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ANTIMICROBIAL AND FREE RADICAL SCAVENGING ACTIVITIES OF FIVE PALESTINIAN MEDICINAL PLANTS

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Abstract

Extracts from five indigenous Palestinian medicinal plants including *Rosmarinus officinalis, Pisidium guajava, Punica granatum* peel, grape seeds and *Teucrium polium* were investigated for antimicrobial and free radical scavenging activities against eight microorganisms, using well diffusion method. The microorganisms included six bacterial isolates (i.e. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginos, Klebsiella pneumonia*, *Bacillus subtilis* and *Micrococcus luteus)* and two fungal isolates (i.e. *Candida albicans* and *Aspergillus niger*). A standard antioxidant assay was performed on the plant extracts to assess their capability in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH). Of the five tested plant extract, only *Rosmarinus offcinalis* extract contained significant antimicrobial activity against all eight microbial isolates including *Pseudomonas aeruginosa*. Extracts from other four plants exhibited a variable antimicrobial activity against all microorganisms, except *Pseudomonas aeruginosa.* Significant antioxidant activity was detected in all plant extracts. However, extracts from *Pisidium guajava* leaves contained significantly higher antioxidant activity compared to the other extracts tested. The antimicrobial and scavenging activities detected in this in vitro study in extracts from the five Palestinian medicinal plants suggest that further study is needed to identify active compounds to target diseases caused by a wide-spectrum pathogens.

Key words: Antimicrobial activity, plant extract, scavenging activity

Introduction

The increased use of antibiotics in medicine, agriculture and animal care has contributed largely to the antibiotic-resistant microorganisms, urging the search for new and effective drugs. Emerging and re-emerging pathogens are also other reasons to screen for suitable drugs (Darwish et al., 2010; Nascimento et al., 2000a; Nimri et al., 1999). Medicinal plants contain active compounds that are effective in treatment of chronic as well as infectious diseases. Herbal plants are being used worldwide for medicinal purposes, particularly in the rural areas of the developing countries due to its availability, low cost, safety, as well as its effectiveness (Hassawi and Kharma, 2006; Nimri et al., 1999). Screening of herbal plant extracts for compounds with antimicrobial and free radical scavenging activities may yield new leads to target and combat infectious diseases (El Astal et al., 2005). Active antimicrobial compounds from medicinal plants as reported in the literature include phytochemicals as anthocyanins, phenolics, gallic acid, thiols, and caratenoids which work against the free radicals by ameliorating their oxidative damage (Awah et al. 2012; Jayaprakasha et al., 2003).

Palestine is rich in medicinal plants that have been used for centuries by many practitioners and clinicians to effectively treat patients with chronic diseases and infections (Jaradat, 2005; Essawi and Srour, 2000). Among the most common plants used in folk medicine in Palestine include *Rosmarinus officinalis* (*R. officinalis*), *Pisidium guajava* (*P. guajava*), *Punica granatum* (*P. granatu),* grape seeds, and *Teucrium polium* (*T. polium*). For example, *R.officinalis* in folk medicine is used in treating respiratory disorders, renal colic (as antispasmodic), rheumatoid arthritis (as antirheumatic) as well as general analgesic, diuretic, expectorant and others (Al-Sereitia et al., 1999). It is known to have antimicrobial and antioxidant activities (Wang et al., 2012; Toroglu, 2011 Romano et al., 2009; Elgayyar et al., 2001). Additionally, *P. guajava* has been used to treat wound ulcers, cholera, diarrhoea and bowels. The leaves of *P. guajava* are used in folk medicine as febrifuge and antispasmodic (Sanda et al., 2011). These species have antimicrobial activity against many types of microbes. For example, *P. guajava* was reported to have a strong antimicrobial activity against many types of microbes as well as antioxidant capability (Jiménez-Escrig et al., 2001; Nascimento et al., 2000). Moreover, *P. granatum* has been used in folk medicine for the treatment of diarrhoea, dysentery, respiratory, and hemorrhagic diseases. It also has antioxidant activity, antibacterial, antiviral and anti-atherosclertic properties (Choi et al., 2011; Al-Zoreky, 2009). Other anciently used plants in medicine in Palestine include *T. polium.* The latter has been used for its anti-inflammatory, anti-diabetic, antispasmodic, analgesic and antioxidant properties. It has been found to possess antimicrobial properties against various clinical pathogens (Darabpour et al., 2010). Finally, grape seed is a rich source of phenolics which are known to have antioxidant, antimutagenic, and immunomodulatory properties. Grape seed has been reported to carry out antibacterial activity against gram-positive bacteria, including methicillin-resistant *staphylococcus aureus* (MRSA) as well as gram-negative and some fungal species (Cheng et al., 2012; Al-Habib et al., 2010; Baydar et al., 2004; Jayaprakasha et al., 2003).

