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Chemical characterization and antimicrobial activity of Moroccan *Pelargonium asperum* essential oil

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ABSTRACT

In the aim of valorizing aromatic and medicinal plants from Morocco, this work focuses on the chemical characterization and antimicrobial activity of *Pelargonium asperum* essential oil against 11 microbial strains causing problems in the medical and food domain. The chemical profile of the volatile oil was investigated by GC/MS. The major compounds were citronellol (26.98%), geraniol (14.12%), isomenthone (8.80%), linalool (4.97%), citronellylformate (3.1%), followed by geranylformate (4.07%) and guai-6,9-diene (4.24%). The results of antimicrobial activity by using the broth microdilution method indicated that essential oil of *Pelargonium asperum* exhibited significant antimicrobial activity against all tested microorganisms with the strongest inhibitory effect against yeasts. The MICs values ranged from 0.003% to 0.25% (v/v) for all strains, except *Pseudomonas aeruginosa*, which was least susceptible and inhibited by 2% (v/v). These results suggest that *Pelargonium asperum* oil could be used for the development of new antimicrobial agents.

INTRODUCTION

The microbiological quality of food is one of the essential foundations or basis of its ability to satisfy consumer safety. Foods exposed to deterioration by bacteria and fungi could reduce their sensory, nutritional and health characteristics (Guiraud, 2003). Despite improved food preservation techniques, natural food preservatives remain one of the most important issues for public health (Burt, 2004). In fact, several synthetic food preservatives have been limited in many countries, because of their long term side effects, including carcinogenicity (Chahardehi *et al.*, 2010). Furthermore, antimicrobial resistance is now a global concern, which reached a crisis point in many hospitals worldwide. Indeed, there is an urgent need to replace our arsenal of anti-infective agents (Cushnie and Lamb, 2011), by developing new antimicrobial agents. In this context, a great interest has been focused on the natural products, in particular essential oils (Bakkali *et al.*, 2008). Essential oils are volatile components containing complex mixtures of oxygenate hydrocarbon substances having for general formula $(C_5H_8)n$: monoterpenes and sesquiterpenes (Deans and Svoboda, 1990). One of their apparent properties is their antiseptic power, linked to their activity against pathogenic bacteria, including some antibiotic-resistant strains. Several previous data already described many recipes of herbal and aromatic oils that priests and doctors employed. Currently, the use of essential oils is carried out on the scientific and rational basis, in order to develop new products for various fields: food, medical, veterinary and cosmetic. Furthermore, in the literature, several research studies have proved the antimicrobial, antiviral and insecticide properties of terpenes (Nsambu *et al.*, 2014; Sadiki *et al.*, 2014; Salah-Fatnassi *et al.*, 2010). In addition, chemical complexity of the essential oils prevents the decoding from pathogens and thus reduces the risk of developing microbial resistance.

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Indeed the use of antimicrobial properties of essential oils does not start today (Beylier-Maurel, 1976), while nowadays the need to their applications became urgent. Therefore, the present study aims to determine the chemical composition of *Pelargonium asperum* essential oil and to investigate its antimicrobial activity by micro-dilution methods against 11 selected spoiling and pathogenic microorganisms, in an attempt to contribute to the use of these as alternative products for microbial control and food preservation.

MATERIAL AND METHODS

Plant material

Fresh aerial part of *Pelargonium asperum* was harvested from the garden of National Institute of the Medicinal and Aromatic Plants (NIMAP). The botanical identification was performed, and then the voucher specimen was deposited at the Herbarium of NIMAP (Morocco).

Essential oil extraction

The fresh aerial part of *P. asperum* (leaves and stems) was hydrodistilled for 3 h using a Clevenger-type apparatus. The essential oil was then kept in dark at 4°C until further use.

