard and tough, used as posts for rural house buildings. In the Philippines, Syria, India, Pakistan, Nigeria and many other countries, guava fruit is freely eaten for its good taste and nutritional benefits.

The leaves and bark of *P. guajava* L. tree have a long history of medicinal uses that are still employed today (Nwinyi *et al*., 2008). Leaves and shoot of *P. guajava* exhibited their antibacterial activity against the both Gram-positive and Gram-negative bacteria such as *S. aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Proteus vulgaris*, *Shigella dysenteriae* and *E. coli* (Rattanachaikunsopon and Phumkhachorn, 2010). Moreover, Shruthi *et al*. (2013) reported the significant antibacterial activity of *P. guajava* L. and also its antifungal and antimalarial actions.

Mailoa *et al*. (2014) reported that flavonoids and tannins, out of the phenolic components, were present in majority in *P. guajava* L. leaves extracts and these compounds act as antimicrobial agent. It has achieved a very long history of traditional use for treating a wide range of diseases (Elekwa *et al*., 2009; Nwinyi *et al*., 2008).

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The long history of guava's use has led modern-day researchers to study guava extracts. Thereby, the current study aimed to investigate the inhibitory effect of methanol, ethanol, acetone, ethyl acetate and hot water extracts of leaves, seeds, fruits and twigs fractions of *P. guajava* L. against some bacterial pathogens using antibiotic ciprofloxacin as a standard reference.

Materials and Methods

Preparation of plant materials. Plant materials of fresh leaves, fruits, seeds and twigs (< 1cm diameter and > 1cm diameter) of the *P. guajava* L. were harvested from Lattakia-Syria. Samples collection was carried out in Spring with an annual rainfall ranging from 650 to 700 mm. The twigs and leaves were dried under shade for one week, powdered by special electric mill and stored separately in polyethylene bags until needed for analysis.

Preparation of plant extracts. Different plant crude extracts were prepared by using different solvents such as methanol, ethanol, acetone, ethyl acetate and hot water. The extractions were performed in a Soxhlet apparatus successively as reported by Saleh *et al*. (2015). The extracts were dried using rotary evaporator at 40 °C under reduced pressure. All dried extracts were kept in air-tight bottles and stored at 4 °C. The concentration of extract was considered 100 mg/mL (stock solution).

Micro-organisms and growth conditions. Eight pure clinical bacterial isolates (*Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Salmonella typhimurium*, *Escherichia coli* O157, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were collected from the Microbiology and Immunology division, Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS) in Damascus - Syria.

The cultures were activated in trypticase soy broth (TSB, Difco, BD, Spars, MD) at 37 °C for 24 h. The medium was centrifuged (1000 x g/15 min/4 °C) after growth and the pellets were resuspended in sterile phosphate-buffered saline (PBS). Prior to antimicrobial sensitivity test, a bacterial suspension was obtained from overnight cultures. The turbidity of each bacterial suspension was adjusted equivalent to a no. 0.5 McFarland standard and then inoculated on Mueller-Hinton agar (Oxoid, UK). The bacterial cultures standardized to approximately 10^6 CFU/mL (Saleh *et al*., 2015). The exact counts were assessed retrospectively by viable counts on trypticase soy agar plates (TSA, Difco, BD, Spars, MD) at 37 °C for 18 h.

Antibacterial activity evaluation. *The disc-diffusion method.* Ciprofloxacin (100 mg/mL) antibiotic was used as standard for antimicrobial activity of bacteria. Filter paper discs (Whatman no.1) of 6 mm diameter were prepared and sterilized. The discs impregnated with 100 µL of extract dilutions (100 mg/mL) were applied over each of the culture plates previously seeded with the 10^6 CFU/mL cultures of bacteria. Bacterial cultures were then incubated at 37 °C for 18 h, while the paper discs impregnated with 20 µL of extracts on each disc or solution of 10 mg/mL of ciprofloxacin were used as standard antimicrobials for comparison. Negative control was prepared using methanol or acetone (the highest concentration of solvent in the plant extract was tested). Diameter of inhibition zone (ZI in mm) was measured after incubation at 37 ºC for 18-24 h. For each extract, duplicate trials were conducted against each microorganism.

Activity index (A.I). Activity index (A.I) of *P. guajava* L. plant extracts was calculated as previously reported using the following formulae (Egharevba *et al*., 2010): Activity index (A.I) = Inhibition zone of sample / Inhibition zone of standard.

