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Chemical composition and biological activities of the essential oils of *Psidium guajava* leaf

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ABSTRACT

As a herbal medicine, essential oil is used for the treatment of several chronic diseases, however, the clinical evidence of essential oil is insufficient. In this context, we have made an effort to quantify its percentage of chemical composition and the biological potency of essential oil of the leaves of *Psidium guajava*. The content of essential oil was found in 0.38% (v/w) and the oil was isolated by using hydro distillation from the leaves of the selected plant. The isolated oil was analyzed by using gas chromatography–mass spectrometry (GC–MS) equipped with a HP-5MS fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 μm). The antimicrobial and cytotoxic activities of the isolated leaves oil at different concentrations were determined by agar gel diffusion and brine shrimp lethality bioassay. The antimicrobial activity of the essential oil at various concentrations was determined against three Gram (+) *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus aureus* and three Gram (−) *Escherichia coli*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* bacterial strains. A total of 54 chemical compounds were detected by using GC–MS which is representing 98.172% of the total oil. The dominating ingredients in the leaves oil were *iso*-caryophyllene (33.53%), veridiflorene (13.00%), farnesene (11.65%), *dl*-limonene (9.84%), δ-cadinene (1.75%), α-copaene (2.80%), α-humulene (3.74%) and τ-cadinol (0.08%). The oil showed significant antimicrobial activity against both Gram (+ and −) bacterial strains with an inhibition range of 0–13 mm. However, the isolated leaves oil did not show any activity at any concentration against brine shrimp lethality bioassay. The isolated oil showed significant antimicrobial activity and it could be used as natural antibiotics.

1. Introduction

Since the ancient time, natural products have been used as therapeutic agents for the treatment different diseases. A good number of traditional herbal drugs with significant potential are available in the global market (Martins, 2013). Most of them are derived from natural sources and most of them are using the traditional medical system. Recently, scientists and researcher are involving to find the new drugs from natural sources with significant potential. A simple, though the incomplete definition of essential oil is the predominantly volatile material possessing odor and other characteristic properties of the plant which is isolated by some physical process. They are normally organic with several classes of structural product oils such as non-volatile or fixed oils, including fatty oils which are completely different in composition from the essential oils since they contain mainly glycerides (fatty acid esters of glycerol). The most important and well-known oil on earth is petroleum, which is a product derived from prehistoric forests. Essential oil and its related products are composed of liquid and tiny-solid hydrocarbons. The components of essential oils usually contain fifteen carbon atoms or less. Some small droplets of non-volatile liquids may be swept into the receiver during the distillation. Essential oils are made up of three elements almost exclusively carbon, hydrogen, and oxygen. By far the most common component class is the terpenes. The structure of terpenes can be rationalized as the joining together of identical branched units each of which comprises of five carbon atoms. Recently, the

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essential oil has been approved by the US Food and Drug Administration department as a food additive (WHO, 2006). Traditionally, the essential oils are used for the treatment of insomnia, convulsions, epilepsy, bronchitis, asthma attacks, wound healing, pain relief, obesity and to control of diabetes mellitus. More recently, sustainability and making eco-friendly product is the focus of the medicine and biomedicine industry. Microencapsulation can be an important tool to protect unstable or non-substantive biodegradable essential oil (Samanta et al., 2014).

Psidium guajava (*P. guajava*) is a medicinal plant belongs to the family Myrtaceae (Wagner et al., 1990). Its common name is *guajava* and Arabic name is Jewafa. The selected plant has several species and the majority of these species are essential oil-bearing plants. About 75 genera and nearly 3000 species are available belong to the family. Most of the species are evergreen trees and shrubs and mainly grown tropical and subtropical countries (Wagner et al., 1990). The selected plant is indigenous to Central America and some part of South America between Colombia and Peru (Sahu et al., 2016) and largely available in Mexico down to Sao Paolo state and Brazil (Almaguer et al., 1997). The plant is widely available in many countries like Nigeria, Philippines, Amazonia, Cuba, Ghana, Haiti, Malaya, India, Trinidad, and Pakistan (Sanda et al., 2011). It is also grown in many Arabic countries like Saudi Arabia, Egypt, including Oman (Milyani and Ashy, 2012). The leaves and fruits are very important due to food and nutritional values throughout the world (Joseph and Priya, 2011) (Fig. 1). All parts of the selected plant possess well-known economic and medicinal value (Belardo et al., 1986; Kumar, 2012). In addition, the fruits, leaves, and roots of this plant are also well-known medicine in the traditional medical system (Shruthi et al., 2013).

