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Bioactivity of essential oil from *Hertia cheirifolia* L. flowers in the control of bacteria

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ABSTRACT

To date, numerous studies have demonstrated beneficial properties of essential oils in the prevention and treatment of a variety of diseases. The current report evaluated, *in vitro*, the antibacterial activity of essential oil from the flowers of *Hertia cheirifolia* L. This oil was obtained by hydrodistillation using a Clevenger apparatus. The antibacterial activity of the water-distilled essential oil of *H. cheirifolia* L. was evaluated against Gram positive microorganisms, namely *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Bacillus licheniformis* and Gram negative bacterial strains, namely *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteric* IPC 8039 and *Salmonella typhimirium* using the microdilution method in sterile 96-well microplates. The numerical values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined. Regarding the obtained results, the highest antibacterial activities of essential oils were found against *Staphylococcus aureus* ATCC 6538 and *Bacillus licheniformis* both having an identical MIC value of 0.078 mg/mL. Our study showed that the use of the essential oil of *H. cheirifolia* flowers could be considered as a natural source to inhibit the growth of tested pathogenic bacteria.

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1. Introduction

Essential oils are usually odorous and volatile liquids at room temperature; this characteristic distinguishes these oils from fixed oils (Abrassat, 1988). Due to their non-polar nature, the essential oils are immiscible with water but are generally quite soluble in most organic solvents (Franchomme et al., 2001). They are formed in a large number of plants as secondary metabolism products (Sanon et al., 2001). They can be stored in all plant organs such as flowers, leaves, barks, woods, roots, rhizomes, fruits or seeds (Bruneton, 1987).

In the nature, essential oils have an important defensive role in protecting plants against natural predators (Sharifi-Rad et al., 2017). They present chemical signal sources that allow the plant to control or regulate its environment such as attraction of insect pollinators, repulsive action on predators, inhibition of seed germination and emission of chemical signals representing the presence of herbivorous animals (Baudoux, 2002).

Essential oils are complex and variable mixtures of natural components belonging to two distinct groups of odorous compounds including the predominant terpene group in most oils and the less frequent group of aromatic compounds which can be derived from phenylpropane or through other pathways (Bruneton, 1993). These oils have many biological properties, especially essential oils rich in monoterpenes which are recognized as potential food preservatives (Ruberto et al., 2000). Thus, monoterpenic essential oils are reported of to natural antioxidants (Yanishlieva et al., 1999), to have anti-cancer effects (Sharma et al., 2009) and antimicrobial activities (Dorman and Deans, 2000).

Hertia cheirifolia L. is known in Tunisia as a medicinal plant having several pharmaceutical and biological activities. In fact, people use the infusion of the leaves and stems to reduce hyperglycemia and to treat rheumatic pains (Majouli et al., 2016a). The extracts of this plant have demonstrated spasmolytic, anti-inflammatory (Ammar et al., 2009), acaricidal (Attia et al., 2012) and antibacterial

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activities (Majouli et al., 2016b), while the essential oils of *H. cheirifolia* have an antioxidant and α-glucosidase inhibition effects (Majouli et al., 2017). The main objective of the present study was to investigate the antibacterial activity of the essential oil from the flowers of *Hertia cheirifolia* L.

2. Experimental

2.1. Plant sampling

The plant sample (*H. cheirifolia*) was collected at the flowering stage (Fig. 1) in February 2012 from the area of Thala, Tunisia (Fig. 2). This region is characterized by calcareous brown soils and a variable continental climate with low temperatures in winter (5 °C) and high temperatures in summer (42 °C). The altitude varies from 750 m to 1200 m and the mean rainfall is about 322 mm/year. A voucher specimen (*H. cheirifolia*) (*Hc* 112) was deposited in the Laboratory of Medicinal Chemistry and Natural Products at the Faculty of Science of Monastir, Tunisia.

Fig. 1. Representation of the plant sample (*Hertia cheirifolia* L.).

