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# Comparative study of the volatile oil content and antimicrobial activity of *Psidium guajava* L. and *Psidium cattleianum* Sabine leaves

**Fathy M. Soliman, Magda M. Fathy, Maha M. Salama, Fatema R. Saber** *

*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr el-Aini Street, Cairo 11562, Egypt*

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**Abstract** The chemical composition of the hydrodistilled oils of the leaves of *Psidium guajava* L. (guava leaf) and *Psidium cattleianum* Sabine (strawberry guava) was determined by GC/MS analysis to identify their chemotypes. Moreover, *in vitro* antimicrobial activity of these volatile oils against selected bacteria, yeast, and mycelia fungi was studied. The yield of the volatile oil hydrodistilled from the leaves of *P. guajava* L. and *P. cattleianum* Sabine was 1.6 and 2.69 g/kg on fresh weight basis, respectively. Limonene was the major identified hydrocarbon in *P. guava* leaves' oil (54.70%), whereas, 1, 8-cineole was the major identified oxygenated monoterpenoid (32.14%) in common guava leaves. The foliar oil of *P. cattleianum* was predominated by the sesquiterpene hydrocarbon; *β*-caryophyllene representing 28.83% of the total oil make-up. The antibacterial activity of guava leaf oil was more pronounced against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* than that of strawberry guava leaves, while *P. cattleianum* showed a higher activity against *ess*. The MIC of the volatile oil of the leaves of *P. guajava* against *S. aureus* was 6.75 μg/ml, while that of *P. cattleianum* exhibited MIC value of 13.01 μg/ml against *Neisseria gonorrhoeae*. Results demonstrated that the volatile oil of both *Psidium* species showed different chemotypes. Moreover, the volatile oils of guava and strawberry guava leaves might be good candidates as antimicrobial agents.


## 1. Introduction

Infectious diseases are major causes of death world-wide. Infections with bacteria are associated with high morbidity and mortality especially with immunocompromised patients.[1,2] The main strategies to prevent and control infectious diseases include public health improvements in sanitation and hygiene, safe water initiatives, as well as vaccines and the use of antimicrobial agents.[3]

Antibiotic resistance has become a global concern.[4] This guided the search for new chemotherapeutic agents to combat the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotics.[5,6] Natural products could be

* Corresponding author.
E-mail address: Fatema.Saber@pharma.cu.edu.eg (F.R. Saber).
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considered as new drug leads owed to their chemical diversity.[7] Therefore, researchers are increasingly paying their attention to herbal medicine to find natural solutions against microbial infections.[8]

Essential oils evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for treatment of many infectious diseases.[9] They possessed antibacterial, antifungal, antiviral, insecticidal and antioxidant properties.[10,11]

*Psidium guajava* (common guava), is native to Mexico.[12] It extends throughout South America, Europe, Africa and Asia. It grows in all the tropical and subtropical areas of the world. It adapts to different climatic conditions but prefers dry climates.[13] *P. guajava* is used in many parts of the world for treatment of many ailments. A decoction of new shoots is taken as febrifuge. An aqueous leaf extract is used to reduce blood glucose level in diabetics.[14] Leaves are applied on wounds, ulcers, while they are chewed to relieve toothache.[15] The leaves are used in China as antiseptic for treatment of diarrhoea.[16]

In USA, guava leaf extracts are used in various herbal formulas for a variety of purposes; from herbal antibiotics and diarrhoea formulas to bowel health and weight loss formulas.[17] In Brazil, the fruit and leaves are considered for anorexia, cholera, diarrhoea, digestive problems, dysentery, gastric insufficiency, inflamed mucous membranes, laryngitis, mouth swelling, skin problems, sore throat, ulcers and vaginal discharge.[18]

*Psidium cattleianum* Sabine (strawberry guava) is a shrub or small tree native to Brazil, popularly known as ''araca''.[19] It is grown throughout the tropics and subtropics for its edible fruits.[20] Its leaves are used in folk medicine as an anti-haemorrhagic, anti-spasmodic and anti-diarrhoeal agent.[21,22]

The aim of our study is to determine the chemical composition of the volatile oils of the leaves of *P. guajava* L. and *P. cattleianum* Sabine cultivated in Egypt in order to identify their chemotypes. Furthermore, to study the *in vitro* antimicrobial activity of these volatile oils against selected Gram positive, Gram-negative bacteria, yeast, and mycelia fungi to justify their use as antimicrobial agents.