The above mentioned plants have been used medically to treat patients in critical care unit with diseases caused by micro-organisms such as *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli).* and *Candida albicans* (Edmond et al.,

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1999). Other pathogens including *Pseudomonas aeruginosa* (P. aueruginosa) and *Klebsiella pneumoniae* (K. pneumoniae) are common causes of chronic infections (Costerton et al., 1999; Edmond et al., 1999). *Aspergillus niger* (*A. niger*) was reported to cause infections in haematopoietic stem cell transplant recipients (Marr et al., 2002). *Micrococcus luteus (M.luteus)* was reported to be among the bacteria that may cause recurrent bacteremia (von Eiff et al., 1996).

The purpose of this investigation was to screen for antimicrobial and antioxidant activities in Palestine-native plants extracts from *R. officinalis, P. guajava, P. granatum* Peel, grape seeds, and *T. polium*. These plants are very commonly used in Palestinian folklore medicine. They are used to treat dysentery, chronic diarrhoea, infectious diseases, heart problems, bronchitis, stomach and intestine spasms, smallpox and ear infections as reported by Jaradat (2005) and Nascimento et al., (2000). The anti-microbial activities were screened against the following pathogenic microbes *S. aureus*, *E. coli*, *M. luteus*, *Candida albicans* (*C.albicans*), *A. niger*, *B. subtilis*, *P. aeruginosa*, and *K. pneumoniae*. Such microbes are well known clinical pathogens and represent a good combination of gram-negative, gram-positive and fungal isolates to study antimicrobial properties of plant-based extracts used in traditional or folkloric medicine.

Materials and methods

Plant extracts

The medicinal plants used in this study were collected from different locations in Palestine between July and November of 2011. *R. officinalis* was supplied from Bait Al-Maqdes for Medical Herbs Production Company at Jenin-Seer. *P. guajava* leaves and *P. granatum* peel were collected from trees in Yabad-Jenin. *R. officinalis, P. granatum* oeel, and *P. guajava* leaves were air dried in the shade at room temperature. Dry *T. polium* was purchased from Al-Attariene market at Jerusalem. Grape was collected from a farmer's vineyard at Hebron city; seeds were washed with distilled water and air dried in the shade at room temperature. The Palestinian Ministry of Agriculture Bureau at Jenin has kindly confirmed the identity of *T. polium, R. officinalis, P. granatum* peel, and *P. guajava.* The dried samples were grounded in a grinder (brand: C200G). Fifty gram of the ground sample was mixed with 250 ml of 96% ethanol and shaken at $25C^0$ and 75 RPM using a Lab-Line Max Q4000 shaker for five days. The ethanolic extract was filtered using Watman filter paper then the solvent was removed by rotary evaporator. The residue was dried and stored at $-20C^0$ until further use (El Astal et al., 2005; Essawi and Srour, 2000; Nimri et al., 1999).

Determination of anti-microbial activity

Microorganisms used in this study include *S. aureus* (ATCC: 29213), *E. coli* (ATCC: 10536), *M. luteus* (ATCC: 10240), *Candida albicans* (*C. albicans*) (ATCC: 10231), *Bacillus subtilis* (*B. subtilis*) (ATCC: 6633) and *A. niger* (ATCC: 16404) which were kindly provided by the Palestinian Health Central Laboratory (Ramallah, Palestine). Other microorganisms including *P. aeruginosa*, and *K. pneumoniae* were confirmed for identity and kindly provided by the diagnostic laboratory at Suliman Khaleel Hospital at Jenin. The crude extract was serially diluted using 10 % (v/v) dimethyl sulfoxide (DMSO) to a final concentration of 50%, 25%, 12%, and 6% and sterilised by filtration using 0.45 µm Millipore filters (Sampaio et al., 2009; Nimri et al., 1999; NCCLS, 1993). Using Mueller-Hinton agar plates, the antibacterial activity of the extracts were tested against the microbes by the well diffusion method according to NCCLS. Wells of 6 mm in diameter were made in the Mueller-Hinton agar plates aseptically. The microbes were cultured in nutrient broth tubes until their suspension reached turbidity similar to 0.5 McFarland standards. A swap of this suspension was streaked evenly on the surface of the Mueller-Hinton agar plates (NCCLS, 1993). For each extract, 50 µl of the above extract-10% DMSO solutions was added to the wells containing 25, 12.5, 6, and 3 mg of the extract respectively in each cultured Mueller-Hinton agar plates and incubated at $37C^0$ for 24 hours for all microbes, except *A. niger* and *C. albicans* which were incubated up to 72 hours. 10% DMSO was used as negative control. Discs containing different concentrations of eight antibiotics Gentamycin (GM) 10 UI, Kanamycin (K) 30 µg, Ampicillin (Am) 10 µg, Streptomycin (S) 10 µg, Penicillin (P) 10 µg, Ciproflaxin (CIP) 5 µg, Cefalixine (CN) 30 µg, and Bacitracin (B) 10 UI were used as positive controls.