Minimum inhibitory concentration (MIC). Stock solutions of the above mentioned antibiotic was prepared according to manufacture. Determination of MIC by the microdilution broth method was carried out according to NCCLS approved standards. Microdilution broth susceptibility assay was used (Saleh *et al*., 2015). Three replicates of serial dilutions of extract (100 mg/mL) or of antibiotic (128 µg/mL) were prepared in TSB medium in 96-well microtiter plates. One hundred microliters of freshly grown cultures containing 10^6 CFU/mL in TSB were added to each well. Positive control was achieved with the same conditions but without extract or antibiotics, negative control was also made with the same conditions but without adding the bacteria. The MIC was defined as the lowest concentration of each antimicrobial agent that inhibited visible growth of the tested isolate was recorded and interpreted as the MIC.

Statistical analysis. Results were expressed as mean of three replicates/treatment. Data were subjected to one way analysis of variance (ANOVA) for determining level of differences among variables. Differences between means were tested for significance by Fisher's Least Significant Difference (PLSD) test. The means were separated by using the student's t-test with $P \leq 0.05$ was considered to be significant.

Results and Discussion

Zone of inhibitions (ZI). Inhibitory effect (ZI) of extracts of leaves (LE), seeds (SE), fruits (FE) and twigs (TE) of *P. guajava* L. against 8 bacterial isolates are presented Table 1. Analysis of variance using PLSD test at $P \leq 0.05$ revealed statistically significant differences in ZI against all tested bacterial isolates among all examined solvents.

Inhibitory effect of *P. guajava* extracts varied according to the plant fractions, bacterial isolates and the solvents. Methanol extract, among the different solvents used, was the most potent against the eight tested bacterial isolates in this investigation. The zone of inhibition (ZI) observed for this extract ranged between 9 – 18 mm. The highest ZI value was recorded to be 18 mm for LE and TE < 1 against *S. typhimurium* and LE against *P. mirabilis* isolate. Whereas, the lowest value was found to be 9 mm for TE > 1 against *K. pneumoniae*. The ethanol, acetone, ethyl acetate and hot water extracts were active against only *S. typhimurium* isolate (Table 1).

Previously, Abdelrahim *et al.* (2002) reported that *P. guajava* aqueous and methanolic bark extracts exhibited antibacterial activity.

In our case study, ethanolic *P. guajava* extracts were non active against all tested isolates except *S. typhimurium.* Whereas, This value was ranged between 19.4-21.9 mm against *L. monocytogenes* strain and between 10.7-15.3 mm against *S. aureus* strain with ethanolic *P. guajava* LE (Mahfuzul Hoque *et al*., 2007). While, this value was 25, 28, 26 and 25 mm with 10, 25, 50 and 100% methanolic *P. guajava* leaf extracts, respectively against *S. typhi* isolate (Ahmed and Yagoub, 2007).

Dhiman *et al*. (2011) reported antibacterial effect of methanolic *P. guajava* leaf extracts (1, 3, 5, 10 and 20 µg/mL) against 3 bacterial pathogens. Their study revealed that the highest ZI was recorded to be 10.6 mm against *E. coli*, 9.6 mm against *S. aureus* and 12.6 mm against *B. subtilis* at the highest concentration (20 µg/mL). in our case study this value was recorded to be 13, 15 and 16 mm with methanolic TE < 1cm and to be 11, 13 and 11 mm with methanolic TE > 1cm against *P. aeruginosa*, *S. aureus* and *E. coli* isolates, respectively, whereas, this value was 28.5, 20 and 22.5 mm against *P. aeruginosa*, *S. aureus* and *E. coli* isolates, respectively using methanolic stem *P. guajava* extracts (Pandey and Shweta, 2012).

In our case study, ZI was recorded to be 13, 16 and 15 mm against *S. aureus* isolate with methanolic TE >1 cm, LE and TE< 1cm extracts, respectively. Whereas it ranged between 5-15 mm (20 mg/mL extract) and between 6-20 mm (40 mg/mL extract) against 8 methicillin-resistant *S. aureus* (MRSA) isolates using *P. guajava* stem bark methanolic extracts (Esimone *et al*., 2012).

In the current study, this value was recorded to be 17 and 16 mm again*st B. cereus* and *S. aureus*, respectively using methanolic *P. guajava* LE extract. Whereas, ethanolic one had no activity against all tested isolates except *S. typhimurium* isolate. While, this value was 8.27 and 12.3mm with methanolic *P. guajava* leaf extracts and to be 6.11 and 11.0 mm with ehanolic ones against *B. cereus* and *S. aureus*, respectively (Biswas *et al*., 2013).