## 2. Materials and methods

### 2.1. Plant material

Samples of *P. guajava* L. leaves were collected in December 2012 from El-Behera Governorate, Egypt, while, *P. cattleianum* was cultivated and collected in the same period from the experimental station of medicinal plants, Faculty of Pharmacy, Cairo University. Samples were kindly identified by Dr. Mohamed El-Gebaly (Senior Botanist). Voucher specimens (PG-12-12-2012) and (PC-12-12-2012) were kept at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

### 2.2. Microbial strains

A series of bacterial and fungal strains (available in stock culture of Micro-analytical Centre, Faculty of Science, Cairo university) were used for antibiotic sensitivity testing: Gram positive bacteria *Bacillus subtilis* (ATCC 6051), *Streptococcus faecalis* (ATCC 19433), and *Staphylococcus aureus* (ATCC 12600); Gram negative bacteria [*Pseudomonas aeruginosa* (ATCC 10145), *Neisseria gonorrhoeae* 19424 and *Escherichia coli* (ATCC 11775)]; Filamentous fungi [*Aspergillus flavus* (ATCC 15517)]; and yeast [*Candida albicans* (ATCC 7102)].

### 2.3. Reference drugs

Ampicillin (Sigma Pharmaceutical industries, Monofiya, Egypt) and amphotericin B (Bristol-Myers Squibb, Switzerland) were used.

### 2.4. Preparation of the volatile oil

Five hundred grams of the leaves of each *Psidium* species under investigation was hydrodistilled separately in a Clevenger-type apparatus for 4 h, according to the procedure described in the Egyptian pharmacopeia (2005).[23] The obtained oils were dehydrated by filtration through anhydrous sodium sulfate and kept in a refrigerator for GC/MS analysis and antimicrobial screening.

### 2.5. Determination of physical characters of the volatile oil

The yield of the volatile oils was calculated as weight/weight (g/kg), on fresh weight basis. Colour, odour and specific gravity were determined according to the Egyptian Pharmacopeia method (2005).[23] Results were listed in Table 1.

### 2.6. GC/MS analysis of the volatile oil content

Volatile oil prepared from *P. guajava* L. and *P. cattleianum* sabine leaves were subjected to GC/MS analysis. The injection volume was 1 μL. The instrument was controlled by the Shimadzu Class-5000 Version 2.2 software containing a NIST62 (National Institute of Standards and Technology) MS library. Volatiles were separated on a DB5-MS column (30 m length, 0.25 mm inner diameter, and 0.25 μm film (J&W Scientific, Santa Clara, California). Injections were made in the split mode for 30 s, and the gas chromatograph was operated under the following conditions: injector 220 °C and column oven 40 °C for 3 min, then programmed at a rate of 12 °C/min to 180 °C, kept at 180 °C for 5 min, and finally ramped at a rate of 40 °C/min to 220 °C and kept for 2 min, He carrier gas at 1 mL/min. The transfer line and ion–source temperatures were adjusted at 230 and 180 °C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at 40–500 m/z.

**Table 1** Yield, sensory characters and specific gravity of the volatile oil of the leaves of *P. guajava* L. *and P. cattleianum* Sabine.

| Item | *Psidium guajava* L. | *Psidium cattleianum* Sabine |
|---|---|---|
| Yield (w/w, g/kg) | 1.6* | 2.69* |
| Color | Faint yellow | Yellow |
| Odour | Pleasant odour | |
| Specific gravity | 0.844* | 0.867* |

* Results are average of three determinations.

**Table 2** Constituents identified by GC/MS analysis of the volatile oil of the leaves of *P. guajava* L. and *P. cattleianum* Sabine.