The histochemical and phytochemical analyses showed that the plant contains several chemical compounds such as flavonoids, tannins, phenols, triterpenes, saponins, carotenoids, lectins, vitamins, fiber and fatty acids, resins, glycosides (Joseph and Priya, 2011; Gutierrez et al., 2008). Some previous studied showed that the essential oil of the selected plant contains β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humlene, selinene, cardinene, curcumene in the high percentage (Satyal et al., 2015). In addition, the essential oil also contains β-caryophyllene, α-pinene and 1,8-cineeole (Chen and Yen, 2007). The leaves extract was found to possess anticestoidal, analgesic, anti-inflammatory, cough sedative, anti-diarrheic, hepato-protective, antioxidant, anti-hypertension properties (Gutierrez et al., 2008). Traditionally, in Oman, the selected plant is used for the treatment of insomnia, convulsions, epilepsy, bronchitis, asthma attacks, wound healing, pain relief, obesity and to control of diabetes mellitus (Metwally et al., 2010; Barbalho et al., 2012). Nowadays, it is commercially cultivated worldwide, including Oman due to its medicinal importance and phytochemicals. From the literature survey reveals that no scientific research has been done on essential oil, which was isolated from Omani species. The lack of information or data in regards to antimicrobial and cytotoxic activities of the essential oil of the selected plant. So far our knowledge, this report is the first report on the analysis of chemical composition of the selected oil and its antimicrobial and cytotoxic activity of the leaves essential oil of *P. guajava* leaves collected from Oman. Therefore, the present study was designed to isolate essential oil by hydro distillation and to determine their chemical composition, antimicrobial and cytotoxic activities of leaves essential oil of *P. guajava* native to Sultanate of Oman.

2. Materials and methods

2.1. Chemicals and reagents

Most of the reagents and solvents used in the present experiment were obtained Sigma-Aldrich Company, UK. Filter paper discs of diameter 6 mm were obtained from Whatmann Company. Nutrient ager and plastic petri dishes were from Sharlau Chemie Company. Brine shrimp eggs (Artemia cysts) were purchased from Goaqua, Taiwan. Sea salt was obtained from Al-Qurum, Muscat, Oman. Ciprofloxacin and dimethyl sulphate (DMSO) were collected from Merck, Germany. All the glassware used in this present experiment was from Borosil, India.

2.2. GC–MS instrument and condition

The analyses of the isolated leaves oil were performed using a sensitive Perkin Elmer gas chromatography-mass spectrometry (Model: Clarus 600) which was equipped with a capillary silica column (HP-5MS; 30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thickness). The program of the injector, transfer line, and ion source were set at 290, 260 and 260 °C, respectively. The oven temperature was from 50 to 280 °C at 3 °C/min and then held isothermal for 10 min. and finally raised to 280 °C at 5 °C/min. As the carrier gas, inert gas (Helium, purity 99.9999%) was used with a flow rate of 1 ml/min.

Fig. 1. Plant leaves picture of *P. guajava*.

One microliter oil was injected by using a Hamilton syringe into the injector with a split mode. An electron ionization mode was used for MS detection with ionization energy of 70ev. The chemical compounds of the leaves oil were identified and quantified by comparison of their spectral data with reference spectra in the databases (Wiley AccessPak V7, May 2003 and NIST2005 version 2.1.0). *n*-alkanes (C_7-C_{26}) was used as a standard to get the retention indices.

2.3. Microbes

The selected culture three Gram (+) bacterial strains (+): *Enterococcus faecalis* (*E. faecalis*), *Staphylococcus aureus* (*S. aureus*) and *Streptococcus aureus* (*St. aureus*) and three Gram (−) bacterial strains: *Escherichia coli* (*E. coli*), *Haemophilus influenzae* (*H. influenzae*), and *Pseudomonas aeruginosa* (*S. aeruginosa*) were collected on October 2012 from the Department of Microbiology, Nizwa Hospital, Nizwa, Oman. All the collected microbes are clinically isolated bacterial strains.

