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Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid contents

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ABSTRACT

Objective: To investigate antioxidant and antibacterial activities of *Callistemon viminalis* (*C. viminalis*) leaves. **Methods:** The essential oil of *C. viminalis* leaves obtained by hydro-distillation was analyzed by GC/MS. Different extracts were tested for total phenolic and flavonoid contents and *in vitro* antioxidant (DPPH assay) and antibacterial (agar disc diffusion and 96-well micro-plates methods) actives. **Results:** Fourteen components were identified in the essential oil, representing 98.94% of the total oil. The major components were 1,8-cineole (64.53%) and α-pinene (9.69%). Leaf essential oil exhibited the highest antioxidant activity of (88.60±1.51)% comparable to gallic acid, a standard compound [(80.00±2.12)%]. Additionally, the biggest zone of inhibitions against the studied bacterial strains was observed by the essential oil when compared to the standard antibiotic (tetracycline). The crude methanol extract and ethyl acetate fraction had a significant antibacterial activity against the tested bacterial strains. **Conclusions:** It can be suggested that *C. viminalis* is a great potential source of antibacterial and antioxidant compounds useful for new antimicrobial drugs from the natural basis. The present study revealed that the essential oil as well as the methanol extracts and ethyl acetate fraction of *C. viminalis* leaves exhibited highly significant antibacterial activity against the tested bacterial strains.

1. Introduction

In ancient Rome, Greece and Egypt, the essential oils and extracts have been used as perfumes, food flavors, deodorants and pharmaceuticals[1]. For thousands of years, the aromatic plants have been reported as rich sources for secondary chemical products and their derivatives. Even today, about 80% of the world's population relies predominantly on plants and plant extracts for health care and sources for clinically useful drugs[2].

Bacteria cause serious infections in humans as well as other animals. For example, it was found that *Staphylococcus aureus (S. aureus)* causes superficial skin lesion and food poisoning[3]. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a nosocomial pathogen accounting for a significant percentage of hospital-acquired infections and health care centers because there are a little effective antimicrobial agents against it[4]. Thus, the medicinal and herbal plants have assumed greater importance in recent days, due to the tremendous potential that they offer in formulating new drugs against many diseases and illnesses that affect the humankind[5].

Callistemon species belonging to family Myrtaceae are widely used for forestry, ornamental horticulture, essential

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oil production, windbreak plantings and degraded-land reclamation[6]. The most species of *Callistemon* are known in traditional medicine for its anticough activity and the essential oils have been used as antimicrobial and antifungal agents[7-9]. Among them, *Callistemon viminalis* (Sol. ex. Gaertn) G. Don. (*C. viminalis*), shrub or small tree, native to New South Wales, Australia[10], is used in traditional Chinese medicine pills for treating hemorrhoids[11].

Previously studies have been showed that aqueous extract of flowers and leaves had an antibacterial activity against the Gram-positive bacteria[8]. The water and ethanol extracts of inflorescence of *C. viminalis* have shown strong anti-quorum sensing activity against *Chromobacterium violaceum* and *Agrobacterium tumefaciens*, while the ethanol extract showed anti-quorum activity sensing against Agrobacterium tumefasciens[12]. Extracts from other species (*Callistemon citrinus*) have been observed to have the most antibacterial activity[13]. On the other hand, the essential oils of *Callistemon lanceolatus*, *Callistemon citratus*, *Callistemon viridiflorous* and *Callistemon pendulous* have been reported to show strong antimicrobial activities[14-16].

Chemically, 1,8-cineole has been reported as a major constituent in the essential oils of some *Callistemon* species such as *C. lanceolatus* and *C. viminalis*[9,17,18], *C. speciosus*[19], *C. comboynensis*[20] and *C. linearis*[21]. C-methyl flavonoids, triterpenoids and phloroglucinol derivatives were identified in the genus of *Callistemon*[14,22-24]. GC and GC/MS analysis of *C. viminalis* leaf essential oil resulted in the identification of 42 constituents, representing 99.5% of the oil. Among them, 1,8-cineole (61.7%), α-pinene (24.2%) and methyl acetate (5.3%) were the major components[8].