Quantitative DPPH radical-scavenging assay

The free-radical scavenging activity of plant extracts was analysed by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test (Awah et al., 2012; Souza et al., 2012). The scavenging activity of extracts on the DPPH free radicals were evaluated according to the method reported by Awah et al. (Awah et al., 2012). The extracts were diluted at different concentrations (2-125 µg/ml) in methanol. Two ml of the above extract concentrations were mixed with 1.0 ml of 0.3 mM DPPH in methanol, shaken vigorously for few seconds and incubated in dark at room temperature for 25 min. For each sample solution, a blank was prepared as 2.0 ml of the sample solution and 1.0 ml of methanol. The negative control was 2.0 ml methanol plus 1.0 ml of 0.3 mM DPPH. At the end of the incubation time, the absorbance of the reaction mixtures was measured at 518 nm against each blank using Spectro 23 spectrophotometer. Radical-scavenging activity of DPPH was calculated using the following equation:

% Scavenging activity= 100 - [$((\text{Abs sample} - \text{Abs blank})/(\text{Abs control})) \times 100\%$]

Three replicates were used to evaluate the radical scavenging activity as well as the EC_{50} (the concentration of the sample leading to 50% reduction in initial DPPH concentration) (Awah et al., 2012). The EC_{50} was calculated by linear regression plots where the average of the scavenging capacities was represented by the ordinate and the concentration of the tested samples was represented by the abscissa.

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Results and discussion

Antimicrobial activity

The five plant extracts (Grape seed, *R. officinalis*, *P. guajava*, *P. granatum* Peel, and *T. polium*) showed different antimicrobial activities against a good combination of clinical pathogens: three Gram positive (*S. aureus*, *B. subtili*, and *M. luteus*), three Gram negative (*E. coli*, *P. aueruginosa* and *K. pneumonia*), and two fungal (*C. albicans* and *A. niger*) isolates.

The antimicrobial activity of the grape seed extract against the tested microbes was illustrated in Table 1. The extract of grape seeds inhibited the growth of *S. aureus*, *E. coli*, *B. subtili*, and *C. albicans*. Such results are consistent with results reported by Cheng et al. (2012) and Jayaprakasha et al. (2003). However, the growth of *P. aueruginosa* and *K. pneumonia* was not inhibited by the grape seed extract, contradicting results reported by Jayaprakasha et al. (2003) and Bayder et al. (2004). To the best of the author's knowledge, *M. luteus* and *A. niger* sensitivity to the extract of the grape seed was not investigated earlier. As shown in Table 1, *M. luteus* exhibited high susceptibility, while *A. niger* was resistant.

Table 1: Zone of Inhibition (mm) for Grape Seed Ethanolic Extract at Various Concentrations against some Microbes

	mg/well			
Microbes	25	12.5	6	3
S. aureus	22.3±1.5	15.7±2.1	9.7±0.6	R
E. coli	17.0±2.0	11.3±1.5	8.0±1.0	R
C. albicans	23.7±1.2	20.7±1.5	16.0±1.7	9.7±1.2
P. aueruginosa	R	R	R	R
K. pneumoniae	R	R	R	R
A. niger	R	R	R	R
M. luteus	26.3±2.1	21.3±0.6	15.7±1.5	8.0±1.0
B. subtilis	18.3±1.5	12.0±2.0	R	R

Data represented as means ± SD (n=3), R= absence of susceptibility (inhibition zone < 7.0 mm)

As shown in Table 2, The *R.offcinalis* extract showed antimicrobial activity against all eight microbial isolates tested. Such findings support the already widespread use of this plant in Palestinian traditional medicine to treat bronchitis, heart diseases, bacterial infections and infertility as reported by Jaradat (2005). The above results are consistent with earlier studies of Moreno et al. (2006), Toroglu (2011), Elgayyar et al. (2001) and Wang et al. (2012). *K. pneumoniae* was the least susceptible microbe and was only inhibited at 25.0 mg/well.