Moreover, Mailoa *et al*. (2014) reported the antimicrobial activity of extracted tannins (different concentrations) from *P. guajava* L. against 3 bacterial and 2 fungal pathogens. This investigation suggested that, antimicrobial activity increased as tannins concentration increased. In this respect, for bacteria ZI at 30% ranged between 12 mm (*E. coli*) and 14.5 mm (*P. aeruginosa*); while this value at the same tannins concentration varied between 9 mm (*C. albicans*) and 9.5 mm (*A. niger*) for fungal pathogens. Saleh *et al*. (2015) reported antimicrobial effects of methanol, ethanol, acetone, ethyl acetate and hot water (leaves and twigs fractions) *P. guajava* L. crude extracts against three bacterial and two fungal pathogens. they reported that hot water twigs < 1 cm diameter extract displayed the highest ZI of 20 mm with hot water twigs < 1 cm against *C. albicans* fungi. Whereas, for bacteria, the highest ZI was recorded to be 19 mm with methanolic LE against *Acinetobacter baumannii* bacterial isolate.

In the current investigation ZI value was recorded to be 12, 13 and 16 mm against *K. pneumoniae*, *P. aeruginosa* and *S. aureus* isolates, respectively using methanolic LE. Whereas, this value was 8, 10 and 10 mm with 480 µg/disc *P. guajava* leaves at 45 °C against *Klebsiella* spp*, Pseudomonas* spp and *Staphylococcus* spp, respectively (Taura *et al*., 2014). While, it was 21, 23, 20, 18, 22, 14 and 17 mm against *Bacillus subtilis, Micrococcus luteus*, *S. aureus*, *Streptococcus* sp., *E. coli*, *P. aeruginosa* and *S. typhimurium,* respectively using *P. guajava* L. leaves extracts (methanol, ethanol and aqueous extracts (Gitika1 and Kumar 2016). Whereas, it was 13, 16, 18, 19 and 20 mm with methanolic leaf extracts and to be 22, 15, 19, 23 and

Table 1. Antibacterial activity of the *P. guajava* L. extracts using disc-diffusion test

Solvent	Tested micro-organisms	Zone of inhibition (mm) LE	TE < 1 cm	TE > 1 cm	Control
Methanol	*S. aureus*	16 ± 0.2^{Aa}	15 ± 0.09^{Ba}	13 ± 0.07^{Ab}	24 ± 0.2
	L. monocytogenes	14 ± 0.19^{Bb}	16 ± 0.24^{Aa}	11 ± 0.09^{Ac}	19 ± 0.12
	B. cereus	17 ± 0.28^{Aa}	15 ± 0.19^{Bb}	12 ± 0.12^{Ac}	21 ± 0.07
	S. typhimurium	18 ± 0.0^{Aa}	18 ± 0.0^{Aa}	13 ± 0.0^{Ab}	34 ± 0.2
	E. coli O:157	17 ± 0.03^{Aa}	16 ± 0.1^{Aa}	11 ± 0.06^{Ab}	27 ± 0.15
	P. mirabilis	18 ± 0.17^{Aa}	14 ± 0.26^{Bb}	13 ± 0.24^{Ab}	19 ± 0.25
	P. aeruginosa	13 ± 0.08^{Bb}	13 ± 0.17^{Ba}	11 ± 0.13^{Ab}	15 ± 0.06
	K. pneumoniae	12 ± 0.11^{Bb}	13 ± 0.29^{Ba}	9 ± 0.08^{Bb}	18 ± 0.32
Ethanol	*S. aureus*	na	na	na	24 ± 0.2
	L. monocytogenes	na	na	na	19 ± 0.12
	B. cereus	na	na	na	21 ± 0.07
	S. typhimurium	11 ± 0.0^{Cb}	16 ± 0.0^{Aa}	9 ± 0.0^{Bc}	34 ± 0.2
	E. coli O:157	na	na	na	27 ± 0.15
	P. mirabilis	na	na	na	19 ± 0.25
	P. aeruginosa	na	na	na	15 ± 0.06
	K. pneumoniae	na	na	na	18 ± 0.32
Acetone	*S. aureus*	na	na	na	24 ± 0.2
	L. monocytogenes	na	na	na	19 ± 0.12
	B. cereus	na	na	na	21 ± 0.07
	S. typhimurium	12 ± 0.0^{Bb}	14 ± 0.0^{Ba}	11 ± 0.0^{Ab}	34 ± 0.2
	E. coli O:157	na	na	na	27 ± 0.15
	P. mirabilis	na	na	na	19 ± 0.25
	P. aeruginosa	na	na	na	15 ± 0.06
	K. pneumoniae	na	na	na	18 ± 0.32
Ethyl acetate	*S. aureus*	na	na	na	24 ± 0.2
	L. monocytogenes	na	na	na	19 ± 0.12
	B. cereus	na	na	na	21 ± 0.07
	S. typhimurium	17 ± 0.11^{Aa}	na	na	34 ± 0.2
	E. coli O:157	na	na	na	27 ± 0.15
	P. mirabilis	na	na	na	19 ± 0.25
	P. aeruginosa	na	na	na	15 ± 0.06
	K. pneumoniae	na	na	na	18 ± 0.32
Hot water	*S. aureus*	na	na	na	24 ± 0.2
	L. monocytogenes	na	na	na	19 ± 0.12
	B. cereus	na	na	na	21 ± 0.07
	S. typhimurium	13 ± 0.0^{Ba}	13 ± 0.0^{Ba}	12 ± 0.0^{Aa}	34 ± 0.2
	E. coli O:157	na	na	na	27 ± 0.15
	P. mirabilis	na	na	na	19 ± 0.25
	P. aeruginosa	na	na	na	15 ± 0.06
	K. pneumoniae	na	na	na	18 ± 0.32