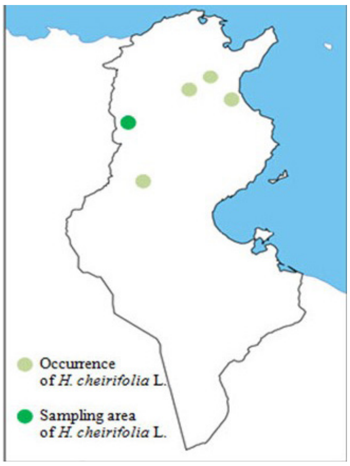

Fig. 2. Distribution of *Hertia cheirifolia* L. in Tunisia.

2.2. Extraction process of essential oils

There are several methods of extracting essential oils of which the most effective one is the classical approach based upon the use of Clevenger, invented in 1928 (Clevenger, 1928). In fact, hydrodistillation using the Clevenger is the most accepted procedure of distillation of essential oils by the scientific community.

This method consists of immersing the plant material (*H. cheirifolia* L.) in a balloon filled with water and placed on a heat source. When this assemble boils, the applied heat allows the bursting of plant cells and/or secretory glands and the subsequent release of fragrant molecules occurs. The vapors are condensed in a condenser and the essential oils are separated due to the differences in their densities.

The obtained essential oils are recovered, dried with anhydrous sodium sulfate (Na_2SO_4) and accumulated in glass vials in a refrigerator.

2.3. Bacterial strains

The antibacterial activities of *H. cheirifolia* essential oils were individually tested against bacterial ATCC reference strains namely *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Salmonella enteric* IPC 8039, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 and clinical strains namely *Bacillus licheniformis* and *Salmonella typhimirium* which were isolated from the Microbiology Laboratory of University Hospital Fattouma Bourguiba of Monastir.

2.4. Determination of MIC and MBC

The minimum inhibitory concentrations (MICs) of the samples were determined by the microdilution method on a plate divided into 96 wells. The MIC was defined as the lowest concentration of each sample that inhibited the microbial growth after incubation at 37 °C for 18 to 24 h (Cintia et al., 2007). The minimum bactericidal concentration (MBC) was determined by taking a volume of 10 µL from the well corresponding to the MIC, which was then spread on the agar and incubated at 37 °C. After 24 h, if there are no surviving microbial colonies, it can be confirmed that this concentration accounts for MBC. Gentamicin was used as positive controls against the bacterial strains.

3. Results and Discussion

3.1. Chemical profile of water-distilled essential oil from the flowers of *H. cheirifolia*

The analysis of the essential oil from flowers of *H. cheirifolia* by gas chromatography/mass spectrometry (Majouli et al., 2016a) showed qualitative and quantitative variability in its chemical composition (Table 1). In fact, the comparison of the data obtained from the same essential oil showed the predominance of α-pinene (70.4%) along with minor quantities of other compounds such as germacrene D (6.7%), α-cadinol (3.2%) and sabinene (2.3%). However, according to Afsharypuor et al. (2000), the main constituents of the essential oils from the aerial parts of *H. angustifolia* (DC.) *O. Kuntze* from Iran were β-pinene (51.5%), β-phellandrene (16,5%), α-pinene (13,9%) and α-thujene (2,7%).

Table 1

Identified components in the essential oil from flowers of *Hertia cheirifolia* L.

Compound[a]	L.R.I[b]	Flowers (%)[c]
α-Pinene	941	**70.4**
Camphene	955	tr [d]
Sabinene	978	2.3
β-Pinene	982	1.3
Myrcene	993	0.9
α-Terpinene	1020	Tr
p-Cymene	1028	tr
Limonene	1032	0.8
γ-Terpinene	1064	tr
Terpinolene	1090	0.1
α-Pinene oxide	1097	tr
cis-verbenol	1141	tr
4-Terpineol	1179	0.8
α-Terpineol	1191	tr
Verbenone	1205	tr
α-Terpinyl acetate	1352	tr
(E)-β-damascenone	1383	0.1
β-Elemene	1392	0.9
β-Caryophyllene	1419	0.6
α-Humulene	1456	0.6
Germacrene D	1482	**6.7**
Valencene	1493	0.6
Bicyclogermacrene	1496	0.8
β-Bisabolene	1508	0.8
δ-Cadinene	1524	0.9
Spathulenol	1577	0.6
Caryophyllene oxide	1582	0.7
Globulol	1584	0.7
epi-10-γ-eudesmol	1622	0.7
1-*Epi*-cubenol	1629	0.6
T-Cadinol	1641	1.0
α-Cadinol	1654	**3.2**
Acorenone	1688	0.8
Manoyl oxide	1991	0.7
Abietadiene	2082	0.5
Identified Compounds		**98.1**
Monoterpene hydrocarbons		75.8
Oxygenated monoterpenes		0.8
Sesquiterpene hydrocarbons		11.9
Oxygenated sesquiterpenes		8.3
Diterpenes		1.2
Others		0.1

