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herba polonica
From Botanical to Medical Research
Vol. 61 No. 3 2015
DOI: 10.1515/hepo-2015-0025

SHORT COMMUNICATIONS

In vitro leaves and twigs antimicrobial properties of *Psidium guajava* L. (*Myrtaceae*)

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Summary

The current study aimed to investigate antimicrobial activity of methanol, ethanol, acetone, ethyl acetate and hot water (leaves and twigs fractions) *Psidium guajava* L. crude extracts against three bacteria and two fungi pathogens. Antimicrobial activity expressed by disc-diffusion assay (zone of inhibitions - ZIs), activity index (AI) and minimum inhibitory concentration (MICs) that were measured as reported in many investigations. Based upon the estimated ZIs, AI and MICs values, hot water twigs <1 cm diameter extract was the most potent against all tested microorganisms. The MICs value ranged between 4 and 7.2 mg/ml for bacteria, while, it was between 14.5 and 37.3 mg/ml for fungi. Moreover, ethyl acetate had the lowest antimicrobial activity compared to the other tested solvents. From the results obtained herein, it could be concluded that *P. guajava* serve as antibacterial and antifungal agent.

Key words: *activity index, antimicrobial activity, Psidium guajava L., minimum inhibitory concentrations, zone of inhibitions*

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 like Asia, Egypt, Hawaii, Florida, Palestine, Syria, 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 [1]. The most commonly cultivated species of *Psidium* is *P. guajava* L. which is the common guava. *P. guajava* is an important food crop and medicinal plant in tropical and subtropical countries widely used like food and in folk medicine around of the world. The wood is hard and tough, used as posts for rural house buildings.

Many pharmacological investigations have demonstrated the importance of this plant as anti-allergenic, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses [2]. Their biological activity in terms of pharmacological study could be related to its major components such as essential oils, flavonoids, carotenoids, polyphenolic compounds, pentacyclic triterpenoids, esters, aldehydes etc. [2, 3-5].

Development of microbial resistance to available antibiotics have led scientists to introduce the antimicrobial activity of medicinal plants. *P. guajava* (guava) had been previously reported for their antimicrobial activity in many investigations [4-5, 6-12].

In several studies, guava showed significant antibacterial activity and has also demonstrated antifungal and antimalarial actions [13]. *P. guajava* leaves and shoot have been known for their antibacterial activity against the both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella* Enteritidis, *Bacillus cereus*, *Proteus vulgaris*, *Shigella dysenteriae* and *Escherichia coli* [14]. It has achieved a very long history of traditional use for a wide range of diseases [7-8]. In the Philippines, Syria, India, Nigeria and worldwide, guava fruit is freely eaten for its good taste and nutritional benefits.

Thereby, this work was set out to evaluate *P. guajava* extracts for their antimicrobial spectrum of methanol, ethanol, acetone, ethyl acetate and hot water extracts of leaves and twigs fractions against *Streptococcus pneumoniae*, *Acinetobacter baumannii, Brucella abortus* bacteria as well as *Candida albicans* and *Aspergillus niger* fungi strains.

MATERIAL AND METHODS

Plant material preparation

Fresh leaves and twigs (<1cm in diameter and >1 cm in diameter) of the medicinal plant *P. guajava* were collected from Lattakia city located in the coastal regions of Syria. The plants were identified in the Division of Plant Biotechnology at the AECS in Damascus, Syria. Sampling was carried out in spring with an annual rainfall ranging from 650 to 700 mm. Further, twigs were cut into small pieces and leaves fractions were shade-dried for one week, powdered by special electric mill and stored separately in polyethylene bags until analysis.

Plant extract preparation

Methanol, ethanol, acetone, ethyl acetate and hot water extracts were performed as follows: 500 g of shade-dried pulverized plant material was extracted in a Soxhlet apparatus successively with solvent 10 times. The extraction was conducted until no more coloured matter was extracted. Solvent from each extracted mixture was evaporated to dryness using a rotary evaporator under reduced pressure at 40°C. All dried extracts were kept in tightly fitting stopper bottles and stored in 4°C. The concentration of extract was considered to be 100 mg/ml.