2.4. Plant material

The target leaves samples of the selected plant were collected from Alsharqia region, Sultanate of Oman during the month of October 2012 at 8–10 am. The morphological characterization of the collected leaves samples was done by the Ministry of Agriculture and Fisheries and the voucher specimen (00) was kept in their lab. In addition, database and morphological characterization of selected plant species are present in the website (https://en.wikipedia.org/wiki/Guava). The leave samples were separated instantly after harvesting and packed in a polyethylene bag for the protection of volatile oils. The bag was transported to a laboratory at the University of Nizwa for the extraction purpose.

2.5. Extraction of essential oil

The extraction of essential oils from the *P. guajava* leaves was performed by using a Clevenger apparatus. Fresh leaves (117.6 g) were subjected to hydro distillation for three hours by using a Clevenger type apparatus (Rahma et al., 2013). The isolated oil was anhydrous by Na_2SO_4. The water free oil was preserved in a sealed amber colour vial at 4 °C in the refrigerator until analyzed by using a sensitive GC–MS.

2.6. Antibacterial assay

The antimicrobial activity of the isolated leave oil of *P. guajava* was determined by disc diffusion method with minor modification (Hossain et al., 2011). In our present experiment, various concentrations of oil such as 1000, 500, 250 and 125 μg/ml were prepared by DMSO. Ciprofloxacin and DMSO were used as positive and negative controls. The standard (250 μg/ml) was prepared by using the same solvent. The agar plates were prepared by the distribution of each bacteria strain such as Gram (+ and −) over the plates. After that, the filter paper disc (6 mm diameter) was dipped in each prepared concentration and then placed on the agar plates inoculated with the bacteria. All the experimental plates were incubated at less than 37 °C for 24 h. The growth of inhibition of each bacterial strain was calculated manually by the scale. The experiment was run in triplicates.

2.7. Cytotoxic assay

Thirty-eight gram of NaCl was dissolved in 1000 ml of distilled water and filtered to obtain a clear solution (Afaf et al., 2014). The sea salt was placed in a small tank divided into two compartments by perforated polyethylene wall. About 50 mg of GOAQUA brine shrimp eggs were sprinkled at the covered chamber of the duo compartment plastic container. The open compartment was illuminated to attract the shrimp larvae from the dark compartment once were hatched within 24 h. These nauplii were taken into cytotoxic bioassay. Four milligram essential oils were taken in a vial and dissolved in 100 μl of pure dimethyl sulfoxide (DMSO) to get a stock solution (Rehab and Hossain, 2016). Then the solution was serial dilution to 10, 100, 250, 500 and 1000 μg/ml with sea water. From each of these test solutions, 50 μl was added to premarked test tubes containing 5 ml of sea water and 10 nauplii. Five milliliters of seawater was added to each test tube containing 10 brine shrimp nauplii. A magnifying glass used for counting surviving nauplii of each tube. Different concentrations of the test sample were applied to the test tubes containing nauplii. After 24 h, the test tubes were observed and the number of surviving nauplii in each test tube was counted using a magnifying glass and recorded. From the record, the percentage of lethality of brine shrimps was calculated for each concentration of the sample.

3. Results

3.1. Chemical ingredients of essential oils

The oil was isolated from the leaves by using hydro distillation method to give about 0.45 ml (0.38% v/w) of a yellowish oil of pleasant odor. GC–MS analysis of the isolated essential oil from the leaves of *P. guajava* led to the identification of fifty-four compounds which was representing about 98.172% of the total oil. The chemical composition of the essential oil is presented in Table 1 according to their elution order in a silica gel capillary column. The major chemical constituents detected in the fresh leave oil were *iso*-caryophyllene (33.53%), veridiflorene (13.00%), farnesene (11.65%), *dl*-limonene (9.84%), δ-cadinene (1.75%), α-copaene (2.80%), α-humulene (3.74%), aromadendene (1.70%) and τ-cadinol (0.08%). The chemical structures of the major ingredients are presented in Fig. 2.

3.2. Antimicrobial activity

The antimicrobial activity of the isolated essential oil of *P. guajava* at different concentrations was evaluated by agar diffusion bioassay against three Gram (+) bacterial strains: *E. faecalis*, *S. aureus* and *St. aureus* and three Gram (−) bacterial strains: *E. coli*, *H influenzae*, and *P. aeruginosa*. All the prepared concentrations gave potent inhibition against all applied selected Gram (+) bacterial strains with an inhibition range of 0–13 mm (Table 2). The highest activity was found at the concentration of 1000 μg/ml against *E. coli* and second highest was found at the same concentration against *H. influenza* and *S. aureus*.