| No | $R_t$ | Constituent | K.I. calc. | K.I. reported | Percentage | |
|---|---|---|---|---|---|---|
| | | | | | *p. guajava* | *p. cattleianum* |
| 1 | 7.69 | α-Pinene | 937 | 932 | 1.53 | **28.0** |
| 2 | 7.99 | Camphene | 954 | 946 | – | 0.08 |
| 3 | 8.24 | Benzaldehyde | 963 | 952 | 0.83 | – |
| 4 | 8.49 | β-Pinene | 980 | 974 | – | 1.30 |
| 5 | 8.65 | β-Myrecene | 990 | 988 | – | **13.40** |
| 6 | 8.96 | α-Phellandrene | 1007 | 1002 | – | 0.30 |
| 7 | 9.015 | δ-3-Carene | 1013 | 1008 | – | 0.16 |
| 8 | 9.137 | α-Terpinene | 1020 | 1014 | – | 0.22 |
| 7 | 9.26 | *p*-Cymene | 1028 | 1020 | 0.52 | 0.23 |
| 8 | 9.34 | Limonene | 1030 | 1024 | **54.7** | 1.63 |
| 9 | 9.42 | 1, 8-Cineole | 1033 | 1026 | **32.14** | – |
| 10 | 9.50 | β-*trans*-Ocimene | 1037 | 1032 | – | 5.25 |
| 11 | 9.57 | β-*cis*-Ocimene | 1047 | 1044 | 0.28 | 1.25 |
| 12 | 9.79 | γ-Terpinene | 1062 | 1054 | 0.38 | 1.13 |
| 13 | 10.24 | Terpinolene | 1090 | 1086 | – | 2.38 |
| 14 | 11.84 | α-Terpineol | 1204 | 1186 | 1.79 | 0.32 |
| 15 | 13.85 | α-Terpinyl acetate | 1355 | 1346 | – | 0.28 |
| 16 | 14.19 | α-Ylangene | 1380 | 1373 | – | 0.21 |
| 17 | 14.27 | α-Copaene | 1390 | 1374 | – | 0.66 |
| 18 | 14.86 | β-Caryophyllene | 1420 | 1417 | 2.91 | **28.83** |
| 19 | 15.23 | α-Humulene | 1460 | 1452 | 0.77 | 3.03 |
| 20 | 15.49 | γ-Muurolene | 1488 | 1478 | – | 0.49 |
| 21 | 15.55 | α-Amorphene | 1496 | 1483 | – | 0.56 |
| 22 | 15.76 | β-Selinene | 1510 | 1489 | – | 0.98 |
| 23 | 15.84 | α-Selinene | 1516 | 1498 | – | 0.54 |
| 24 | 15.88 | γ-Cadinene | 1519 | 1513 | – | 0.32 |
| 25 | 16.07 | δ-Cadinene | 1531 | 1522 | – | 0.85 |
| 26 | 16.32 | *trans*-Cadina-1,4-diene | 1540 | 1533 | – | 0.24 |
| 27 | 16.44 | α-Calacorene | 1553 | 1544 | – | 0.55 |
| 28 | 16.52 | selina-3,7(11)-diene | 1555 | 1545 | – | 0.55 |
| 29 | 16.81 | Germacrene B | 1565 | 1559 | – | 0.29 |
| 30 | 17.23 | Caryophyllene oxide | 1590 | 1582 | – | 2.33 |
| 31 | 18.17 | δ-Cadinol | 1651 | 1658 | – | 0.72 |
| Total identified constituents | | | | | 95.85% | 97.08% |
| *Hydrocarbons* | | | | | | |
| Monoterpenes | | | | | 58.24 | 55.33 |
| Sesquiterpenes | | | | | 3.68 | 38.1 |
| Total hydrocarbons | | | | | 61.92 | 93.43 |
| *Oxygenated compounds* | | | | | | |
| Monoterpenes | | | | | 33.93 | 0.6 |
| Sesquiterpenes | | | | | 0 | 3.05 |
| Total oxygenated compounds | | | | | 33.93 | 3.65 |

$R_t$ = retention time, K.I. = kovat's index, (–) absent.
Bold values are the major constituents in the volatile oils.

The percentages of different components in each oil sample were determined by computerized peak area measurements relative to each other. Volatile components were identified using the procedure described in Farag and Wessjohann (2012).[24] The peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (RI) relative to *n*-alkanes ($C_6$–$C_{20}$), mass spectrum matching to NIST, WILEY library database. Results are recorded in Table 2.