Microorganisms and growth conditions

The pure clinical isolates of three bacteria strains (*S. pneumoniae, A. baumannii* and *B. abortus*) and two fungi pathogens (*C. albicans* and *A. niger*) were collected from the Microbiology and Immunology Division, Department of Molecular Biology and Biotechnology of Atomic Energy Commission of Syria (AECS), Damascus, Syria.

The cultures were started from the transference of stock cultures for trypticase soy broth (TSB, Difco, BD, Spars, MD) at 37°C for *S. pneumoniae* and *A. baumannii*, for a period of 24 h. Whereas, for *B. abortus* culture period was 48 h.

After growth, the samples were centrifuged (1000 x g/15 min/4°C) and 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 standardize to approximately 10^6 CFU/ml [12]. 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.

Whereas, for fungal inoculation, the standardized culture of each test fungi was prepared by dissolving 3.9 g of Potato Dextrose Agar (PDA) in 100 ml distilled water, autoclaving, pouring and inoculating into plates. The inoculated plates were incubated at 28±3°C for 2 days.

The disc-diffusion method

Commercial antibiotics ciprofloxacin (100 mg/ml) and nystatin (5 μg/ml) were used as standard for antimicrobial activity of bacteria and fungi isolates, respectively.

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) and reconstituted in minimum amount of each tested solvent were applied over each of the culture plates previously seeded with the 10^6 CFU/ml cultures of bacteria. Then, bacterial cultures were incubated at 37°C for 18 h. The paper discs was impregnated with 20 μl of 10 mg/ml ciprofloxacin as a standard antimicrobial comparison.

Negative control was prepared using tested solvents (final concentration of the solvent in the highest concentration of plant extract was tested). Diameter of inhibition zones (ZIs in mm) was measured after incubation at 37°C for 18–24 h for bacteria and at 28±3°C for 2 days for fungi isolates. For each extract, duplicate trials were conducted against each organism.

Minimum Inhibitory Concentrations (MICs)

Stock solutions of the abovementioned antibiotics were prepared according to manufacture. Determination of MICs by the microdilution broth method was carried out according to Clinical and Laboratory Standards Institute (CLSI) approved standards. Microdilution broth susceptibility assay was used [15]. Three replicates of serial dilutions of extract (100 mg/ml) or of antibiotics (128 μg/ml) were prepared in TSB medium in 96-well microliter plates. One hundred microlitres of freshly grown bacteria standardized in 10^6 CFU/ml in TSB were added to each well. Positive control was achieved in the same conditions but without extract or antibiotics, negative control was also made in the same conditions but without adding pathogens. 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_{100}.

Activity Index (AI)

Activity index (AI) of *P. guajava* plant extracts was calculated as previously reported by Egharevba *et al.* [10] using the following formulae:

Activity index (AI) = Inhibition zone of sample/ Inhibition zone of standard.

Statistical methods

All statistical analyses were performed using Graphpad programme at the 5% level of significance ($p=0.05$). Data are expressed as mean of three replicates.

RESULTS AND DISCUSSION

The bioassay results for antimicrobial activity of various solvent extracts from *P. guajava* are presented in table 1. Our data indicated that all extracts inhibited bacteria and fungi growth in different degree. Among various tested extracts, the hot water and methanol extracts, showed the maximum activity against all the tested strains used in the present study followed by acetone extract (tab. 1).

Table 1.

Antimicrobial activity of the *P. guajava* leaf and twigs extracts against tested microorganisms (zone of inhibition in mm)