[a]Identification of compounds was made by the calculation of their L.R.I and by GC-MS analysis.

[b]LRI: Linear Retention indices (HP-5 column).

[c]%: Percentage calculated by GC-FID on non-polar capillary column (HP-5).

[d]tr: Trace (< 0.1%).

3.2. Antibacterial activity of the plant essential oil

The study of the antibacterial activity of essential oil of the plant species (*H. cheirifolia*) shows that this oil has an inhibitory effect on all the used bacterial strains. This effect is highlighted by the measurement of the trends of growth inhibition resulting in an MIC of 0.078 mg/mL. According to our findings, the oil of *H. cheirifolia* possessed a significant inhibitory effect against *Staphylococcus aureus* and *Bacillus licheniformis* with the lowest MIC values (Table 2). However, in general, the different microorganisms do not have a similar sensitivity against essential oils.

Table 2

Antibacterial activity of essential oil from *H. cheirifolia* flowers.

Microorganisms	MIC [a] and MBC [b] (mg/mL)		
	Flowers		Gentamicin
	MIC	MBC	MBC
Staphylococcus aureus (ATCC [c] 6538)	0.078	0.078	0.031
Bacillus subtilis (ATCC 6633)	0.156	0.312	0.062
Bacillus licheniformis	0.078	0.156	0.031
Escherichia coli (ATCC 8739)	1.25	1.25	0.125
Pseudomonas aeruginosa (ATCC 9027)	2.5	5	0.5
Salmonella enteric (IPC [d] 8039)	2.5	>5	0.125
Salmonella typhimirium	1.25	5	0.25

[a]MIC: Minimum inhibitory concentration in (mg/mL).

[b]MBC: Minimum bactericidal concentration in (mg/mL).

[c]ATCC: American Type Culture Collection.

[d]IPC: Institute Pasteur Collection.

Due to the variability in the amounts and profiles of essential oils components, it seems that their antimicrobial activity is not due to a single mechanism and it is most probably related to several sites of action at the cellular level (Carson et al., 2002) such as perturbation of the cytoplasmic membrane, perturbation of the proton motive force and coagulation of the protein content of cells (Davidson, 1997). The mode of action of essential oils depends on the type and characteristics of the active components and microorganisms (Carson et al., 2002; Cox et al., 2000; Dorman and Deans, 2000). Likewise, the antibacterial activity can be reactive with other factors such as species of the plant of interest and the climatic conditions of the sampling area (Mohammadhosseini et al., 2017).

4. Concluding remarks

In the present report, the most abundant natural compound occurring in the essence of *H. cheirifolia* (flowers) was found to be α-pinene (>70%) accounting or the high occurrence of monoterpene hydrocarbons in the respective chemical profile.

The results obtained in this study also showed that the essential oil from the flowers of *H. cheirifolia* exhibits antimicrobial activity against all bacterial strains more specifically versus *Staphylococcus aureus* and *Bacillus licheniformis*.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

Abrassat, J.L., 1988. A Thousand and One Virtues of Essential Oils, Ed. Guy Tredaniel.

Afsharypuor, S., Jahromy, M.M., Rahiminezhad, M.R., 2000. Investigation of the volatile oil of *Hertia angustifolia* (DC.) O. Kuntze. Daru J. Pharm. Sci. 8(1-2), 7-8.

Ammar, S., Edziri, H., Mahjoub, M.A., Chatter, R., Bouraoui, A., Mighri, Z., 2009. Spasmolytic and anti-inflammatory effects of constituents from *Hertia cheirifolia*. Phytomedicine 16(12), 1156-1161.