### 2.7. Evaluation of the antimicrobial activity

#### 2.7.1. In-vitro susceptibility test

The volatile oils of the leaves of both *Psidium* species under investigation were screened for their antimicrobial activity against representatives of; Gram–positive bacteria (*B. subtilis, S. aureus* and *Streptococcus faecalis*), Gram-negative bacteria (*E. coli, P. aeruginosa* and *N. gonorrhoeae*), yeast (*C. albicans*) and mycelia fungi (*A. flavus*) applying the agar disc diffusion according to CLSI guidelines (2009).[25]

The volatile oils were tested by impregnating sterile discs of Whatmann filter paper 1 (5 mm diameter) in twenty µl of the oils. Twenty µl of dimethyl sulfoxide was used as a negative control. The reference standards ampicillin and amphotericin B were dissolved separately in dimethyl sulfoxide at a concentration of 20 µg/µl.

The discs were then placed onto the surface of the plates containing the solid bacterial medium (Mueller–Hinton agar) or the fungal medium (Dox's medium) which has been heavily

**Table 3** Antibacterial activity of the volatile oils of *P. guajava* and *P. cattleianum* leaves.

| Micro-organism | Diameter of zone of inhibition (mm)* (%, potency relative to standard drug) | | |
|---|---|---|---|
| | *P. guajava* oil (20 µl) | *P. cattleianum* oil (20 µl) | Ref. standard ampicillin (20 µg/µl) |
| *Bacillus subtilis* | 13 ± 0.23 (65%) | 13 ± 0.21 (65%) | 20 ± 0.2 (100%) |
| *Staphylococcus aureus* | 16 ± 0.15 (88.89%) | 10 ± 0.28 (55.56%) | 18 ± 0.15 (100%) |
| *Streptococcus faecalis* | 12 ± 0.24 (66.67%) | 11 ± 0.15 (61.11%) | 18 ± 0.17 (100%) |
| *Escherichia coli* | 12 ± 0.18 (54.45%) | 10 ± 0.3 (45.45%) | 22 ± 0.25 (100%) |
| *Pseudomonas aeruginosa* | 13 ± 0.31 (65%) | 11 ± 0.17 (55%) | 20 ± 0.22 (100%) |
| *Neisseria gonorrhoeae* | 12 ± 0.22 (70.59%) | 13 ± 0.29 (76.47%) | 17 ± 0.4 (100%) |

* The results are expressed as mean ± standard error, $n = 3$.

**Table 4** Minimum inhibitory concentration of the volatile oils of *P. guajava* and *P. cattleianum* leaves.

| Sample | MIC (µg/ml) | |
|---|---|---|
| | *Neisseria gonorrhoeae* ($G^-$) | *Staphylococcus aureus* ($G^+$) |
| *P. guajava* oil | – | 6.75 |
| *P. cattleianum* oil | 13.01 | – |

seeded with the spore suspension of the tested micro-organisms. The plates were incubated at 37 °C for 25 h in case of bacteria and at 25 °C for 48 h in case of fungi. After incubation, the inhibition zones were measured in mm. Diameters less than 5 mm indicated no effect. The results are recorded in Tables 3 and 5.

### 2.7.2. Determination of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was evaluated for the volatile oils of the two plants, based on the results obtained for the antimicrobial screening. Accordingly, *N. gonorrhoeae* was selected for the oil of *P. cattleianum* and *S. aureus for P. guajava* oil. In brief, Stationary phase cultures of bacterial strains were prepared at 37 °C and used to inoculate fresh 5.0 ml culture to an $OD_{600}$ of 0.05. The 5.0 ml culture was then incubated at 37 °C until an $OD_{600}$ was achieved from which standardized bacterial suspensions were prepared to a final cell density of $6 \times 10^5$ CFU/ml. Serial dilutions from the volatile oils were prepared and mixed with 5.0 ml of the standardized bacterial suspension then added to the plates and incubated for 24 h at 37 °C. The colony forming units (CFU) were counted for each dilution.[26]

*2.7.2.1. Agar dilution method.* The tested samples were serially diluted in molten medium equilibrated at 50 °C with 2% glucose. One millilitre was added to each well in a 24-well plate with a flat bottom and allowed to solidify. The centre of each well was inoculated with 10 ml of the bacterial suspension. Drug free growth control was included. MIC was determined after 48 h at 35 °C. MICs were defined as the lowest concentration that had granular appearing micro-colonies of growth instead of filamentous radiating colonies on solid agar. Results are shown in Table 4.

## 3. Results and discussion

The yield of the volatile oil of the leaves of *P. cattleianum* Sabine cultivated in Egypt was higher than that obtained from *P. guajava* L. (1.6 and 2.69 g/kg on fresh weight basis, respectively). Slight differences were noted between the two oils in their specific gravity and colors, Table 1.