Although *C. viminalis* is widely cultivated, the information on its biological properties is still scarce. The present work focused on the extracts and essential oil of *C. viminalis* leaves grown in Egypt. Additionally, antioxidant and antibacterial activities as well as the total phenolic and flavonoid contents were reported.

2. Materials and methods

2.1. Plant material

Leaves of *C. viminalis* were collected during the month of August, 2012 from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt and obtained voucher numbers at Egypt barcode of life project (www.egyptbol.org), Faculty of Agriculture, Alexandria University.

2.2. Hydro-distillation apparatus and procedure

One hundred grams of *C. viminalis* fresh leaves were hydro-distillated with a Clevenger-type apparatus according to the European Pharmacopoeia[25] and extracted with 2 L of water for 3 h until no more essential oil was obtained. The essential oil was collected, dried under anhydrous sodium sulphate, kept dry in sealed brown bottles and stored at 4 ℃ before chemical and bioassay analyses. The amount of oil obtained was measured and the oil was calculated based on the fresh weight (mL/100 g fresh weight).

2.3. GC/MS analysis conditions

GC/MS analyses of the oils were performed on a Schimadzu QP-5000 gas chromatograph interfaced with a DB 5-MS mass spectrometer system equipped with a capillary column (30.00 m × 0.25 mm id, film thickness 0.25 μm). The oven temperature was programmed from 70 to 240 ℃ at the rate of 5 ℃/min. The ion source was set at 240 ℃ and electron ionization at 70 eV. Helium was used as the carrier gas (1 mL/min). The split ratio was 1:25 with the scan range of 35 to 425 amu, and 0.2 μL of diluted oil in hexane was manually injected into the GC/MS[9]. The components of the oils were identified on the basis of MS library search (NIST and Wiley) and by comparing with the MS literature data[26,27]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

2.4. Preparation of extracts

Leaves of *C. viminalis* were air-dried at room temperature and then ground at approximately 0.2-0.4 mm. The dried powder was extracted by soaking with 150 mL of 80% (v/v) methanol. After filtration, the residue was processed similarly with the same amount of solvent. The crude methanol extract (MeOH extract) was concentrated to dryness under reduced pressure at 45 ℃ with a rotary evaporator, lyophilized and stored at 4 ℃ until further use. Five grams from the methanol extract was further fractionated by successive solvent extraction with ethyl acetate (EtOAc fraction), chloroform ($CHCl_3$ fraction) and then with *n*-butanol saturated water (*n*-BuOH fraction). After organic solvent extraction, the remaining aqueous fraction was also used for activity testing (Aq fraction)[28] and the marc was discarded.

The chloroform fraction ($CHCl_3$ fraction) which contains alkaloids[28,29] was determined. The sample of about 1 g from the lyophilized MeOH extract was dissolved in 50 mL of 99% (v/v) methanol and treated with an equal volume of 1% (v/v) aqueous HCl. Then, the alkaloids were precipitated by drop-wise addition of 10% (v/v) NH_4OH.

The solvents were removed under reduced pressure and the extracts were concentrated under vacuum at 40-60 ℃ and weight of the dried mass was recorded. All the dried extracts were dissolved in specific reagents through standard procedures and tested for phytoconstituents using standard

methods[28-30].

2.5. Determination of total phenolics

The total phenolic content (TPC) was determined with the Folin-Ciocalteau assay[31]. Briefly, 1 mL of extracts (MeOH extract and EtOAc, $CHCl_3$, *n*-BuOH and Aq fractions) or a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to a volumetric flask, containing 9 mL of distilled deionized water (dd H_2O). One milliliter of the Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 70 g/L Na_2CO_3 solution was added to the mixture. The solution was diluted to 25 mL with dd H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm with a UV scanning spectrophotometer (Unico® 1200, Alexandria, Egypt). The data for the TPCs of *C. viminalis* leaves were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams extract (mg GAE/g dry extract).