Solvent	Microorganism	Zones of inhibition [mm]			
		LE	TE <1 cm	TE >1 cm	Control
Methanol	*S. pneumoniae*	13±0.08^{6i}	12±0.05^{**1}	10±0.1^{C}	10±0.09
	A. baumannii	19±0.25^{6ei}	12± 0.22	13±0.18***	25±0.14
	B. abortus	16±0.12^{6i}	17±0.19^{1}	12±0.087^{e}	17±0.1
	C. albicans	19±0.4^{*****6ei}	13±0.22^{1}	13±0.09	10±0.1
	A. niger	16±0.22 ^{*****6ei}	13±0.12^{1}	10±0.15^{e}	11±0.19
Ethanol	*S. pneumoniae*	11±0.13^{*****7j}	10±0.08^{2}	7±0.04^{4f}	10±0.09
	A. baumannii	12±0.097^{7a}	12±0.09	10±0.08^{4a}	25±0.14
	B. abortus	11±0.07 $^{***** j}$	12±0.06	8±0.04^{4f}	17±0.1
	C. albicans	9±0.07^{7fj}	12±0.1^{2}	13±0.05^{4}	10±0.1
	A. niger	6±0.05^{7fj}	9±0.09^{2}	ND	11±0.19
Acetone	*S. pneumoniae*	13±0.07^{8gb}	11±0.05^{3g}	8±0.05*	10±0.09
	A. baumannii	13±0.13^{8}	12± 0.13	13±0.1*	25±0.14
	B. abortus	13±0.076^{g}	12±0.06	10±0.04^{*b}	17±0.1
	C. albicans	12±0.12^{8}	13± 0.08^{3}	12±0.2	10±0.1
	A. niger	13±0.08^{d}	12±0.1^{3}	11±0.12	11±0.19
Ethyl acetate	*S. pneumoniae*	17±0.27	ND	ND	10±0.09
	A. baumannii	13±0.12	ND	ND	25±0.14
	B. abortus	12±0.1	ND	ND	17±0.1
	C. albicans	12±0.15	ND	ND	10±0.1
	A. niger	13±0.14	ND	ND	11±0.19
Hot water	*S. pneumoniae*	16±0.12^{k}	16±0.16	11±0.09^{5h}	10±0.09
	A. baumannii	16±0.075^{9hk}	12± 0.08	11±0.05	25±0.14
	B. abortus	13±0.07	13± 0.09	12±0.09	17±0.1
	C. albicans	17±0.19^{9h}	20±0.22	16±0.07^{5h}	10±0.1
	A. niger	14±0.15^{h}	18±0.17	13±0.07^{5h}	11±0.19

Observations are expressed as mean±standard deviation (SD), n=3. LE: leaf extract; TE: twig extract ND: not determined
Comparing the effect of different extracts from the same part of the plant by using different solvents:
* $p<0.05$ vs TE>1 using methanol, ethanol, or hot water for *S. pneumoniae, B. abortus,* and *A. baumannii*. ** $p<0.05$ *vs* TE<1 using ethanol for *S. pneumoniae*. *** $p<0.05$ vs TE>1 using hot water for *A. baumannii*. **** $p<0.05$ vs LE using methanol, acetone, or hot water for *S. pneumoniae*, and *B. abortus*. ***** $p<0.05$ vs LE using hot water for *A. niger*, and *C. albicans*.
^{1} $p<0.001$ *vs* TE<1 using ethanol, acetone, or hot water for *S. pneumoniae, B. abortus, A. niger,* and *C. albicans*. ^{2} $p<0.001$ vs TE<1 using acetone, or hot water for *S. pneumoniae, A. niger,* and *C. albicans*. ^{3} $p<0.001$ *vs* TE<1 using hot water for *S. pneumoniae, A. niger,* and *C. albicans*. ^{4} $p<0.001$ *vs* TE>1 using methanol, acetone, or hot water for *S. pneumoniae, B. abortus, C. albicans,* and *A. baumannii*. ^{5} $p<0.001$ *vs* TE>1 using methanol, or acetone for *A. niger, C. albicans, S. pneumoniae*. ^{6} $p<0.001$ vs LE using all other solvent (ethanol, acetone, ethyl acetate) or hot water for all microorganisms. ^{7} $p<0.001$ *vs* LE using acetone, ethyl acetate,or hot water for *S. pneumoniae, A. niger, C. albicans, A. baumannii*. ^{8} $p<0.001$ *vs* LE using ethyl acetate, or hot water for *S. pneumoniae, C. albicans, A. baumannii*. ^{9} $p<0.001$ *vs* LE using hot water for *C. albicans, A. baumannii*.
Comparing the effect of the same solvent by using extracts from different parts of the plant:
^{a} $p<0.05$ vs TE>1 for *A. baumannii*. ^{b} $p<0.05$ vs TE<1 for *S. pneumoniae,* and *S. pneumoniae*. ^{c} p<0.05 vs TE<1 for *S. pneumoniae*. ^{d} $p<0.05$ vs TE>1 for *A. niger*. ^{e} $p<0.001$ vs TE<1 for *B. abortus, , C. albicans, A. baumannii,* and *A. niger*. ^{f} $p<0.001$ vs TE<1 for *S. pneumoniae*, *A. niger*, *B. abortus,* and *C. albicans*. ^{g} $p<0.001$ vs TE>1 for *S. pneumoniae,* and *B. abortus*. ^{h} $p<0.001$ vs TE<1 for *S. pneumoniae*, *A. niger*, *A. baumannii,* and *C. albicans*. ^{i} $p<0.001$ vs LE for *S. pneumoniae*, *B. abortus*, *A. niger*, *C. albicans*, *A. baumannii*. ^{j} $p<0.001$ *vs* TE>1 for all microorganisms. ^{k} $p<0.001$ *vs* TE>1 for *S. pneumoniae, B. abortus, A. niger, C. albicans*. ^{l} $p<0.001$ *vs* TE>1 for *S. pneumoniae, A. baumannii*.