Results of GC/MS analyses of the oils, displayed in Figs. 1 and 2 and Table 2 revealed both qualitative and quantitative variations in the oil composition of both *Psidium* species.

Ten compounds were identified in *P. guajava* oil accounting for 95.85% of the volatile oil of guava leaves. Meanwhile, thirty-one compounds were identified representing 97.08% of the volatile oil of the leaves of *P. cattleianum*.

Both oils were rich in hydrocarbons in the leaves of *P. guajava* L. and *P. cattleianum* Sabine, (61.92% and 93.43%) respectively. Limonene was the major identified monoterpene hydrocarbon in the foliar oil of *P. guajava* L. (54.70%), accounting for the lemon-like odour of the oil. On the other hand, α-pinene was the major identified hydrocarbon in *P. cattleianum* Sabine leaf oil (28.00%).

β-caryophyllene was the main identified sesquiterpene hydrocarbon in *P. cattleianum* Sabine volatile oil (28.83%), while a much lower percentage was present in *P. guajava* L. (2.91%).

The oxygenated constituents were higher in *P. guajava* oil than that of *P. cattleianum* (33.93% and 3.65%, respectively)., 8-cineole (eucalyptol) was only detected in high percentage in the foliar oil of *P. guajava* L. (32.14%). However, oxygenated sesquiterpenes [caryophyllene oxide (2.33%) and δ-cadinol (0.72%)] were only found in the foliar oil of *P. cattleianum* Sabine.

**Table 5** Antifungal activity of the volatile oils of *P. guajava* and *P. cattleianum* leaves.

| Micro-organism | *P. guajava* volatile oil (20 µl) | *P. cattleianum* volatile oil (20 µl) | Ref. standard Amphotericin B (20 µg/µl) |
|---|---|---|---|
| *Candida albicans* | 9 ± 0.1* (42.86%) | 9 ± 0.12* (42.86%) | 21 ± 0.23* (100%) |
| *Aspergillus flavus* | – | – | 19 ± 0.38* (100%) |

* The results are expressed as mean ± standard error, $n = 3$.