Target strains

Tested bacteria include seven isolates of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, *Micrococcus luteus* ATCC 14452, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Salmonella enterica* (serovar *typhimurium*) and *Bacillus cereus*. Before use, strains were revivified by subcultures in Luria-Bertani (LB) plates at 37°C for 24 h. As regards the tested fungi, they include *Candida albicans*, *Candida tropicalis*, *Aspergillus niger* and *Penicillium expansum*. Revivification of molds was made by subcultures in malt extract-agar plates (malt extract 30 g/L and agar 20 g/L) at 25°C for 7 days. After incubation, their spores were harvested by scraping the culture surface in sterile Tween 20 (1%) solution. Then the spore suspension was concentrated by centrifugation at 10000 g for 15 min at 4°C until a concentration of 10^6 spores/mL (counted with an hemocytometer).While, yeast strains were inoculated in yeast-peptone-glucose agar (YPG) (yeast extract 10 g/L, peptone 20 g/L, glucose 20 g/L and agar 20 g/L) and incubated at 30°C for 48 h.

Determination of minimum inhibitory concentration against bacteria

The minimum inhibitory concentration was determined in 96 well-microplate using the microdilution assay according to the protocol previously described by (Balouiri *et al.*, 2016) with slight modifications. Bacteriological agar at 0.15 % (w/v) was used as an emulsifier of the essential oil in the culture medium. For bacteria, the essential oil was serially diluted in Muller Hinton broth supplemented with agar to obtain the final concentrations ranging between 8% and 0.007% (v/v). The 12^{th} well was considered as growth control (free-essential oil control). Then, 50 µL of bacterial inoculum, previously prepared and adjusted to 0.5 McFarland, were added to each well to reach the final concentration of 10^6CFU/mL. After incubation at 37°C for 24 h, 10 µL of resazurin were added to each well as a bacterial growth indicator.

After further incubation at 37°C for 2 h, the bacterial growth was revealed by the change of coloration from purple to pink. Experiments were carried out in triplicate.

Determination of Minimum inhibitory concentration against fungal strains

To investigate the antifungal activity of the studied essential oil against *Aspergillus niger* and *Penicillium expansum* a modified microdilution technique described by (Daouk *et al.*, 1995) was used. Firstly, 50 µl of malt extract broth were added from the second to the 12^{th}well. The essential oil was diluted in Tween 20 1% (v/v) at a final concentration of 40% (v/v), then 100 µL of this solution were deposed in the first well. Afterwards, scalar dilution was made by transferring 50 µL from the 1^{st} to the 11^{th} well. The 12^{th} well was considered as growth control. Thereafter 50 µL of the fungal spore suspension was added to each well to reach a final concentration of 10^6 spores/mL. The microplate was sealed and incubated for 72 h at 30°C. The lowest essential oil concentration that prevents visible fungal growth was defined as the MIC. Likewise, the MIC determination against *Candida albicans* and *Candida tropicalis*, was performed in 96 well-microplate according to the protocol previously described (Balouiri *et al.*, 2016) with slight modifications. The essential oil was also serially diluted in YPG broth supplemented with agar at 0.15% (w/v). The 12^{th} well was also considered as growth control. Then, 50 µL of fungal inoculum were added to each well at a final concentration of 10^3 CFU/mL. Finally, the microplate was sealed and incubated at 30°C for 48 h. Experiments were carried out in triplicate. Similarly, the lowest essential oil concentration that prevents visible fungal growth was defined as the MIC.

RESULTS AND DISCUSSION

The studied essential oil was previously subjected to a gas chromatography-mass spectrometry analysis. This analysis revealed 61 different compounds accounting for 99.96% of the whole *Pelargonium asperum* essential oil, where the major constituents were citronellol (26.98%), geraniol (14.12%), isomenthone (8.80%), linalool (4.97%), geranylformiate (4.07%), guaï-6,9-diene (4.24%) and citronellyl formiate (3.1%). Likewise, several previous studies have found citronellol as the principal major component of this essential oil (Boukhatem *et al.*, 2013; Boukhris *et al.*, 2013; Bouzenna and Krichen, 2013; Gomes *et al.*, 2007; Jalali-Heravi *et al.*, 2006).