Analysis of variance showed that the effect of different extracts from the same part of the plant on ZIs by using different solvents was significantly ($p<0.05$) different. Indeed, the effect of the same solvent on ZIs by using extracts from different parts of the plant was also significantly ($p<0.05$) different (tab. 1).

As mentioned in table 1, the highest zone of inhibition was recorded for hot water TE<1 cm against *C. albicans* fungi (20 mm), followed by methanol LE (19 mm) against the same pathogen. Whereas, Egharevba *et al.* [10], reported lower values for this parameter (0, 15, 15 and 0 mm) for 70% methanol, methanol, erythromycin and hexane *P. guajava* leaf extracts, respectively, against *C. albicans.* Whereas, for bacteria, the highest ZIs value was pronounced for methanol LE against *A. baumannii* (19 mm). Thereby, the later pathogen could be declared as the highest sensitive pathogen among the tested bacteria. In this respect, this value was found to be 19 mm, 16 mm, 15 mm and 12 mm with methanol LE, hot water LE, acetone TE <1 cm, ethanol LE & TE <1 cm, respectively, for the later pathogen.

Activity index (AI) was also estimated to confirm the observed inhibitory effect of *P. guajava* extracts. This parameter varied according to tested microorganisms, examined plant parts and solvents (tab. 2). Our data indicated that the hot water was also more potent than other tested extracts. The difference in activity could be related to different secondary metabolites present in each extract. Otherwise, different solvents would have various degrees of solubility for different phytochemical compounds [16]. This indicates that the secondary metabolites act as an antimicrobial compounds, which either inhibit or kill the pathogens by different mechanisms.

Our data mentioned that hot water TE <1 cm exhibited the highest AI against *C. albicans* fungi (2), followed by methanol LE (1.9) against the same fungi. Whereas, Egharevba *et al.* [10] reported lower values for this parameter (0, 0.63, 0.63 and 0) for 70% methanol, methanol, erythromycin and hexane *P. guajava* leaf extracts against *C. albicans.*

While, for bacteria microorganisms, LE ethyl acetate exhibited the highest AI against *S. pneumoniae* (1.7), followed by LE hot water (1.6) and TE <1 cm (1.6) against the same isolate.

Antimicrobial activity of the extracts was tested; then, minimum inhibitory concentration (MIC) was recorded. The examined solvent extracts revealed comparable activity against the tested microorganisms (tab. 3). This variance could be related to plant part, solvent tested and microorganism strain examined in this study.

Analysis of variance showed that the effect of different extracts from the same part of the plant on MICs with use of different solvents was significantly different ($p<0.05$) (tab. 3).