Table 2: Zone of Inhibition (mm) for *R. officinalis* Ethanolic Extract at Various Concentrations against some Microbes

	mg/well			
Microbes	25	12.5	6	3
S. aureus	25.0±1.7	20.3±0.6	14.2±1.2	11.3±1.5
E. coli	14.7±1.5	9.0±1.7	R	R
C. albicans	25.0±2.0	21.7±0.6	17.7±1.5	10.7±2.5
P .aueruginosa	16.0±1.0	13.3±1.5	9.7±1.2	R
K. pneumoniae	9.7±0.6	R	R	R
A. niger	18.7±1.5	12.0±1.7	8.7±1.2	R
M. luteus	27.3±1.5	23.0±1.0	19.3±2.1	10.0±2.0
B. subtilis	20.0±1.7	14.3±1.2	12.0±1.0	8.3±0.6

Data represented as means ± SD (n=3), R= absence of susceptibility (inhibition zone < 7.0 mm)

The antimicrobial activity of *P. guajava* ethanolic extract against microbes used in this study has been illustrated in Table 3. The growth of *S. aureus* and *C. albicans* was inhibited, in well-agreement with results reported by Nascimento et al. (2000). However, *K. pneumoniae*, *B. subtilis*, *E. coli* and *P. aueruginosa* were resistant, contradicting results obtained by Nascimento et al. (2000). *A. niger* growth was not inhibited contradicting the results reported by Dhiman et al. (2011).

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Table 3: Zone of Inhibition (mm) for *P. guajava* Ethanolic Extract at Various Concentrations against some Microbes

	mg/well			
Microbes	25	12.5	6	3
S. aureus	21.3±1.2	17.3±1.5	14.0±1.0	9.7±1.1
E. coli	R	R	R	R
C. albicans	23.7±1.5	21.0±1.0	16.3±1.2	10.7±2.1
P. aueruginosa	R	R	R	R
K. pneumoniae	17.0±1.7	11.7±1.5	8.3±1.2	R
A. niger	R	R	R	R
M. luteus	18.7±1.5	15.3±0.6	13.3±2.1	10.0±1.0
B. subtilis	19.3±1.1	15.7±1.5	13.0±1.0	9.0±2.0

Data represented as means ± SD (n=3), R= absence of susceptibility (inhibition zone < 7.0 mm)

In *P. granatum* peel, the growth of all microbes tested was inhibited, except that of *P. aueruginosa* as shown by Table 4. Such antimicrobial activities are in agreement with results reported by Al-Zoreky (2009) with only one contradiction in the effect against *P. aueruginosa.* Overall, *P. granatum* peel extract has shown a significant antimicrobial activity and ranked as the second (after *R. officinalis*) among the extracts tested.

Table 4: Zone of Inhibition (mm) for *P. granatum* Peel Ethanolic Extract at Various Concentrations against some Microbes

	mg/well			
Microbes	25	12.5	6	3
S. aureus	28.3±2.5	21.0±2.0	13.7±1.2	9.3±1.5
E. coli	27.3±2.1	22.0±1.0	14.7±1.5	10.0±1.7
C. albicans	17.0±1.0	13.3±1.5	9.3±1.5	R
P. aueruginosa	R	R	R	R
K. pneumoniae	20.7±1.5	15.7±0.6	9.0±1.0	R
A. niger	18.7±1.5	13.7±1.5	8.7±1.2	R
M. luteus	21.3±2.5	15.3±0.6	12.3±1.5	8.0±1.0
B. subtilis	28.0±1.7	24.0±1.0	20.0±2.0	11.3±1.5

Data represented as means ± SD (n=3), R= absence of susceptibility (inhibition zone < 7.0 mm)

E. coli and *S. sureus* growths were inhibited using extracts from *T. polium* conforming previous findings by Darabpour et al. (2010), while *P. aueruginosa* was resistant (Table 5) as confirmed by Dababneh (2008). Also, *C.albicans* growth was inhibited, contradicting results obtained by Kunduhoglu et al. (2011). To the best of the author's knowledge, this is the first time to report the antimicrobial activity of *T. polium* extract against *K. pneumoniae, M. luteus, A. niger,* and *B. subtilis.*

