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# *In vitro* antibacterial activity of the *Hertia cheirifolia* L. essential oils

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ABSTRACT

**Objective:** To investigate the antibacterial activity of essential oils from *Hertia cheirifolia* L. flowers, vegetative part (leaves + stems) and roots against a panel of bacterial strains.
**Methods:** The essential oils from *Hertia cheirifolia* L. flowers, vegetative part (leaves + stems) and roots were analyzed by gas chromatography and gas chromatography–mass spectrometry. The antibacterial activity of essential oils was evaluated *in vitro* by the broth dilution method.
**Results:** The results showed that the essential oil of flowers exhibited a strong antibacterial activity against *Staphylococcus aureus* with minimal inhibitory concentration of 0.078 mg/mL.
**Conclusions:** The essential oils of *Hertia cheirifolia* can be a good source of antibacterial compounds. They can be used for pharmaceutical treatments.

## 1. Introduction

Since the last several years, the development of scientific research and industrial applications require the use of plant extracts and natural components[1]. Essential oils are characterized by their aromatic and volatile substances. They have several biological properties[2]. The essential oils are used in various fields such as food conservation and pharmacotherapy[3]. However, to be more effective, the concentration of essential oils should be specified during application[4]. Lamiaceae, Apiaceae and Asteraceae were the most studied families for the extraction of essential oils[5-7].

The Asteraceae family includes the genus *Hertia* which is an aromatic herb used in traditional medicine and contains a plurality of species[8]. In Tunisia, the species *Hertia cheirifolia* L. (*H. cheirifolia*) was found.

Previous studies on *H. cheirifolia* showed the presence of sesquiterpenoid compounds and have examined some biological activities. The spasmolytic and anti-inflammatory effects of the crude extracts of the vegetative part of *H. cheirifolia* were reported by Ammar *et al*.[9] and the acaricidal activity reported by Attia *et al*.[10]. However, according to literature survey, there are no reports on the antibacterial activity of *H. cheirifolia* essential oil. The present research aimed to study the main compounds and the biological activity of the essential oils extracted from different parts of *H. cheirifolia*.

## 2. Materials and methods

### 2.1. Plant material

*H. cheirifolia* was collected from Thala in Tunisia. The different parts of this plant including flowers, vegetative part (leaves + stems) and roots were separated. A voucher specimen (Hc 112) was deposited in the Laboratory of Medicinal Chemistry and Natural Products at the Faculty of Science, University of Monastir, Tunisia.

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### 2.2. Extraction of essential oils

The fresh flowers, vegetative part (leaves + stems) and roots of *H. cheirifolia* were subjected to hydrodistillation[11]. Then, the essential oils were collected and accumulated in glass vials in a refrigerator.

### 2.3. Analysis of the essential oils

The essential oils of fresh flowers, vegetative part (leaves + stems) and roots of *H. cheirifolia* were analysed by gas chromatography and gas chromatography–mass spectrometry[12].

### 2.4. Antibacterial activity

#### 2.4.1. Bacterial strains

The antibacterial activities of the essential oils of flowers, vegetative part (leaves + stems) and roots were individually tested against the Gram positive bacteria [*Staphylococcus aureus* ATCC 25923 (*S. aureus*) and *Enterococcus faecalis* ATCC 29212], the Gram negative bacteria [*Escherichia coli* ATCC 25922 (*E. coli*) and *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*)] and clinical strain [*Acinetobacter baumanii* (*A. baumanii*)].

#### 2.4.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC is the lowest sample concentration able to inhibit visible microbial growth; this concentration does not specify if the bacteria were killed in part or in full. While, the MBC is the concentration able to kill 99.9% of the bacterial population[13].

The MIC values of various samples were evaluated by microdilution in a 96-well plate[14]. The MIC of each sample was defined as the lowest concentration of essential oil that inhibited the bacterial growth after incubation at 37 ˚C for 18 to 24 h. The MBC was determined by subculture on blood agar. Indeed, a volume of 10 µL was taken from the well corresponding to the MIC, then streaked 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 was regarded as MBC[15]. Thymol was used as a positive control due to its known antimicrobial properties[16].

## 3. Results

### 3.1. Analysis of the essential oils

The major compounds of the essential oils extracted from the different fresh parts were α-pinene, valencene, β-caryophyllene, germacrene D, germacrene A and α-terpinyl acetate.