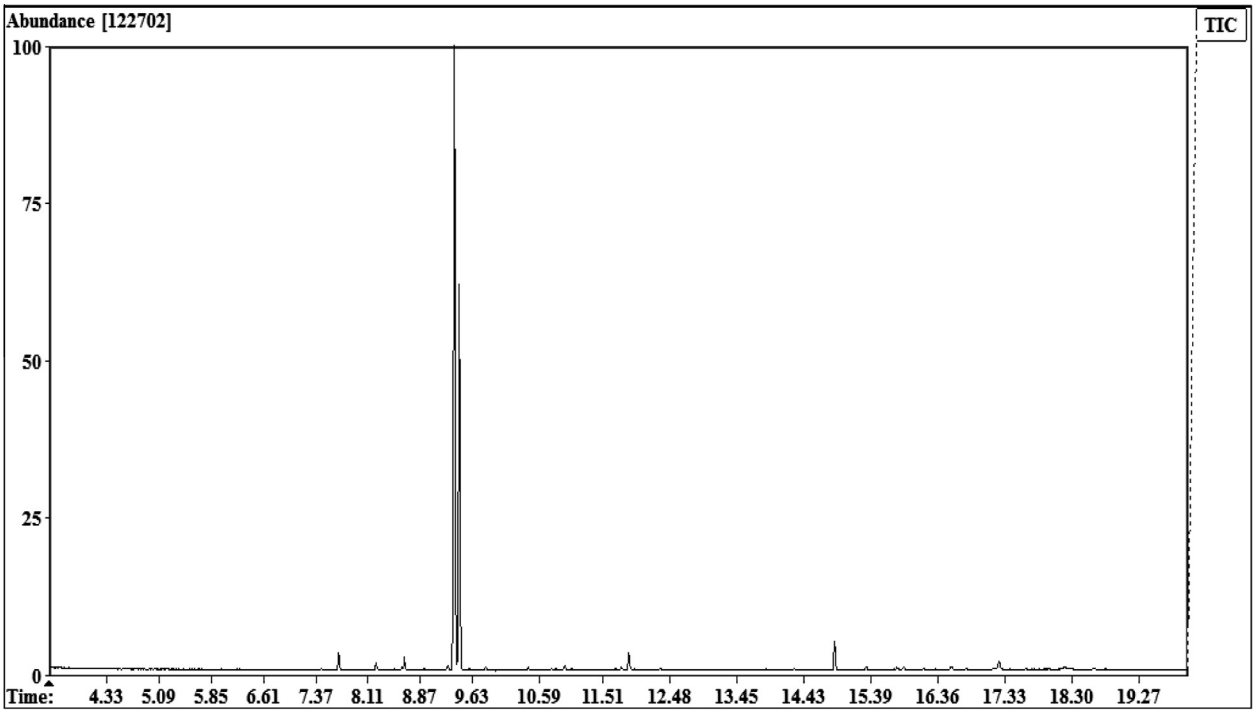


**Figure 1** GC/MS chromatogram of the essential oil of *P. guajava* L. leaves.

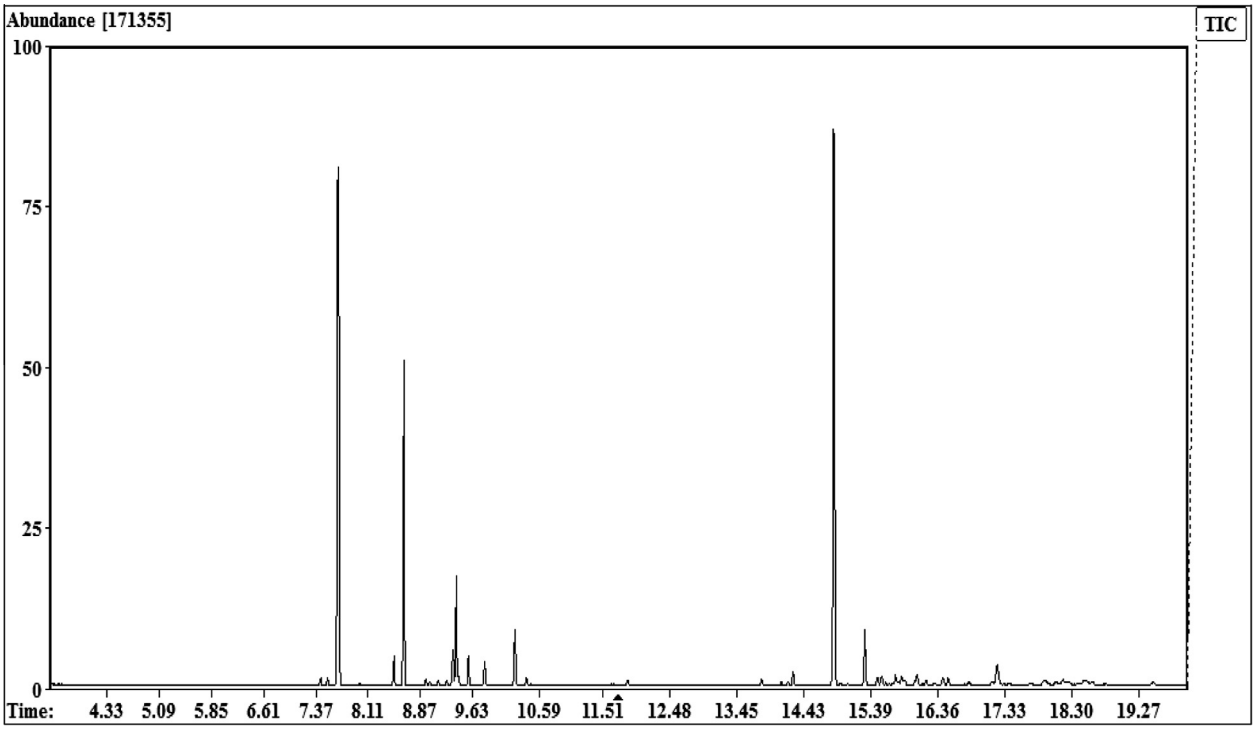


**Figure 2** GC/MS chromatogram of the essential oil of *P. cattleianum* Sabine leaves.

Reviewing previous data regarding *P. guajava* leaves cultivated in Egypt, qualitative and quantitative variations were found. Guava leaf oil studied by El-Ahmady et al. (2013) consisted mainly of *β*-caryophyllene (16.9%), 4 α-selin-7(11)-enol (8.3%), *β*-caryophyllene oxide (6.5%) and α-selinene (6.5%).[27] Also, Karawya et al. (1999) found that guava leaf oil constituted mainly of sesquiterpene hydrocarbons *viz*; caryophyllene, aromadendrene, α and *β*-selinene and *β*-bisabolene.[28]