LE= Leaf extracts; TE= Twig extracts; na= no activity; Figures sharing same capital letter (column) and lowercase letter (row) are not significantly different at P= 0.05 probability by Fisher's PLSD test. LSD 0.05 solvent 2.200, plant part 1.362.

18 mm with ethanolic leaf extracts against *P. aeruginosa*, *K. pneumonia*, *Streptococcus pneumonia*, *S. aureus* and *E. coli* pathogens, respectively, while, for bark once, it was recorded to be 20, 14, 13, 23 and 19 mm with methanolic bark extracts and to be 19, 18, 16, 18 and 19 mm with ethanolic bark extracts against *P. aeruginosa*, *K. pneumonia*, *Streptococcus pneumonia*, *S. aureus* and *E. coli* pathogens, respectively (Ifeanyichukwu *et al*., 2015).

Recently, Farhana *et al*. (2017) reported that this value was 12.67, 9.83, 13.5, 17 and 8.67 mm against *S. aureus, S. typhi*, *E. coli*, *B. cereus* and *Shigella sonnei* isolates, respectively using 100% fresh guava extracts.

Activity index (A.I). Activity index (A.I) was also estimated to investigate *P. guajava* L. inhibitory effect (Table 2). Data presented herein showed that this parameter varied according to plant fraction, examined solvent and tested microorganism (Table 2). Statistical variance analysis revealed that the effect of plant parts extracts expressed as A.I on all tested bacterial isolates among all examined solvents were significantly ($P \leq 0.05$) different (Table 2).

It was noticed that, ethanol, acetone, ethyl acetate and hot water were active against only *S. typhimurium* with A.I ranged between 0.3 -0.5 , 0.3 – 0.4, 0.5 and 0.4 for the previous tested solvents, respectively. Whereas, for

Table 2. Activity index (A.I) of the *P. guajava* L. extracts against tested isolates

Solvent	Tested micro-organisms	Activity index (A.I) LE	TE < 1 cm	TE > 1 cm
Methanol	*S. aureus*	0.7 ± 0.01^{Ca}	0.6 ± 0.01^{Db}	0.5 ± 0.01^{Ec}
	L. monocytogenes	0.7 ± 0.01^{Cb}	0.8 ± 0.01^{Ba}	0.6 ± 0.01^{Dc}
	B. cereus	0.8 ± 0.01^{Ba}	0.7 ± 0.01^{Cb}	0.6 ± 0.01^{Dc}
	S. typhimurium	0.5 ± 0.01^{Ea}	0.5 ± 0.01^{Eb}	0.4 ± 0.01^{Fb}
	E. coli O:157	0.6 ± 0.01^{Da}	0.5 ± 0.01^{Eb}	0.4 ± 0.01^{Fc}
	P. mirabilis	0.9 ± 0.02^{Aa}	0.7 ± 0.01^{Cb}	0.7 ± 0.01^{Cb}
	P. aeruginosa	0.9 ± 0.02^{Aa}	0.9 ± 0.02^{Aa}	0.7 ± 0.01^{Cb}
	K. pneumoniae	0.7 ± 0.01^{Ca}	0.7 ± 0.01^{Ca}	0.5 ± 0.01^{Eb}
Ethanol	*S. aureus*	-	-	-
	L. monocytogenes	-	-	-
	B. cereus	-	-	-
	S. typhimurium	0.3 ± 0.00^{Gb}	0.5 ± 0.01^{Ea}	0.3 ± 0.00^{Gb}
	E. coli O:157	-	-	--
	P. mirabilis	-	-	-
	P. aeruginosa	-	-	-
	K. pneumoniae	-	--	-
Acetone	*S. aureus*	-	--	--
	L. monocytogenes	-	--	--
	B. cereus	-	--	-
	S. typhimurium	0.4 ± 0.01^{Fa}	0.4 ± 0.01^{Fa}	0.3 ± 0.00^{Gb}
	E. coli O:157	-	-	-
	P. mirabilis	-	-	-
	P. aeruginosa	-	-	-
	K. pneumoniae	-	-	-
Ethyl acetate	*S. aureus*	-	-	-
	L. monocytogenes	-	-	-
	B. cereus	-	-	-
	S. typhimurium	0.5 ± 0.01^{Ea}	-	-
	E. coli O:157	-	-	-
	P. mirabilis	-	-	-
	P. aeruginosa	-	-	-
	K. pneumoniae	-	-	-
Hot water	*S. aureus*	-	-	-
	L. monocytogenes	-	-	-
	B. cereus	-	-	-
	S. typhimurium	0.4 ± 0.01^{Fa}	0.4 ± 0.01^{Fa}	0.4 ± 0.01^{Fa}
	E. coli O:157	-	-	-
	P. mirabilis	-	-	-
	P. aeruginosa	-	-	-
	K. pneumoniae	-	-	-