2.6. Determination of total flavonoids

The total flavonoids content was measured with an aluminum chloride colorimetric assay[31]. One mililiter of extracts or a standard solution of (+)-catechin (20, 40, 60, 80 and 100 mg/L) was added to a 10 mL volumetric flask, containing 4 mL of dd H_2O. Subsequently, 0.3 mL of 50 g/L $NaNO_2$ was added to the flask. After 5 min, 0.3 mL of 100 g/L $AlCl_3$ was added. At the sixth minute, 2 mL of 1 mol/L NaOH was added and the total volume was made up to 10 mL with dd H_2O. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510 nm with a UV scanning spectrophotometer (Unico® 1200, Alexandria, Egypt). The data of the total flavonoid contents were expressed as milligrams of (+)-catechin equivalents (CE) per gram extract (mg CE/g dry extract).

2.7. DPPH radical-scavenging assay

Free radical scavenging activity of the samples was determined using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method[32] with some modifications. Briefly, an aliquot of 2 mL of stock solution of 0.1 mmol/L DPPH (Sigma-Aldrich) reagent dissolved in pure methanol was added to a test tube with 2 mL of the sample solution in methanol (200 μg/L). The reaction mixture was mixed for 10 s and left to stand in fibber box at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer (Unico® 1200). Pure methanol (Sigma-Aldrich) was used to calibrate the spectrophotometer. The decrease in the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. Total antioxidant activity (TAA, %) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation:

$$TAA\ (\%) = (A_0 - A_s/A_0) \times 100 \quad (1)$$

where, *TAA* is the total antioxidant activity, A_0 is the absorbance of DPPH solution in methanol and A_s is the absorbance of a DPPH solution with a tested fraction solution (test) or gallic acid (positive control) solution. The control contained 2 mL of DPPH solution and 2 mL of methanol.

2.8. Antibacterial activity test

The antibacterial activity was carried out on the extracts with concentration of 2 000 μg/mL against the Gram-positive bacteria including *Bacillus subtilis (B. subtilis)* ATCC 6633, *Bacillus cereus (B. cereus)* ATCC 14579, *Micrococcus luteus* ATCC 4698, *Sarcina lutea* (*S. lutea*) ATCC 9341 and *S. aureus* ATCC 6538 and the Gram negative bacteria including *Escherichia coli* (*E. coli*) ATCC 8739, *Serratia marcescens* (*S. marcescens*) ATCC 13880, *Salmonella typhi* (*S. typhi*) ATCC 6229, *Proteus vulgaris* ATCC 6509 and *P. aeruginosa* ATCC 9027.

2.8.1. Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oil[33]. A suspension of the tested bacteria (0.1 mL of 1×10^8 cells/mL) was spread on the solid media plates. Filter paper discs (5 mm in diameter) were loaded with 20 μL of the oil and placed on the inoculated plates. After staying at 4 ℃ for 2 h, the plates were incubated at 37 ℃ for 24 h. The diameters of the inhibition zones (IZs) were measured in millimeters. Negative control was prepared using respective solvent. Tetracycline (20 μg/disc) was used as a positive control with the tested bacteria.

2.8.2. Determination of minimum inhibitory concentrations (MICs)

MICs were performed in 96-well micro-plates[32-34]. The serial dilution of essential oil, MeOH extract and EtOAc, $CHCl_3$, *n*-BuOH and Aq fractions at 250, 500, 1 000, 1 500, 2 000, 3 000, 4 000 and 5 000 μg/mL was prepared. All wells were filled with 50 μL sterile Mueller Hinton broth. After adding 50 μL of the bacterial suspension (1×10^5 CFU/mL), the micro-plate was covered and incubated at 37 ℃ at 100% relative humidity overnight, and 50 μL of a 0.2 mg/mL solution of *p*-iodonitrotetrazolium violet (INT, Sigma-Aldrich) was added to each well. All the extracts were dissolved in 10% (v/v) dimethyl sulfoxide (DMSO, Sigma-Aldrich) and made up as a stock solution (5 000 μg/mL) with distilled water.

2.9. Statistical analysis

The measurements of antibacterial activity, total phenolic compounds, total flavonoids and DPPH radical scavenging activity were carried out for three replicates. The results are

expressed as mean values ± standard deviation (SD).

3. Results

3.1. Quantification and phytochemical analysis of extracts

The MeOH extract of *C. viminalis* leaves yielded 23% based on oven dry weight of the leaves. The percentage quantities of *C. viminalis* MeOH extract with different solvents was 8.80% for EtOAc, 12.30% for *n*-butanol and 16.56% for water. The percentage quantity of $CHCl_3$ fraction was 6.00% as separated from the lyophilized MeOH extract. The phytochemical screenings of *C. viminalis* leaves (Table 1) were reported to contain tannins, flavonoids, saponins, phenolics and traces of steroids and alkaloids.