Antibacterial effect of *Pelargonium asperum* essential oil

This study focused on the *Pelargonium asperum* essential oil bioactivity. Results found of its antimicrobial activity

evaluated against 11 microbial strains are compiled in Tables (Tab. 1, 2 and 3).

As regards to the antibacterial effect, it can be seen in the Tab. 1 that all tested bacterial strains were susceptible to the studied essential oil. The antibacterial effect unveiled seems to be strain- dependent. In fact, strong inhibitory effect has been shown against all Gram-positive bacteria, especially *Bacillus cereus* and *Micrococcus luteus*, since they were inhibited by very low concentrations 0.007% and 0.015% (v/v) respectively. In addition, the concentration of 0.031% (v/v) was sufficient to inhibit the growth of *S. aureus* and *B. subtilis*. Against Gram-negative bacteria the tested essential oil was more active against *E. coli* and *S. enterica* serovar *typhimurium* with a minimum inhibitory concentration of 0.125% (v/v). In contrast, *P. aeruginosa* was the most resistant strain with MIC value of 2% (v/v). Previous studies confirmed that this bacterial strain was the most resistant (Boukhatem *et al.,* 2013; Ghannadi *et al.,* 2012; Haloui *et al.,* 2015). Usually, the Gram-positive strains were more susceptible to the essential oils than the Gram-negative bacteria. This difference is closely related to their cell wall compositions, since the antibacterial activity of the essential oils has been explained by molecular interactions of the functional groups of their components and the bacterial wall, which inflict several damages to the cell (Calo *et al.,* 2015).

In fact, the outer membrane of Gram-negative bacteria is characterized by the presence of lipopolysaccharides (75%), which have hydrophilic character that makes the outer membrane of these bacteria invulnerable to the most hydrophobic molecules (i.e. Hydrocarbons terpenes). Thus, this structural particularity is in part responsible for the intrinsic resistance of Gram-negative compared to the Gram-positive bacteria to the essential oils constituents (Gachkar *et al.,* 2007; Trombetta *et al.,* 2005). Furthermore, concerning the antifungal activity against molds and yeasts (Tab. 3 and 4), the screening test revealed that all tested fungal strains were susceptible to the *Pelargonium asperum* oil. Indeed, the strongest inhibitory effect was exerted against yeasts (both *Candida* species) with MIC values of 0.003% and 0.007% (v/v) against *Candida albicans* and *Candida tropicalis* respectively. These results are in agreement with those of previous works (Boukhatem *et al.,* 2013; Hassane *et al.,* 2011). As regards to the molds they were inhibited with MIC values of 0.312% and 0.15% (v/v) against *Penicillium expansum* and *Aspergillus niger* respectively. The broad spectrum and the significant antimicrobial activity of the tested essential oil may be attributed to its richness in terpenic alcohols (citronellol, geraniol, linalool), which represent 46.07% of its total composition. In fact, these molecules are well-known for their greater efficiency as antimicrobials (Hammer *et al.,* 2003; Inouye *et al.,* 2001; Satrani *et al.,* 2006). In addition, among the identified compounds in this oil, some molecules were previously reported to exhibit antimicrobial activity such as limonene (Mazzanti *et al.,* 1998), geraniol (Araújo *et al.,* 2003), carvacrol and citronellol (Sacchetti *et al.,* 2005).

In addition, this antimicrobial outcome could also be attributed to the synergistic interaction between the various components of this oil. In fact, it has been reported in previous studies that the inhibitory activity of an essential oil results from a complex interaction between its different constituents (Burt, 2004; Viuda-Martos *et al.,* 2008; Xianfei *et al.,* 2007).

Table 1: Chemical composition of Geranium (*Pelargonium asperum)* essential oil.