LE= Leaf extracts; TE= Twig extracts; no activity Figures sharing same capital letter (column) and lowercase letter (row) are not significantly different at P= 0.05 probability by Fisher's PLSD test. LSD 0.05 solvent 0.073, plant part 0.057.

methanol one, this value ranged between 0.4 - 0.9. In this respect the highest value was recorded to be 0.9 for both LE and TE < 1 against *P. aeruginosa* isolate and LE against *P. mirabilis*. Previously, Egharevba *et al.* (2010) reported lower values for this parameter (0, 0.63, 0.63 and 0) using 70% methanol, methanol, erythromycin and hexane *P. guajava* LE against *C. albicans.* Whereas, Saleh *et al*. (2015) reported that hot water TE <1 cm exhibited the highest A.I against *C. albicans* fungi (2). While, for bacterial isolates, LE ethyl acetate exhibited the highest A.I against *S. pneumoniae* (1.7), followed by LE hot water (1.6) and TE <1 cm (1.6) against the same isolate. Our data were in coherent with findings of Saleh *et al*. (2015), who

Table 3. Minimum inhibitory concentration (MIC) of the *P. guajava* L. extracts against tested isolates

	Minimum inhibitory concentration values (mg/mL)			
Solvent	Tested microorganisms	LE	TE < 1 cm	TE > 1 cm
Methanol	*S. aureus*	10.3 ± 0.58^{Da}	8.3 ± 0.58^{Fa}	16.7 ± 1.15^{Fa}
	L. monocytogenes	8.3 ± 0.58^{Da}	7.3 ± 0.58^{Fa}	12.5 ± 2.52^{Ga}
	B. cereus	10.3 ± 0.58^{Da}	5.0 ± 0.00^{Fa}	16.7 ± 1.15^{Fa}
	S. typhimurium	5.0 ± 0.00^{Db}	5.0 ± 0.00^{Fb}	33.3 ± 0.58^{Fa}
	E. coli O:157	7.3 ± 0.58^{Db}	7.3 ± 0.58^{Fb}	41.7 ± 1.53^{Ea}
	P. mirabilis	5.0 ± 0.00^{Db}	5.0 ± 0.00^{Fb}	29.3 ± 0.58^{Fa}
	P. aeruginosa	8.3 ± 0.58^{Db}	8.3 ± 0.58^{Fb}	20.7 ± 1.15^{Fa}
	K. pneumoniae	14.3 ± 3.06^{Db}	9.3 ± 0.58^{Fb}	29.3 ± 0.58^{Fa}
Ethanol	*S. aureus*	16.7 ± 1.15^{Db}	7.3 ± 0.58^{Fb}	33.3 ± 0.58^{Fa}
	L. monocytogenes	16.7 ± 1.15^{Da}	5.0 ± 0.00^{Fb}	25.0 ± 0.00^{Fa}
	B. cereus	20.7 ± 1.52^{Db}	4.0 ± 0.00^{Fc}	33.3 ± 0.58^{Fa}
	S. typhimurium	20.7 ± 1.52^{Db}	7.3 ± 0.58^{Fc}	58.3 ± 3.06^{Ea}
	E. coli O:157	25.0 ± 0.00^{Cb}	8.3 ± 0.58^{Fc}	50.0 ± 0.00^{Ea}
	P. mirabilis	20.7 ± 1.52^{Db}	10.3 ± 0.58^{Fb}	58.3 ± 3.06^{Ea}
	P. aeruginosa	20.7 ± 1.52^{Db}	10.3 ± 0.58^{Fb}	50.0 ± 0.00^{Ea}
	K. pneumoniae	29.3 ± 0.58^{Ca}	14.3 ± 3.06^{Fb}	41.7 ± 1.53^{Ea}
Acetone	*S. aureus*	29.3 ± 0.58^{Ca}	8.3 ± 0.58^{Fb}	20.7 ± 1.15^{Fa}
	L. monocytogenes	14.3 ± 3.06^{Da}	7.3 ± 0.58^{Fa}	14.3 ± 3.06^{Ga}
	B. cereus	12.3 ± 0.58^{Da}	8.3 ± 0.58^{Fa}	10.3 ± 0.58^{Ga}
	S. typhimurium	10.3 ± 0.58^{Db}	8.3 ± 0.58^{Fb}	29.3 ± 0.58^{Fa}
	E. coli O:157	14.3 ± 3.06^{Db}	7.3 ± 0.58^{Fb}	41.7 ± 1.53^{Ea}