Table 1
Phytochemical analysis of crude methanol extract and its fractions from *C. viminalis* leaves.

Extract	Tannins	Flavonoids	Alkaloids	Saponins	Phenols	Steroids
MeOH	++	+++	−	++	+++	+
EtOAc	++	+++	−	−	+++	−
$CHCl_3$	−	−	+	−	−	−
n-BuOH	+	+	−	++	+	+
Aq	−	+	−	−	+	−

+++ strong; ++ medium; + poor; − absence. MeOH, methanol crude extract; EtOAc, ethyl acetate fraction; $CHCl_3$, chloroform fraction; *n*-BuOH, *n*-butanol fraction; Aq, aqueous fraction. The measurements were repeated four times and the classification was based on the color intensity of the precipitate.

3.2. Total phenolic and flavonoid contents and antioxidant activity of extracts

The estimation of TPCs, total flavonoids content and antioxidant activity of *C. viminalis* extracts is presented in Table 2. The TAA was ranged between (8.70±1.15)% (Aq fraction) and (88.60±1.51)% (essential oil).

Table 2
Total phenolics and flavonoids contents of extracts and essential oil from *C. viminalis* leaves and their antioxidant activity (TAA).

Extract	Total phenolic (mg GAE/g extract)	Total flavonoids (mg CE/g extract)	TAA (%)
EO	NA	NA	88.60±1.51
MeOH	44.30±3.78	45.36±2.03	70.13±1.43
EtOAc	69.10±3.50	28.55±2.06	85.12±1.42
$CHCl_3$	–	–	30.12±1.20
n-BuOH	14.32±2.32	10.12±1.33	15.32±2.41
Aq	17.21±1.13	18.34±1.36	8.70±1.15
GA			80.00±2.12

All values are mean±SD of three replicates; GAE, gallic acid equivalents; CE, (+)-catechin equivalents; NA, not tested; EO, essential oil; MeOH, crude methanol extract; EtOAc, ethyl acetate fraction; $CHCl_3$, chloroform fraction; *n*-BuOH, *n*-butanol fraction; Aq, aqueous fraction; GA, gallic acid.

3.3. Essential oil composition

The amount of oil obtained was 0.44 mL/100 g fresh weight. The essential oil of *C. viminalis* leaves was comprised of 14 compounds (Table 3) and the main compound was 1,8-cineole (64.53%) followed by α-pinene (9.69%) and α-terpineol (7.90%). The bioactive compounds may not be limited to those already identified in the essential oils but could be related to the secondary compounds.

Table 3
Essential oil composition of *C. viminalis* leaves.

Constituent	Retention time (min)	Content in oil (%)[a]
α-pinene	3.430	9.69
β-pinene	4.512	2.43
Myrcene	4.625	0.10
α-terpinene	4.775	0.83
1,8-cineole	4.977	64.53
Linalool	5.829	0.28
Pinocarvone	7.761	3.45
Terpinen-4-ol	8.183	2.86
α-terpineol	8.382	7.90
Carvone	9.728	1.50
Eugenol	13.867	0.36
Methyl acetate	14.234	2.21
Spathulenol	15.918	1.23
Caryophyllene oxide	18.054	1.57

[a]Percentage of total FID area obtained on HP-5 capillary column.

3.4. Antibacterial activity of leaf essential oil and extracts

The present study investigated the *in vitro* antibacterial activity of six different solvent leaves extracts, i.e., essential oil, MeOH extract and EtOAc, $CHCl_3$, *n*-BuOH and Aq fractions at 2 000 μg/mL of *C. viminalis*. The potency of the essential oil, MeOH extract and the fractions and their antibacterial sensitivity were assessed quantitatively by determining the IZs and MICs as given in Table 4. The average IZ of the methanol extract were highest against the growth of *P. aeruginosa* [(19±1.2) mm] with MIC of 500 μg/mL and lowest against *S. marcescens* [(7±1.5) mm] but with MIC 250 μg/mL. The EtOAc fraction showed the highest activity against *E. coli* [(19±1.6) mm] and the lowest against *S. marcescens* [(10±1.0) mm] with MIC<250 μg/mL for the two bacteria. Additionally, the EtOAc fraction of *C. viminalis* leaves showed good activity against the other studied bacteria with IZs ranged from 13 to 18 mm.