From estimated MIC values, hot water TE <1 cm was the most potent against all tested microorganisms. Whereas, MICs values ranged between 4 and 7.2 mg/ml for bacteria; while it ranged from 14.5 to 37.3 mg/ml for fungi; followed by methanol

LE, where the estimated MIC values varied between 6 and 8.2 mg/ml for bacteria, and from 7.2 to 42.7 mg/ml for fungi. In this respect, MIC values were recorded to be 7.2, 10.3, 6.2, 20.3 and 9.3 mg/ml for LE methanol, ethanol, acetone, ethyl acetate and hot water extracts, respectively against *C. albicans*. Egharevba *et al.* [10] reported that these values were 0, 5, 5 and 0 mg/ml for 70% methanol, methanol, erythromycin and hexane *P. guajava* leaf extracts respectively, against *C. albicans*. In the later study, it was also mentioned that all solvents of leaf extracts have no antimicrobial activity against *A. flavus* and *A. fumigatus* strains.

Table 2.

Activity index (AI) of the *P. guajava* extracts against tested microorganisms

Solvent	Microorganism	Activity Index (AI)		
		LE	TE < 1 cm	TE > 1 cm
Methanol	*S. pneumoniae*	1.3	1.2	1.0
	A. baumannii	0.8	0.5	0.5
	B. abortus	0.9	1.0	0.7
	C. albicans	1.9	1.3	1.3
	A. niger	1.5	1.2	0.9
Ethanol	*S. pneumoniae*	1.1	1.0	0.7
	A. baumannii	0.5	0.5	0.4
	B. abortus	0.6	0.7	0.5
	C. albicans	0.9	1.2	1.3
	A. niger	0.5	0.8	–
Acetone	*S. pneumoniae*	1.3	1.1	0.8
	A. baumannii	0.5	0.5	0.5
	B. abortus	0.8	0.7	0.6
	C. albicans	1.2	1.3	1.2
	A. niger	1.2	1.1	1.0
Ethyl acetate	*S. pneumoniae*	1.7	–	–
	A. baumannii	0.5	–	–
	B. abortus	0.7	–	–
	C. albicans	1.2	–	–
	A. niger	1.2	–	–
Hot water	*S. pneumoniae*	1.6	1.6	1.1
	A. baumannii	0.6	0.5	0.4
	B. abortus	0.8	0.8	0.7
	C. albicans	1.7	2.0	1.6
	A. niger	1.3	1.6	1.2

Observations are expressed as mean of 3 replicates.
LE: leaf extract; TE: twig extract.

Table 3.

Minimum inhibition concentrations (MIC_s) values of the *P. guajava* extracts against tested microorganisms

Solvent	Microorganism	Minimum inhibitory concentration values [mg/ml]		
		LE	TE <1 cm	TE >1 cm
Methanol	*S. pneumoniae*	8.2	7.2	14.5
	A. baumannii	8.2	9.3	58.3
	B. abortus	6.0	7.2	37.5
	C. albicans	7.2	6.2	20.8
	A. niger	42.7	53.3	85.3
Ethanol	*S. pneumoniae*	16.7	5.0	29.2
	A. baumannii	20.8	16.7	54.2
	B. abortus	16.7	7.2	66.7
	C. albicans	10.3	10.3	29.2
	A. niger	96.0	53.3	133.3
Acetone	*S. pneumoniae*	8.2	5.0	14.5
	A. baumannii	16.7	10.3	50.0
	B. abortus	7.2	9.3	41.7
	C. albicans	6.2	8.2	16.7
	A. niger	53.3	48.0	76.0
Ethyl acetate	*S. pneumoniae*	54.2	29.2	66.7
	A. baumannii	116.7[c]	100.0	166.7[bh]
	B. abortus	216.7[f]	233.3[p]	133.3
	C. albicans	20.8[+dg]	233.3[a]	266.7[e]
	A. niger	85.3[*+f]	333.3[a]	333.3[e]
Hot water	*S. pneumoniae*	5.0	4.0	7.2
	A. baumannii	10.3	7.2	41.7
	B. abortus	16.7	5.0	66.7
	C. albicans	9.3	14.5	13.5
	A. niger	48.0	37.3	53.3