	P. mirabilis	10.3 ± 0.58^{Db}	10.3 ± 0.58^{Fb}	33.3 ± 0.58^{Fa}
	P. aeruginosa	8.3 ± 0.58^{Db}	10.3 ± 0.58^{Fb}	33.3 ± 0.58^{Fa}
	K. pneumoniae	10.3 ± 0.58^{Db}	8.3 ± 0.58^{Fb}	41.7 ± 1.53^{Ea}
Ethyl acetate	*S. aureus*	41.7 ± 1.53^{Ca}	37.3 ± 3.06^{Eb}	58.3 ± 3.06^{Ea}
	L. monocytogenes	66.7 ± 5.8^{Ba}	54.3 ± 1.15^{Db}	50.0 ± 0.00^{Eb}
	B. cereus	116.7 ± 15.28^{Aa}	66.7 ± 5.8^{Dc}	83.3 ± 2.89^{Db}
	S. typhimurium	83.3 ± 2.89^{Bc}	116.7 ± 15.28^{Cb}	233.3 ± 15.28^{Ba}
	E. coli O:157	100.0 ± 0.00^{Ab}	116.7 ± 15.28^{Ca}	116.7 ± 15.28^{Ca}
	P. mirabilis	116.7 ± 15.28^{Ac}	133.3 ± 11.55^{Bb}	266.7 ± 28.87^{Aa}
	P. aeruginosa	116.7 ± 15.28^{Ac}	150.0 ± 0.00^{Bb}	266.7 ± 28.87^{Aa}
	K. pneumoniae	83.3 ± 2.89^{Bb}	233.3 ± 15.28^{Aa}	233.3 ± 15.28^{Ba}
Hot water	*S. aureus*	7.0 ± 0.00^{Db}	4.0 ± 0.00^{Fb}	25.0 ± 0.00^{Fa}
	L. monocytogenes	5.0 ± 0.00^{Da}	4.0 ± 0.00^{Fa}	10.3 ± 0.58^{Ga}
	B. cereus	6.0 ± 0.00^{Da}	$5.0 \pm 0.00Fa$	8.3 ± 0.58^{Ga}
	S. typhimurium	13.3 ± 0.58^{Db}	4.0 ± 0.00^{Fb}	50.0 ± 0.00^{Ea}
	E. coli O:157	14.3 ± 0.58^{Db}	6.3 ± 0.58^{Fb}	41.7 ± 1.53^{Ea}
	P. mirabilis	13.3 ± 0.58^{Db}	4.0 ± 0.00^{Fb}	50.0 ± 0.00^{Ea}
	P. aeruginosa	14.3 ± 0.58^{Db}	8.3 ± 0.58^{Fb}	66.7 ± 5.8^{Da}
	K. pneumoniae	16.7 ± 1.15^{Db}	13.3 ± 0.58^{Fb}	83.3 ± 2.89^{Da}

LE= Leaf extracts; TE= Twig extracts; Figures sharing same capital letter (column) and lowercase letter (row) are not significantly different at P= 0.05 probability by Fisher's PLSD test. LSD 0.05 solvent 18.089, plant part 12.202.

reported that TE < 1 cm exhibited the highest A.I value against bacterial isolates.

Minimum inhibitory concentration (MIC). MIC was also estimated (Table 3) to investigate inhibitory activity of *P. guajava* extracts. In this respect, MIC value of methanolic extract ranged between 5- 116.7 mg/mL, while, it was 4 – 233.3 mg/mL for TE < 1. Whereas, it was varied between 8.3 – 266.7 mg/mL for TE > 1 (Table 3). It worth noting that, the hot water TE < 1 extract exhibited the lowest MIC value (4 – 13.3 mg/mL) (Table 3). Analysis of variance using PLSD test at P ≤ 0.05 revealed statistically significant differences in MIC against all tested bacterial isolates among all examined solvents (Table 3). In this regards, MIC estimated values for hot water TE < 1 extract were as follows: 4 mg/ml against *S. aureus*, *L. monocytogeneses*, *S. typhimurium* and *P. mirabilis* < 5 mg/mL against *B. cereus* < 6.3 mg/mL against *E. coli* O:157 < 8.3 mg/mL against *P. aeruginosa* < 13.3 mg/mL against *K. pneumoniae* isolate.