The data revealed that MeOH extract and EtOAc fraction showed good antibacterial activities against the tested bacteria. Additionally, it was shown noteworthy antibacterial activities against *B. cereus*, *B. subtilis*, *P. aeruginosa*, *S. typhi*, *E. coli* and *S. lutea* which is comparable to that of the antibiotic used (tetracycline with 20 μg/disc). The *n*-BuOH and $CHCl_3$ fractions failed to express a good activity against the tested bacterial strains. On the other hand, the Aq fraction exhibited weak activity against *B. subtilis* and

Table 4

Antibacterial activity of extracts from *C. viminalis* leaf using agar disc diffusion and minimum inhibitory concentration assays.

Bacterial strain	EO	MeOH extract	EtOAc	$CHCl_3$	*n*-BuOH	Aq	Negative control	Positive control[a]
Gram-positive								
B. subtilis	18±1.1^A	14±1.3^A	16±1.4^C	8±0.9^D	7±0.6^A	8±1.2^D	R	18
B. cereus	20±1.4^A	15±2.1^C	15±1.1^B	R^E	R^E	R^E	R	17
M. luteus	12±1.1^C	12±1.6^C	13±1.2^D	6±0.4^D	R^E	R^E	R	19
S. lutea	21±1.4^A	19±1.1^A	18±1.8^A	8±1.7^A	12±0.9^A	R^E	R	20
S. aureus	15±1.2^C	16±1.2^C	14±1.7^C	7±0.7^C	8±0.8^C	R^E	R	23
Gram-negative								
E. coli	17±1.3^A	13±0.8^A	19±1.6^A	8±1.5^A	10±0.8^A	R^E	R	18
S. marcescens	10±1.1^D	7±1.5^D	16±1.0^D	8±1.2^D	R^E	R^E	R	20
S. typhii	6±0.4^A	14±0.8^A	11±1.7^A	7±1.4^D	R^A	R^E	R	20
P. vulgaris	12±1.2^C	10±1.2^B	13±1.4^D	R^E	8±0.7^D	9±1.2^D	R	23
P. aeruginosa	20±1.5^C	19±1.2^C	18±1.6^C	6±1.6^C	9±0.7^C	R^E	R	22

Diameter of inhibition zone (mm), including disc diameter of 5 mm at 2 000 μg/mL, is expressed as mean±SD of three replicates. The superscript letters show minimum inhibitory concentrations (MICs) of the extracts against the tested strains. A, B, C, D and E represent MICs of <250, 250, 500, 1 000, 1 500 and >5 000 μg/mL, respectively. EO, essential oil; MeOH, crude methanol extract; EtOAc, ethyl acetate fraction; $CHCl_3$, chloroform fraction; *n*-BuOH, *n*-butanol fraction; Aq, aqueous fraction. R: Resistance at 2 000 μg/mL. [a]Tetracycline (20 μg/disc). The measurements were repeated four times and the classification was based on the color intensity of the precipitate.

Proteus vulgaris.

The results also revealed that *S. lutea, B. cereus and P. aeruginosa* had to be the most sensitive microorganisms to the essential oil with the biggest IZs of (21±1.4), (20±1.4) and (20±1.5) mm and with MICs <250, <250 and 500 μg/mL, respectively, followed by *E. coli* and *S. aureus* with IZs of (15±1.2) and (17±1.3) mm and with MIC values <250 and 500 μg/mL, respectively. On the other hand, the smallest IZs were found by *S. typhi* [(6±0.4) mm] and *S. marcescens* [(10±1.1) mm], and even the essential oil gave the smallest IZs against *S. typhi* but the MIC value was <250 μg/mL.