Table 5: Zone of Inhibition (mm) for *T. polium* Ethanolic Extract at Various Concentrations against some Microbes

	mg/well			
Microbes	25	12.5	6	3
S. aureus	18.3±1.5	11.0±1.0	7.7±0.6	R
E. coli	8.7±1.5	R	R	R
C. albicans	19.7±2.1	14.3±0.6	12.0±0.6	8.3±1.5
P. aueruginosa	R	R	R	R
K. pneumoniae	15.0±2.0	7.7±1.2	R	R
A. niger	R	R	R	R
M. luteus	12.3±1.5	8.3±0.6	R	R
B. subtilis	R	R	R	R

Data represented as means ± SD (n=3), R= absence of susceptibility (inhibition zone < 7.0 mm)

All of the extracts tested had concentration dependent antimicrobial effect on the test organisms as illustrated in Table 1, Table 2, Table 3, Table 4, and Table 5.

As a negative control, antimicrobial activity effect of 10% DMSO was investigated against the microbes used in this study. Fifty µl of 10 % DMSO was added to wells in Muller Hinton already streaked with the microbes used in this study. After incubation of 24 hours at $37C^0$ for all microbes except *C. albicans* and *A. niger* which were incubated for 72 hours at 37^0C, results showed no inhibition zones around the wells of the 10% DMSO against all tested microbes. Such a result shows that 10% DMSO could be used as diluents for the above extracts without affecting the growth of the microbes tested.

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The antimicrobial effects of several antibiotics against the tested microbes were investigated as a positive control. The diameter of the inhibition zones around the effective antibiotics is comparable to those found around the wells of most extracts tested. While some microbes were sensitive to few antibiotics and resistant to the others (Table 6), *S. aureus* and *M. luteus* growth was inhibited by all of the antibiotics tested, whereas *P. aueruginosa* growth was resistant to all of the above antibiotics. *P. aueruginosa* is a well-known resistant bacterium for most of the commercial antibiotics as reported by Lambert (2002).

Table 6: Zones of inhibition of several antibiotics against some microbes
Data represented as means ± SD (n=3), R= absence of susceptibility (inhibition zone < 7.0 mm)

	Antibiotics							
Microbes	GM	K	Am	S	P	CIP	CN	B
S. aureus	15.7±1.2	12.3±0.7	24.7±1.5	12.7±1.5	29.3±2.3	23.0±1.7	13.0±2.0	11.7±1.2
E. coli	21.3±1.5	23.7±1.5	14.7±1.2	17.0±1.7	R	R	R	R
C. albicans	33.7±2.1	R	R	R	34.0±1.7	18.7±1.2	20.7±1.5	R
P. aueruginosa	R	R	R	R	R	R	R	R
K. pneumoniae	22.0±2.0	27.7±2.5	R	R	R	22.7±2.1	16.7±1.5	R
A. niger	R	R	R	R	R	R	R	R
M. luteus	23.3±2.1	18.5±1.7	19.7±2.5	26.3±2.1	31.3±1.5	23.7±1.5	22.3±2.3	14.7±1.5
B. subtilis	20.3±1.5	16.7±1.2	11.0±1.0	20.7±1.5	21.7±1.5	31.0±2.6	20.7±1.2	R

Each of the above extracts had inhibited the growth of at least one gram positive bacterium, one gram negative bacterium, and one fungus (as shown in Table 1, Table 2, Table 3, Table 4, and Table 5). Such a finding reflects the ability of the above extracts to play different mechanisms to destroy different pathogens. Also, it justifies the widespread use of the above plants in traditional medicine against many diseases. However, further research is needed to identify the active ingredients and their modes of actions in the extracts used in this study.

Except for *T. polium*, all tested extracts had shown antibacterial activity against *B. subtilis*. Such a result is very interesting, because this bacterium is a spore former and shows a strong resistance to environmental conditions (Nascimento et al., 2000). Also, this result is in agreement with the considerable use of the tested plant extract in folklore medicine.

Among all the plant extracts tested, *R. officinalis* had shown a remarkable performance as it was the only type of extract to inhibit the growth of *P. aueruginosa*, a bacterium that is very difficult to control through therapeutic means (Lambert, 2002). Contradictions between the antimicrobial activities that were shown by this study and some of the previous studies could be due to many reasons, including different microbial strains, species of herbs, extraction processes and concentrations of extracts and microbes.