3.3. Cytotoxic activity

The cytotoxic activity of various concentrations of essential oil of *P. guajava* was evaluated by brine shrimp lethality bioassay. Five concentrations such as 10, 100, 250, 500 and 1000 μg/ml were used in the present experiment. After 24 h incubation, the number of surviving nauplii in each test tube was counted by using a magnifying glass. We did not find any mortality at any of our working concentration. That means, all the prepared concentrations did not show any activity against by brine shrimp lethality bioassay.

Table 1
Percentage composition of the leaves essential oil of *P. guajava*.

Sl No.	Retention time (min)	Compound Name	KI	Area%
1	5.54	α-Thujene	934	0.08
2	6.21	Phenylmethanal	959	0.08
3	7.021	β-Myrcene	990	0.10
4	8.251	*dl*-Limonene	1028	9.85
5	8.356	1,8-Cineole	1032	0.35
6	8.511	*cis*-Ocimene	1036	0.23
7	8.877	β-Ocimene	1047	0.10
8	10.752	Linalool	1101	0.04
9	22.042	α-Copaene	1379	2.80
10	23.423	U.I	1406	0.03
11	23.833	Iso-Caryphyllene	1411	33.54
12	24.158	U.I	1415	0.03
13	24.323	U.I	1417	0.03
14	24.584	Aromadendene	1421	1.80
15	24.759	U.I	1423	0.04
16	25.169	α-Humulene	1428	3.48
17	25.454	Alloaromadendrene	1432	0.60
18	26.089	α-Amorphene	1440	0.23
19	26.459	U.I	1445	0.09
20	26.549	U.I	1446	0.04
21	26.819	Seychellene	1449	0.19
22	27.03	α-Muurolene	1452	0.14
23	27.115	α-Caryophyllene	1453	0.09
24	27.34	α-Bergamotene	1456	0.11
25	27.555	U.I	1459	0.19
26	27.925	δ-Cadinene	1464	1.75
27	28.25	U.I	1468	0.47
28	28.65	U.I	1473	0.06
29	28.995	U.I	2002	0.08
30	29.281	U.I	1481	0.78
31	29.441	Farnesene	1483	11.65
32	29.571	U.I	1485	0.23
33	29.681	U.I	1486	0.18
34	29.981	U.I	1490	0.17
35	30.206	Veridiflorene	1493	13.00
36	30.511	U.I	1497	0.42
37	30.591	U.I	1498	0.12
38	30.956	U.I	1506	2.13
39	31.166	U.I	1512	0.42
40	31.281	U.I	1515	0.03
41	31.442	U.I	1519	0.37
42	31.662	δ-Guaiol	1525	0.23
43	31.872	U.I	1531	2.33
44	32.017	U.I	1535	1.25
45	32.162	U.I	1539	3.55
46	32.362	U.I	1552	2.07
47	32.532	U.I	1549	1.38
48	32.647	U.I	1553	0.08
49	32.817	τ-Cadinol	1557	1.08
50	32.937	U.I	1561	0.34
51	33.237	U.I	1569	0.13
52	33.417	U.I	1574	1.02
53	33.542	U.I	1577	0.03
54	33.688	U.I	1581	0.23
	Total			98.17

[a]Retention index relative to *n*-alkanes on HP-5MS capillary column.