In the current study, ethanolic extract was non active against all tested isolates except against *S. typhimurium.* Whereas, the lowest lowest MIC value was recorded to be 0.1 mg/mL with ethanol *P. guajava* extracts against each of *L. monocytogenes*, *S. aureus* and *Vibrio parahaemolyticus* isolates (Mahfuzul Hoque *et al*., 2007). Moreover, MIC value was recorded to be 10.3 and 7.3 mg/mL against *S. aureus* with methanolic and hot water LE extracts, respectively. Other study revealed that MIC value was ranged between 625 µg/mL -7.5 mg/mL, respectively and minimum bactericidal concentration (MBC) ranged between 1.25-12.5 mg/mL, for respectively against resistant *S. aureus* isolates using leaves methanol and aqueous *P. guajava* extracts (Anas *et al*., 2008). Indeed, MIC value was 10.3 and 7.3 mg/mL against *S. aureus* and *E. coli* isolates, respectively. Whereas, it was 25 µg/mL against *S. aureus* and 0.78 µg/mL against both *E. coli* and *B. subtilis* pathogens using methanolic *P. guajava* leaf extracts (Dhiman *et al*., 2011). While, it was ranged between 0.125-> 0.250 mg/mL and between 0.062-> 0.250 mg/mL against the tested isolate with aqueous and methanolic extracts, respectively against 8 *S. aureus* (MRSA) isolates with *P. guajava* stem bark aqueous and methanolic extracts (Esimone *et al*., 2012). Whereas, Saleh *et al*. (2015) reported that the estimated MIC values varied between 4 and 7.2 mg/mL for bacterial, whereas, it ranged between 14.5 and 37.3 mg/mL for fungal isolates. While, ethyl acetate had the lowest antimicrobial activity compared to the other tested solvents. Moreover, Taura *et al*. (2014) reported that MIC values for *P. guajava* leaves were recorded to be 250 and 500 µg/mL against *Salmonella* spp. and *Pseudomonas* spp. respectively at 45 °C , whereas, they recorded to be 500, 1000 and 1000 µg/mL against *Klebsiella* spp*., Pseudomonas* spp. and *Salmonella* spp. isolates , respectively at 60 °C. In the current study, MIC value was recorded to be 10.3 and 25 mg/mL against *S. aureus* and *E. coli;* whereas, it was 16.7 and 25 mg/mL; while, it was 7 and 14.3 mg/mL against the two isolates using methanolic, ethanolic and hot water LE extracts.Whereas, Gitika and Kumar (2016) reported that the lowest MIC value was recorded to be 12.5 mg/mL against *B. subtilis*, *M. luteus*, *S. aureus* and *E. coli* with methanolic leaves and with ethanolic leaves against *M. luteus*, and *E. coli*; and also with aqueous leaves against *M. luteus* isolate using *P. guajava* leaves

Conclusion

Antimicrobial activity of LE, SE, FE and TE *P. guajava* (methanol, ethanol, acetone, ethyl acetate and hot water) extracts was investigated against 8 bacterial isolates. Data presented herein showed that seeds (SE) and fruits (FE) extracts have no activity against all tested pathogens regardless tested solvent. Otherwise, ethyl acetate extract was the lowest potent against all tested isolates regardless studies plant fractions. Overall, methanolic LE and hot water TE < 1cm diameter extracts were the most potent against all tested microorganisms by showing the highest ZI and A.I value and lowest MIC values. The current investigation showed that *P. guajava* L. could use as an antibacterial agent with low cost and potential source in future researches. Further research however, on separately chemical components inhibitory activity of methanolic LE and hot water TE < 1cm extracts is requested.

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C**onflict of Interest.** The authors declare that there is no conflict of interest

References

Abdelrahim, S.I., Almagboul, A.Z.,Omer, M.E.A., Elegami, A. 2002 Antimicrobial activity of *Psidium guajava* L. Fitoterapia, **73:** 713-715.

Ahmed, R.S., Yagoub, S.O. 2007. *In vitro* anti-microbial

activity of *Psidium guajava* extracts. *Research Journal of Microbiology,* **2:** 845-850.

Anas, K., Jayasree, P.R., Vijayakumar, T., Manish-Kumar, P.R. 2008. *In vitro* antibacterial activity of *Psidium guajava* Linn. leaf extract on clinical isolates of multidrug resistant *Staphylococcus aureus*. *Indian Journal of Experimental Biology*, **46:** 41-46.

Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., Yadav, A. 2013. Antimicrobial activities of leaf extracts of Guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. *International Journal of Microbiology*, **6:** 136-141.

Dhiman, A., Nanda, A., Ahmad, S., Narasimhan, B. 2011. *In vitro* antimicrobial activity of methanolic leaf extract of *Psidium guajava* L. *Journal of Pharmacy and Bioallied Sciences*, **3:** 226-229.

Egharevba, H.O., Iliya, I., Ibekwe, N., Abdullahi, M.S., Okwute, S.K., Okogun, J.I. 2010. Broad spectrum antimicrobial activity of *Psidium guajava* Linn. leaf. *Nature and Science*, **8:** 43-50.

Elekwa, I., Okereke, S.C., Ekpo, B.O. 2009. Preliminary phytochemical and Antimicrobial investigations of the stem bark and leaves of *Psidum guajava* L. *Journal of Medicinal Plant Research*, **3:** 045-048.

Esimone, C.O., Attama, A.A., Mundi, K.S., Ibekwe, N.N., Chah, K.F. 2012. Antimicrobial activity of *Psidium guajava* Linn. stem extracts against methicillin-resistant *Staphylococcus aureus*. *African Journal of Biotechnol*ogy, **11:** 15556-15559.

Farhana, J.A., Hossain M.F., Mowlah, A. 2017. Antibacterial effects of Guava (*Psidium guajava* L.) extracts against food borne pathogens. *International Journal of Nutrition and Food Sciences*, **6:** 1-5.

Gitika, Kumar, M. 2016. Antibacterial activity of *Psidium guajava* L. leaves extracts against some Gram-negative bacteria. *European Journal of Pharmaceutical and Medical Research*, **3:** 261-266.

Hassimotto, N.M., Genovese, M.I., Lajolo, F.M. 2005. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *The Journal of Agricultural and Food Chemistry*, **53:** 2928-2935.

Ifeanyichukwu, I., Chika, E., Emmanuel, N., Anthonia, O., Ngozi, A., Agabus, N. 2015. Preliminary investigation of the antibacterial activity of *Psidium guajava* extracts. *European Journal of Medicinal Plants*, **7:** 26-30.

Mahfuzul Hoque, MD., Bari, M.L., Inatsu, Y., Juneja,V.K., Kawamoto, S. 2007. Antibacterial activity of guava (*Psidium guajava* L.) and Neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathogens and Disease*, **4:** 481-488.

Mailoa M., Mahendradatta, M., Laga, A., Djide, N., 2014. Antimicrobial activities of tannins extract from guava leaves (*Psidium guajava* L) on pathogens microbial. *International Journal of Scientific and Technology Research*, **3:** 236-241.

Mani, A., Mishra, R., Thomas, G. 2011. Elucidation of diversity among *Psidium* species using morphological and SPAR methods. *Journal of Phytol*ogy, **3:** 53-61.

Nwinyi, O.C., Chinedu, N.S., Ajani, O.O. 2008. Evaluation of antibacterial activity of *Psidium guajava* and *Gongronema latifolium*. *Journal of Medicinal Plant Research*, **2:** 189-192.

Ofodile, N.L., Nwakanma, N.M.C., Mordi, M., Ademolu, O., Ezimoke, I., Owoso, J. 2013. Genotoxic and antimicrobial studies of the leaves of *Psidium guajava*. *Europian Asian journal of Bio Sciences*, **7:** 60-68.

Pandey, A. Shweta, C. 2012. Antibacterial properties of *Psidium guajava* leaves, fruits and stems against various pathogens. *International Journal of Pharmaceutical Research and Development*, **3:** 15-24.

Rattanachaikunsopon, P., Phumkhachorn, P. 2010. Contents and antibacterial activity of flavonoids extracted from leaves of *Psidium guajava. Journal of Medicinal Plant Research*, **4:** 393-396.

Saleh, B., Al-Halab, L., Al-Mariri, A. 2015. *In vitro* leaves and twigs antimicrobial properties of *Psidium guajava* L. (Myrtaceae). *Herba Polonica*, **61:** 93-104.

Shruthi, S.D., Roshan, A., Timilsina, S.S., Sunita, S. 2013. A review on the medicinal plant *Psidium guajava* Linn (*Myrtaceae*). *Journal of Drug Delivery and Therapeutics*, **3:** 162-168.

Taura, D.W., Yusha'u, M., Bello, U.A., Hassan, A., Saidu, J., Panda, T.W. 2014. Antibacterial activity of *Psidium guajava* in clinical isolates. *Academia Journal of Microbiology Research*, **2:** 79-83.