Observations are expressed as mean of 3 replicates.
LE: leaf extract; TE: twig extract.
[*] $p<0.01$ *vs* TE<1 in *A. niger,* and [+] $p<0.01$ *vs* TE>1, in *A. niger* and *C. albicans*. [a] $p<0.001$ *vs* TE<1 for *A. niger* and *C. albicans* using all other solvent (methanol, ethanol and acetone) or hot water. [b] $p<0.05$ *vs* TE>1 for *A. baumannii* using all other solvent. [c] $p<0.05$ *vs* LE for *A. baumannii* using methanol or hot water. [d] $p<0.05$ *vs* LE for *C. albicans* using ethanol or hot water, and for *A. niger* using ethanol. [e] $p<0.001$ *vs* TE>1 for *C. albicans* and *A. niger* using all other solvent (methanol, ethanol and acetone) or hot water. [f] $p<0.001$ *vs* LE for *B. abortus* and *A. niger* using methanol, ethanol and hot water; and *vs B. abortus* using acetone. [g] $p<0.01$ *vs* LE for *C. albicans* using methanol or acetone. [h] $p<0.01$ *vs* TE>1 for *A. baumannii* using hot water.

Phytochemical analysis proved that the alkaloids, flavanoids, carbohydrates, phenols and glycosides abundance in *P. guajava* leaf methanolic and aqueous (hot

water) extracts compared to other extracts examined (results not shown here) could explain the difference in their biological activity. These secondary metabolites have been successfully identified in plant extracts and investigated on its antimicrobial inhibitory and used against some microorganisms resistant to commercial antibiotics [17].

Previously, Nair and Chanda [6] reported that methanol and acetone extracts showed comparable activity against the fungal strains, with ZIs diameters of 7.5 to 18 mm against *Candida* spp. and *C. neoformans* (9 mm), respectively. Baby and Mini [9] investigated the activity of *P. guajava* leaf extract against Gram-positive and Gram-negative bacterial strains as well as fungal strains. The later investigation showed that the antibacterial activity was more effective in acetone and methanol extracts against both Gram-positive and Gram-negative bacterial strains and fungal strains. The ZIs was recorded to be 1.4, 0.6, 0.6 cm for leaf acetone extract, while it was of 1.5, 0.6, 0.8 cm for leaf methanol extract against *C. tropicalis*, *A. niger* and *A. aculeatus* fungi, respectively.

Moreover, Elekwa *et al.* [8] investigated the extracts (ethanol, methanol and aqueous) inhibitory effect of the leaves and stem bark of *P. guajava* against three bacteria isolates (*B. subtilis*, *P. aeruginosa* and *E. coli*) and three fungi strains (*C. albicans*, *Fusarium* spp. and *Geotrichum candidum*). This study mentioned that the aqueous extract of *P. guajava* had inhibitory effects on *Fusarium* spp. and *B. subtilis* with zones of inhibition of 20 and 16 mm, respectively. The ethanolic extracts exhibited no inhibitory effects on these microorganisms. Indeed, the later study indicated that the aqueous extract was highly potent against *Fusarium* spp. and *B. subtilis* microorganisms with MIC value of 5 and 3 mg/ml, respectively.

Nair and Chanda [6] as well as Baby and Mini [9] stated that the *P. guajava* acetone extract was highly active against Gram-positive and fungal strains, e.g. *Aspergillus* species.

Dhiman *et al.* [11] reported the antimicrobial activity of methanolic leaf extract of *P. guajava* against three bacteria isolates (*E. coli, S. aureus* and *B. subtilis)* two fungi strains (*C. albicans* and *A. niger).* The later investigation indicated that the highest antibacterial activity was recorded against *E. coli* with MIC of 0.78 μg/ml, and MBC of 50 μg/ml, and antifungal activity with MIC of 12.5 μg/ml against the two fungi strains tested.

Buvaneswari *et al.* [3] reported that glucose facilitated fungal tip elongation (80 μm/h) and water (8 μm/h) slow down fungal tip elongation in *Aspergillus* spp.