4. Discussion

Microbial infections become a life threat and annually seven million deaths are attributed to antimicrobial resistance (WHO, 2006). The sustainable development goal of the United Nations (UN, 2016) was to ensure health and well-being for all at every stage of life. The aim is to end such infectious diseases; and ensure universal access to safe, affordable and effective antimicrobials. Towards that end, we are devoted to conducting research targeting the development of new antimicrobial molecules using available herbal resources to combat resistance and make antimicrobials effective and affordable in regions like Asia and Africa. Nowadays, the demand of herbal drugs is increasing tremendously in both developing and developed countries due to the safe, cost-effective and availability. Plants are the major natural sources of medicine like antimicrobial, anticancer agents, analgesics and so on. Sultanate of Oman is a hot country which is very rich in rare medicinal plants with potential biological activities and has more than one hundred medicinal plants. An essential oil is a liquid that is generally steam or hydro-distilled from flowers, leaves, bark and roots of plants and trees and are the compounds responsible for the aroma and flavor associated with herbs, spices, and perfumes. Most of the essential oils isolated from nature are used tremendously in perfumery, aromatherapy, homeopathy, cosmetics, Ayurveda medicine, incense, cleaning products, and food and drinks. Essential oil molecules are made up primarily of carbon, hydrogen, and oxygen. The aromatic constituents of essential oils are built from hydrocarbon chains. An essential oil is volatile organic compounds comprised of different types of chemical compounds. Most of the essential oil contains mono terpenes, sesquiterpenes, phenolics hydrocarbons and their derivatives. The chemical ingredients and their derivatives in the essential oil have several physiological and biological benefits such as antibacterial, antifungal, insecticidal and antioxidant capacities. An essential oil is widely used all over the world to flavor food and antimicrobial agent. The essential oil finish products can be used for developing different medicine and pharmaceutical applications. The outcome through this present study is that, this novel technique can be used for the design and development of customized aroma and give the new idea regarding on pharmaceutical finishing products, microbiology, pharmaceutical, chemical research etc. In this study, the essential oil was isolated from the fresh leaves of *P. guajava* by hydro distillation method. The isolated essential oil was analyzed by using sensitive GC–MS had led to the identification of total 54 different organic compounds by using the HP-5MS silica capillary column and instrument conditions, representing 98.172% of the total oil from the fresh leaves samples (Table 1). About 50% chemical compounds in the essential oil were unidentified (U.I) Table 1. The major identified chemical compounds that were found in the isolated essential oil are *iso*-caryophyllene (33.53%), veridiflorene (13.00%), farnesene (11.65%), *dl*-limonene (9.84%), δ-cadinene (1.75%), α-copaene (2.80%), α-humulene (3.74%), aromadendene (1.70%) and τ-cadinol (0.08%). Most of the chemical compounds are terpenoids and their derivatives and almost all the detected compounds are isolated and used as medicine for the treatment of different diseases (Hossain et al., 2011; Rahma et al., 2013). Previous investigations showed that the major constituents of the essential oil are α-pinene, limonene, menthol, terpene lactate, isopropyl alcohol, longicyclene, β-caryophyllene, *e*-nerolidol (Soliman et al., 2016). Most of the isolated major individual compound is used as a traditional system for the treatment of different diseases (Hossain et al., 2011; Rahma et al., 2013). *Iso*-caryophyllene (33.53%) and *dl*-limonene (9.84%) are show significant anticancer activity (Soliman et al., 2016; Rahma et al., 2013). The other major compounds also showed different biological activity (Soliman et al., 2016; Afaf et al., 2014).

The antimicrobial activity of various concentrations of essential oil was determined by disc diffusion method against different Gram (+ and −) bacterial strains. In our results showed that all concentrations essential oil gave potential activity against Gram (+) *St. aureus* and *E. faecalis* bacterial strains within the inhibition range of 0–9 mm. In addition, *S. aureus* bacterial strain also showed significant activity against all concentrations within the inhibition range of 0–12 mm except 500 μg/ml. On the other hand, Gram (−) bacterial strain *H. influenza* showed good activity against all concentrations within the inhibition range of 7–12 mm except the lowest applied concentration (125 μg/ml). However, the other two Gram (−) bacterial strains *E. coli* and *P. aeruginosa* showed the highest activity only at the highest applied concentration (Table 2). The isolated oil did not show antimicrobial activities either due to the low concentration of bioactive compounds or decomposed