Attia, S., Grissa, K.L., Mailleux, A.C., Heuskin, S., Lognay, G., Hance, T., 2012. Acaricidal activities of *Santolina africana* and *Hertia cheirifolia* essential oils against the two-spotted spider mite (*Tetranychus urticae*). Pest Manag. Sci. 68(7), 1069-1076.

Baudoux, D., 2002. The Aromatherapy: To Be Treated by the Essential Oils, Ed. Amyris, Brussels, pp. 17-36.

Bruneton, J., 1987. Element of Phytochemistry and Pharmacognosy. (First Ed.), Tech and Doc, Lavoisier, Paris, pp. 584.

Bruneton, J., 1993. Pharmacognosy, Phytochemistry, Medicinal use the, 2nd Ed. Tech and Doc, Lavoisier, Paris.

Carson, C.F., Mee, B.J., Riley, T.V., 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother. 46(6), 1914-1920.

Cintia, S.G.K., Smania, J.A., Pedrosa, R.C., Ferreira, S.R.S., 2007. Antioxidant and antimicrobial activities of shiitake (*Lentinula edodes*) extracts obtained by organic solvents and supercritical fluids. J. Food Eng. 80(2), 631-638.

Clevenger, J.F., 1928. Apparatus for the determination of volatile oil. J. Am. Pharm. Assoc. 17, 345-349.

Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R., Wyllie, S.G., 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J. Appl. Microbiol. 88(1), 170-175.

Davidson, P.M., Taylor, T.M., 1997. Chemical preservatives and natural antimicrobial compounds. In: Doyle, M.P., Beuchat, L.R., Montville, T.J., (Eds.), Food Microbiology: Fundamentals and Frontiers, ASM press, Washington, pp. 520-556.

Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88(2), 308-316.

Franchomme, P., Jollois, R., Penoel, D., 2001. Aromatherapy Exactly: Encyclopedia of the therapeutic use of aromatic extracts, Ed. Roger Jollois.

Majouli, K., Besbes Hlila, M., Hamdi, A., Flamini, G., Ben Jannet, H., Kenani, A., 2016a. Antioxidant activity and α-glucosidase inhibition by essential oils from *Hertia cheirifolia* (L.). Ind. Crops Prod. 82(1), 23-28.

Majouli, K., Hamdi, A., Msaada, K., Kenani, A., 2016b. A bioactivity guided study on the antibacterial activity of *Hertia cheirifolia* L. extracts. Microb. Pathogen. 1-6.

Majouli, K., Mahjoub, M.A., Rahim, F., Hamdi, A., Wadoode, A., Besbes Hlila, M., Kenani, A., 2017. Biological properties of *Hertia cheirifolia* L. flower extracts and effect of the nopol on α-glucosidase. Int. J. Biol. Macromol. 95(1), 757-761.

Mohammadhosseini, M., Sarker, S.D., Akbarzadeh, A., 2017. Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. J. Ethnopharmacol. 199(1), 257-315.

Ruberto, G., Baratta, M.T., 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 69(2), 167-174.

Sanon, A., Garba, M., Auger, J., Huignard, J., 2001. Analysis of the insecticidal activity of methylisothiocyanate on *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae). J. Stored Prod. Res. 38(2), 129-138.

Sharifi-Rad, J., Sureda, A., Tenore, G.C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M.R., Ademiluryi, A.O., Sharifi-Rad, R., Ayatollahi, S.A., Iriti, M., 2017. Biological activities of essential oils: From plant chemoecology to traditional healing systems: A review. Molecules 22(1), 70.

Sharma, P.R., Dilip, M.M., Muthiah, S., Pal, H.C., Shahi, A.K., Saxena, A.K., Qazi, G.N., 2009. Anticancer activity of an essential oil from *Cymbopogon flexuosus*. Chem. Biol. Interact. 179(2-3), 160-168.

Yanishlieva, N.V., Marinova, E.M., Gordon, M.H., Raneva, V.G., 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem. 64(1), 59- 66.