The well plate diffusion method used in this study was found to be better than the disc diffusion method (El Astal et al., 2005). Although the above extracts had shown an antimicrobial activity against most of the test organisms that were used in this study, it is unfortunate that the extracts were not tested and applied on the ill people suffering from proposed diseases in the folk medicine.

In general, microbial resistance against antibiotics is still debatable and could be due to antibiotic inactivation by enzymatic inactivation, target mutation, altering the efflux pump mechanisms, and decreased uptake of antibiotics, and other factors. On the other hand, bioactive ingredients of some other compounds have the ability to inhibit resistance mechanisms through different ways such as: inhibition of DNA/protein synthesis, inhibition of folate biosynthesis and cell permeability/cell wall inhibition (Premkumar et al., 2010). Nevertheless, the extracts used in this research may contain components with antimicrobial properties that can be used as antifungal and antimicrobial agents in new drugs to treat infectious diseases.

Scavenging effect of herbal extracts on DPPH radicals

T. polium, R. officinalis, P. granatum peel, and *P. guajava* leaves and grape seeds extracts were tested for their ability to scavenge radicals by the DPPH method. All extracts have shown a significant radical-quenching ability as illustrated in Table 7 and Table 8. The most efficient extract was *Pisidium guajava* leaves represented by the lowest EC_{50} value (EC_{50} = 11.25), followed by grape seeds (EC_{50} = 12.7), *Punica granatum* peel (EC_{50} = 16.0), and *Rosmarinus officinalis* (EC_{50} = 18.0). *Teucrium polium* showed the least radical-scavenging activity (EC_{50} = 20.5) among the above extracts. These results are consistent with previous studies of Goulas et al. (2012), Choi et al. (2011), Moreno et al. (2006), Jayaprakasha et al. (2003), and Jiménez-Escrig et al. (2001).

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The ability of the above extracts in scavenging DPPH through hydrogen (H) transferring reactions is strong evidence of their ability in intercepting reactive oxygen species (ROS). As mentioned previously, the plant extracts tested in this study are widely used in folklore medicine to treat various medical disorders, such as atherosclerosis, diabetes mellitus, rheumatoid arthritis, fevers. However, the pathogenesis of such diseases involves oxidative stress which releases free radicals in infected cells of the human body. Such free radicals could be detoxified by the intake of medicinal plant extracts such as those investigated in this study as (Murdaca et al., 2012) .

The scavenging activity is inversely proportional to the EC_{50} value (Awah et al., 2012); suggesting *P. guajava* leaves extract to be the most efficient among the extracts tested in this study.

Table 7: DPPH radical scavenging activity (%) of some plant extracts at different concentrations

	Scavenging activity(%) of extracts				
Concentrations (µg/ml)	*Pisidium guajava*	Grape seeds	*Rosmarinus officinalis*	*Teucrium polium*	*Punica granatum*
2	16.1±1.9	18.9±1.0	9.3±1.6	12.1±1.2	7.3±1.0
4	20.3±0.9	23.1±1.6	12.2±1.0	14.4±0.7	12.4±1.4
8	34.9±0.9	37.8±1.6	26.0±2.4	18.1±0.7	15.6±1.6
16	71.2±1.0	56.0±1.5	45.0±3.3	36.1±2.1	37.4±1.0
32	91.4±0.7	90.3±0.4	66.7±2.5	66.4±2.7	67.1±1.9
64	92.1±0.2	91.5±0.3	90.1±0.8	89.4±1.3	75.7±1.7
125	93.1±0.2	93.8±0.6	94.6±0.5	92.6±0.6	87.4±3.1

Data represented as means ± SD (n=3)

Table 8: DPPH radical scavenging activity represented by EC_{50}

	Plant Extracts				
	Pisidium guajava	Grape seeds	*Rosmarinus officinalis*	*Teucrium polium*	*Punica granatum*
EC_{50}	11.25	12.7	18.0	20.5	16.0

Conclusion

The above findings show that the tested plant extracts have a wide microbial activity against eight species of clinical pathogens, as well as significant antioxidant capability. Such results could be considred for the possible use of the above extracts as natural fungistatic, fungicidal, bacteriocidal, bactriostatic and antioxidant components in natural preservatives as well as pharmaceutical products. The results of this study support the use of these extracts in the traditional medicine against a wide spectrum of clinical disorders once they are proven to be safe after performing a cytotoxicity investigation. The ability of the tested extracts to inhibit the growth of different clinical pathogens suggests that further study is needed to identify active compounds and their modes of action on pathogenic organisms.

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