4. Discussion

The extracts of *C. viminalis* have shown the presence of various chemical groups like phenolic, glycosides, flavanoids, alkaloids, saponins, steroids, tannins and cardiac compounds, which are secondary compounds synthesized and deposited in different tissues of the plant[35,36]. Flavanoids have been reported to own the ability to form complex with extracellular, soluble proteins and bacterial cell walls[37] and possess the antibacterial activity. The purified alkaloids as well as their synthetic derivatives are used as bactericidal agents[38]. The higher plants have alkaloids and flavonoids which control the growth of microbial pathogens. The toxicity of phenolic compounds includes enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins[39,40]. The polar extracts of *C. viminalis* included alkaloids, flavonoids and some phenols and non-polar such as tannins, terpenes and quinines[41]. The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids and saponins[42].

Recently, the biologicals and phytochemicals of plant genus *Callistimon* are reviewed and investigated[43]. It was reported that dichloromethane extract of *C. viminalis* leaves gave the highest extract (4.63%), while ethyl acetate extract gave the lowest amount of crude extract (2.75%) and the afforded betulinic acid[44]. The *C*-methyl flavonoids, triterpenoids and phloroglucinol derivatives were identified in the genus of *Callistemon*[14,22,23]. Tetramethylcyclohexenedione (or similar) moiety and epimeric compounds as well as viminadione A and viminadione B have been reported in *C. viminalis* to be responsible for the activity as insecticidal compounds[45]. The results showed that the EtOAc fraction had the highest TPC than other fraction[15,46]. Previously, it was observed that high TPC values play the main activities and possess potent antioxidant and antimicrobial activities[47].

The essential oil of *C. viminalis* has been shown to contain the bioactive compounds with 1,8-cineole (61%), α-pinene (24%), and methyl acetate (5.3%), and these results are in agreement with the previous studies[8,9].

The results of the antibacterial activity are in agreement with the findings of Abdullah *et al*.[48], where the IZs of MeOH, EtOAc and hexane extracts were 12.75, 8.75 and 11.50 mm against the growth of *B. subtilis*, respectively. On the other hand, the extracts did not show any activity with distilled water. Additionally, all the extracts were not active against the growth of *E. coli*[49], although all plant extracts shown antimicrobial activities against the selected microorganisms[41]. The results revealed that different leaf extracts of *C. viminalis* had a promising antibacterial activity.

The result about the diameter of IZs does not reflect the

antibacterial activity of a compound[50]. Moreover, the IZ values could be affected by the solubility of the oil, diffusion range in the agar, evaporation (it can affect the dose), etc[32]. In addition, the antibacterial activities of the essential oil from *C. viminalis* suggest its importance in the treatment of various infectious diseases caused by the tested human bacterial strains. Moreover, the presence of 1,8-cineole in the essential oil of Myrtaceae family are known for their biological activities[51].

The present results are in comparable with those of Oyedeji *et al.*[9], where the MIC values were 0.08, 5.00 and 5.00 mg/mL against the growth of *S. aureus*, *P. aeruginosa* and *S. marcescena*, respectively. When the IZs were compared, the essential oils from *C. viminalis* showed a good antibacterial activity in comparable with the standard antibiotic (tetracycline).

In some cases, MeOH extract has shown a stronger effect than ethanolic extract, which could be explained by the differences in the compounds between these two extracts[52]. Fractionation of the weakly active MeOH extract results in more active antibacterial partitions. For example, the MeOH extract presented weakly activity against *S. marcescens* with IZ of (7±1.5) mm and MIC of 2 000 μg/mL, while the EtOAc fraction found from the MeOH extract showed a good activity with IZ of (16±1.0) mm and MIC of 1 000 μg/mL).

In general, the Gram-negative bacteria show less sensitivity to plant extracts possibly as a result of their extra lipopolysaccharide and protein cell wall that provides a permeability barrier to the antibacterial agent[53]. Furthermore, the Gram-positive bacteria are more sensitive to the extracts because of the single layer of their cell wall, while the double membrane of Gram-negative bacteria should make them less sensitive[36].

The recourse to naturally occurring products with interesting antimicrobial and eliciting properties such as plant extracts and essential oil have been getting more attention in recent years. Such biopesticides can be used in a number of ways to reduce plant disease levels and prevent the development and spread of pathogens, thus preserving crop yield and quality.

Conflict of interest statement

We declare that we have no conflict of interest.

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