However, predominance of limonene in the leaf oil of *P. guajava* L. was previously recorded from Nigeria amounting to 42.1%.[29] Guava leaf oils from Manila (Philippines) were

found to contain α-pinene, limonene, longicyclene and β-caryophyllene as major compounds followed by β-bisabolene as minor component.[30] The Ecuadorian guava leaf oil recorded high contents of monoterpenes as represented by limonene (33.3%) and α-pinene (29.5%).[31]

In contrast to our results, some authors found that the essential oil of *P. guajava* L. leaves was predominated by sesquiterpenes. In Tunisia, Khadhri et al. (2014) reported that veridiflorol (36.4%) and *trans*-caryophyllene (5.9%) were the major constituents of *P. guajava* L. leaf oil.[32] Moreover, leaf oil of *P. guajava* from French Polynesia contained a mixture of sesquiterpene hydrocarbons (54.9%) and oxygenated sesquiterpenes (20.9%) with β-caryophyllene (18.3%) as the principal sesquiterpene hydrocarbon and selin-11-en-4α-ol (6.9%), α-cadinol (3.6%), and (*E*)-nerolidol (3.2%) as the main oxygenated sesquiterpenes.[33]

Regarding the foliar oil of *P. cattleianum* Sabine., the high percentage of β-caryophyllene (28.83%) is in accordance with those recorded from Brazil[34], Hawaii[35], French Polynesia[36] and California[37], in which, β-caryophyllene was the major constituent (36.8, 59.0, 31.5 & 59.9%, respectively).

Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries. Most of the bacterial species acquire and transmit resistance against currently available antibacterials and became multiresistant to other medications available on the market.[38,39] Consequently, common strategies adopted by pharmaceutical companies to introduce new antimicrobial drugs by changing the molecular structure of the existing medicines in order to make them more effective or restore the activity lost due to the evoked bacterial resistance.[40]

The volatile oils of *P. guajava* and *P. cattleianum* leaves exhibited broad spectrum antibacterial activity at the given concentrations, when compared to ampicillin as a standard. *P. guajava* volatile oil was superior as antibacterial agent to that of *P. cattleianum* against all the tested organisms except *N. gonorrhoeae*. This could be justified by a higher percentage of oxygenated terpenoids in *P. guajava* oil compared to *P. cattleianum*.[41] Referring to Table 4, the MIC of the volatile oil of the leaves of *P. guajava* against *S. aureus* recorded 6.75 μg/ml, while the oil of *P. cattleianum* exhibited MIC value of 13.01 μg/ml against *N. gonorrhoeae*. Thus, these volatile oils could be considered as potent antibacterial agents.

Concerning the antifungal activity (Table 5), the volatile oils of the leaves of *P. guajava* and *P. cattleianum* showed moderate antifungal activity against *C. albicans* with a potency of (9 mm, 42.86%) for both oils, when compared with amphotericin B (19 mm, 100%) as a standard antifungal. This was in accordance with a previous study conducted on *C. albicans*.[30] On the other hand, both investigated oils were inactive against *A. flavus*. The antimicrobial activity found for common guava oil supports the previous reports on its efficacy against *C. albicans* and *S. aureus*.[42,43]

## 4. Conclusion

Different chemotypes recognized in the oil profiles of *P. guajava* and *P. cattleianum* leaves helps in identification and differentiation of the two species. Furthermore, the presence of 1, 8-cineole in a high concentration in *P. guajava* oil is of a chemotaxonomical value as it is a characteristic component of most of myrtaceous oils. The potent antibacterial inhibition of *S. aureus* and *N. gonorrhoeae* suggests the medicinal use and possible therapeutic application of these volatile oils for treatment of infectious diseases such as scarlet fever, upper respiratory infections, urethritis, cervicitis, and pharyngitis, and also to ameliorate the complications resulting from gonorrhea including pelvic inflammatory disease, ectopic pregnancy, and infertility.

## Conflict of interest

All authors have none to declare.

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