Beatriz *et al.* [4] investigated the antifungal properties of leaf hexane, acetone and methanol extracts from *P. guajava* for their antifungal properties against *Trichophyton rubrum*, *T. tonsurans*, *Sporotrix schenckii*, *Microsporum canis*, *Cryptococcus neoformans*, *C. parapsilosis*, and *C. albicans*. The later study mentioned that *C. neoformans* was the most sensitive fungus, and the acetone extract was the most active with ZIs of 18 mm. Where, *C. albicans* exhibited ZIs was found to be 11, 17 and 14 mm for leaf methanol, acetone and hexane extracts, respectively.

Recently, Fagbohun *et al.* [12] investigated the leaves and bark aqueous extracts of *P. guajava* against two bacteria (*S. aureus* and *S. epidermidis*) and two fungi (*Microsporum gypseum* and *Trichophyton mentagrophytes*) isolates. The later study indicated the effectiveness of *P. guajava* as antibacterial and antifungal activity. Moreover, Ofodile *et al.* [5] described the antimicrobial and genotoxic effects of the *P. guajava* leaf extracts. The last work mentioned that the leaf ethanolic and the crude water extracts of guava leaf showed stronger antibacterial than antifungal activity, whereas, *A. fumigatus* was the least susceptible organism to leaf ethanolic extract. The later investigation mentioned that, the lowest ZIs of aqueous extracts of guava leaf was against *C. albicans* at the concentration of 50 mg/500 μl while it was most potent at 100 mg/500 μl.

CONCLUSION

This investigation allowed somewhat to screen different solvents (methanol, ethanol, acetone, ethyl acetate and hot water) antimicrobial activity of *P. guajava* (leaves and twigs fractions) against three bacteria and two fungi pathogens. From the estimated ZIs, AI and MICs values, hot water TE <1 cm was the most potent against all tested microorganisms. Furthermore, ethyl acetate had the lowest antimicrobial activity than the other tested solvents. Overall, antimicrobial inhibitory effect has been revealed using different solvents and plant fractions of *P. guajava*.

ACKNOWLEDGEMENTS

We thank I. Othman (director general of AECS) and N. MirAli (Head of Molecular Biology and Biotechnology Department in AECS) for their support.

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PRZECIWDROBNOUSTROJOWE WŁAŚCIWOŚCI LIŚCI I GAŁĄZEK *PISIDIUM GUAJAVA* L. (*MYRTACEAE*)

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Streszczenie

Celem niniejszych badań było oznaczenie aktywności przeciwdrobnoustrojowej ekstraktu metanolowego, etanolowego, acetonowego oraz uzyskanego za pomocą octanu etylu i gorącej wody z liści i gałązek *Pisidium guajava* L. wobec trzech bakterii i dwóch grzybów

chorobotwórczych. Aktywność przeciwdrobnoustrojową określano za pomocą krążków bibułowych (strefa zahamowania – ZIs), indeksu aktywności (AI) oraz najmniejszego stężenia hamującego (MICs). Na podstawie przeprowadzonych badań stwierdzono, że największą aktywnością, biorąc pod uwagę wszystkie zastosowane metody, odznaczał się ekstrakt uzyskany za pomocą gorącej wody z gałązek o średnicy <1 cm. W metodzie MICs dawał on w odniesieniu do wszystkich testowanych drobnoustrojów strefę zahamowania o średnicy powyżej 1 cm. Wartość wskaźnika MICs waha się w geranicach od 4 do7,2 mg/ml w przypadku bakterii oraz od 14,5 do 37,3 mg/ml dla badanych grzybów. Ponadto wykazano, że najniższą aktywność przeciwdrobnoustrojową wykazywał ekstrakt otrzymany przy użyciu octanu etylu. Przedstawione wyniki badań wskazują, że *P. guajava* może być uważana za roślinę o właściwościach przeciwbakteryjnych i przeciwgrzybiczych.

Słowa kluczowe: *indeks aktywności, działanie przeciwdrobnoustrojowe, Psidium guajava L., najmniejsze stężenie hamujące, strefa zahamowania*