KI	Constituants	Percentages
931	α-Thujene	0.50
1074	Cis-oxidelinalol	0.54
1097	**Linalol**	**4.97**
1111	*Cis*-oxide rose	0.82
1134	Terpinol-1	0.95
1143	Cis-Sabinol	0.59
1164	**Isomenthone**	**8.80**
1189	α-Terpineol	0.70
1228	**Citronellol**	**26.98**
1240	Carvone	0.53
1255	**Geraniol**	**14.12**
1270	Geraniale	0.73
1275	**Citronellyl formiate**	**7.09**
1300	**Geranyl Formiate**	**4.07**
1349	α-Terpinylacetate	0.54
1353	Citronellylacetate	1.24
1384	β- Bourbonene	0.57
1419	β-Caryophyllene	0.76
1443	Guaï-6,9-diene	**4.27**
1474	β-Thujaplicin	1.09
1484	γ-Thujaplicin	0.71
1526	Δ-Cadinene	0.51
1538	α-Cadinene	0.56
1558	1-nor-epi-Bouronanone	1.09
1585	NerylIsovalerate	0.96
1586	Davanone	1.95
1697	(E)-Citronellyltiglate	1.42
	Total	**99.96**

KI: Kovàt's indices, MS: NIST 98 spectra and the literature (Adams 1995), ST: Co injection with authentic standards **% (v/v)**

Table 2: Antibacterial activity of *Pelargonium asperum* essential oil.

Strains	Concentrations v/v											
	8 % (v/v)	4% (v/v)	2% (v/v)	1% (v/v)	0.5% (v/v)	0.25% (v/v)	0.125% (v/v)	0.062% (v/v)	0.031% (v/v)	0.015% (v/v)	0.007% (v/v)	control
S. aureus	-	-	-	-	-	-	-	-	-	+	+	+
Bacillus subtilis	-	-	-	-	-	-	-	-	-	+	+	+
Bacillus cereus	-	-	-	-	-	-	-	-	-	-	-	+
M. luteus	-	-	-	-	-	-	-	-	-	-	+	+
Escherichia coli	-	-	-	-	-	-	+	+	+	+	+	+
P. aeruginosa	-	-	-	+	+	+	+	+	+	+	+	+
Salmonella typhi	-	-	-	-	-	-	-	+	+	+	+	+

S. aureus : Staphylococcus aureus ; M. luteus : Micrococcus luteus ; P. aeruginosa : Pseudomonas aeruginosa.

Table 3: Antifungal activity of *Pelargonium asperum* essential oil against *Candida albicans* and *Candida tropicalis.*

Strains	Concentrations % (v/v)											
	4% (v/v)	2% (v/v)	1% (v/v)	0.5% (v/v)	0.25% (v/v)	0.125% (v/v)	0.062% (v/v)	0.031% (v/v)	0.015% (v/v)	0.007% (v/v)	0.003% (v/v)	Control
C. albicans	-	-	-	-	-	-	-	-	-	-	-	+
C. tropicalis	-	-	-	-	-	-	-	-	-	-	+	+

C. albicans: Candida albicans and *C. tropicalis: Candida tropicalis.*

Table 4: Antifungal activity of *Pelargonium asperum* essential oil against *Aspergillus niger* and *Penicillium expansum.*

Strains	Concentrations v/v											
	20% (v/v)	10% (v/v)	5% (v/v)	2.5% (v/v)	1.2% (v/v)	0.625% (v/v)	0.312% (v/v)	0.15% (v/v)	0.075% (v/v)	0.037% (v/v)	0.018% (v/v)	control
A. niger	-	-	-	-	-	-	-	-	+	+	+	+
P. expansum	-	-	-	-	-	-	-	+	+	+	+	+

A. niger : aspergillus niger ; P. expansum : Penicillium expansum.

CONCLUSION

The findings highlighted that *P. asperum* essential oil was able to inhibit the growth of a wide spectrum of microbial strains, known for their implications in human and animal infections. Its content on several active compounds and their interactions explained well its bioactivity. Hence, this essential oil can be a promising agent to control microbial growth, even if more detailed reports on its toxicity and mechanisms of action are requested to overcome the impediment of its application in several industries.

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