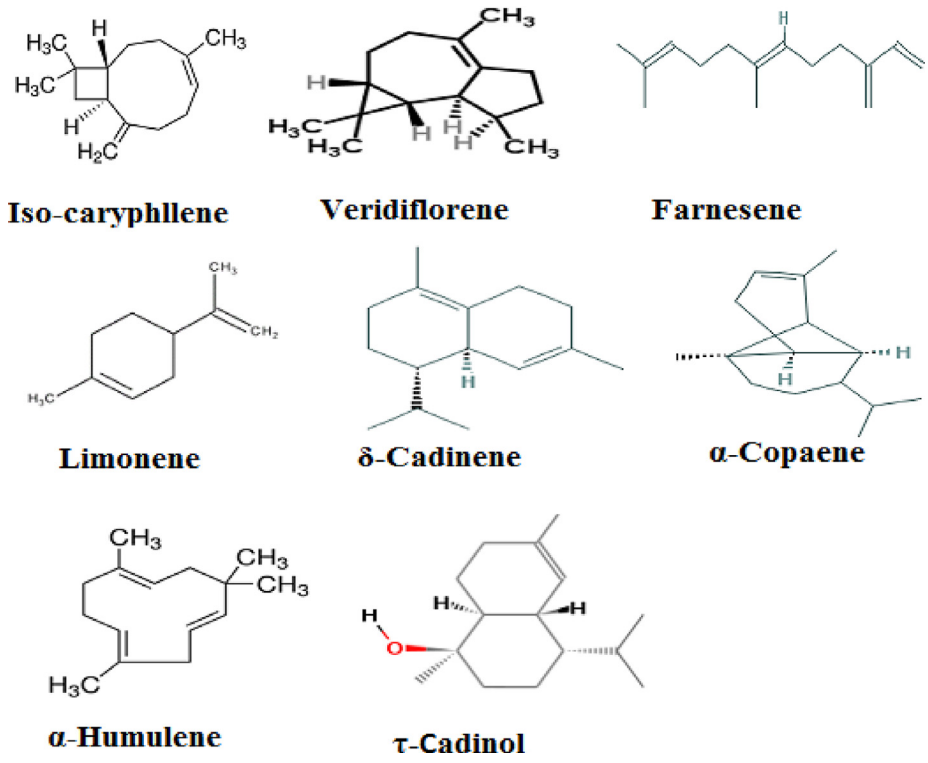

Fig. 2. Chemical structures of the major compounds of leaves essential oil of *P. guajava*.

Table 2
Antimicrobial activity of different concentrations of leave essential oil against selective bacterial strains.

Conc. (μg\ml)	*E. coli*	*H. influenza*	*P. aeruginosa*	*S. aureus*	*St. aureus*	*E faecalis*
1000	13 ± 0.23	12 ± 0.91	6 ± 0.23	12 ± 0.27	9 ± 0.12	6 ± 0.13
500	nd	7 ± 0.19	nd	nd	6 ± 0.15	7 ± 0.23
250	nd	7 ± 0.12	nd	8 ± 0.17	6 ± 0.21	6 ± 0.42
125	nd	nd	nd	9 ± 0.51	6 ± 0.31	6 ± 0.15
Control	28 ± 0.12	29 ± 0.34	13 ± 0.29	29 ± 0.25	31 ± 0.76	32 ± 0.28
DMSO	nd	nd	nd	nd	nd	nd

nd: not detected.

the bioactive compounds during the sample processing or extraction. In addition, it could be due to some volatile components in the selected oil may evaporate from the media, leading to decrease in their concentration. On the other hand, at high concentration, the oil showed significant activity due to bioactive compounds. In addition, the isolated oil is not sensitive to the selected microbes (Goncalves et al., 2008; Penecilla and Magno, 2011; Zhang et al., 2003). Similar, antimicrobial activity report also available elsewhere (Goncalves et al., 2008; Penecilla and Magno, 2011; Rahma et al., 2013. The brine shrimp lethality bioassay is used as a screening tool for the determination of toxicity of essential oil. Many other procedures are available to use for cytotoxicity testing. However, brine shrimp lethality bioassay is the best procedure (Hossain et al., 2009; Afaf et al., 2014). However, in the present experiment, the essential oil did not get any mortality at any concentration. Our results vary from other studies that might be attributed to variation in the environmental conditions, including day length, light intensity, ambient temperature, rainfall, soil or season of collection (Falope et al., 1993).

5. Conclusion

The results of this study showed that there was a great variation in the constituent of the essential oil from the leaves of *P. guajava*. Several terpenoids types of compounds with very high concentration were identified by GC–MS. The essential oil showed significant activity against tested Gram (+ and −) bacterial strains. In addition, the essential oil did not show any mortality against brine shrimp assay at any applied concentrations. In conclusion, the highest antimicrobial activity essential oil could be used as natural antibiotics. This study provides brief scientific information regarding the selected essential oil however, further study needed to isolate and determine the antimicrobial compounds and investigate other pharmacological properties. Therefore, the isolated essential oil could be used as medicine for the treatment of infectious diseases.

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Conflicts of interest

The authors declare no conflicts of interest.

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