The monoterpene hydrocarbons were the most abundant class in the vegetative part and flowers followed by the other classes. In essential oil of roots, sesquiterpene hydrocarbons were dominant (Figure 1).

### 3.2. Antibacterial activity

The antibacterial properties of flowers, vegetative part (leaves + stems) and roots essential oils from *H. cheirifolia* were assessed, and MIC and MBC values were evaluated (Table 1).

The results showed that the essential oils of the flowers and vegetative part (leaves + stems) from *H. cheirifolia* were more active against the Gram positive bacteria (MIC of 0.078 to 0.312 mg/mL) than Gram negative bacteria (MIC of 1.25 to 5.00 mg/mL). The values of MIC and MBC indicated that the essential oils from flowers of *H. cheirifolia* had the strongest antibacterial activities against *S. aureus* (MIC and MBC of 0.078 mg/mL); whereas, the same oil had the weakest activities against *P. aeruginosa* and *A. baumanii*.

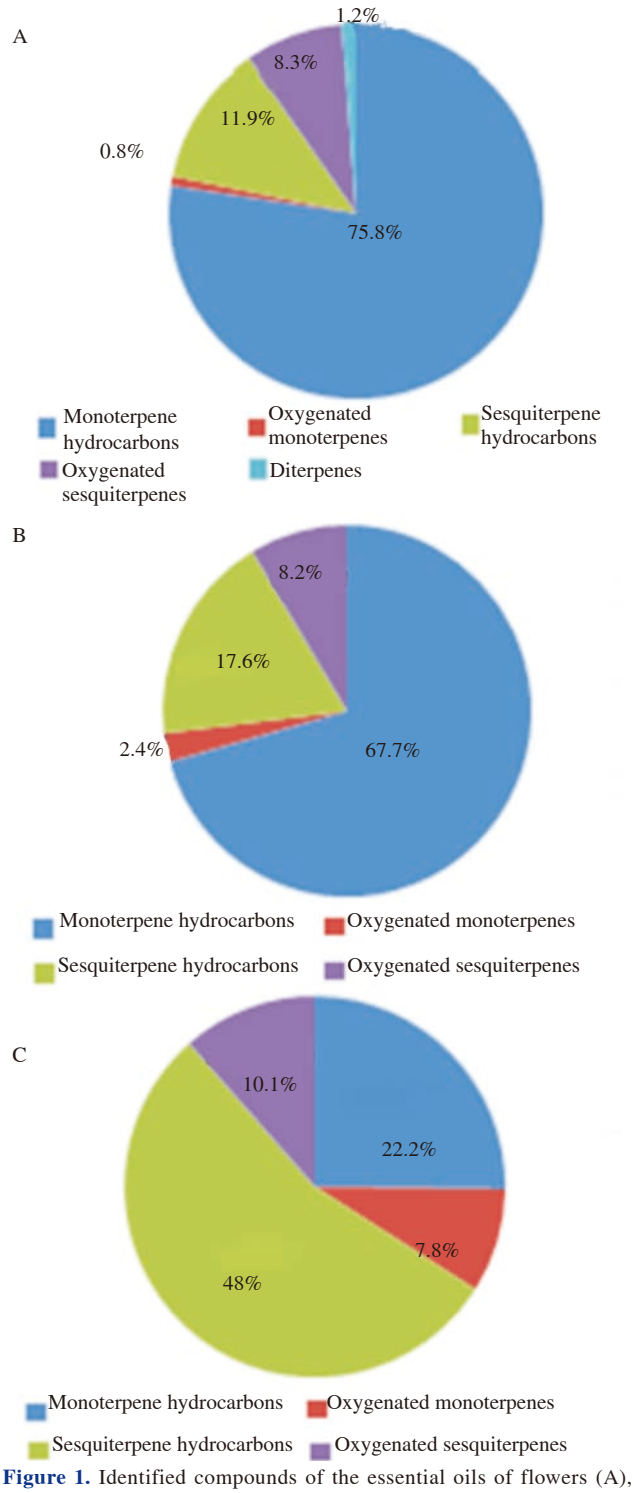


**Figure 1.** Identified compounds of the essential oils of flowers (A), leaves + stems (B) and roots (C) from *H. cheirifolia*.

**Table 1**
Antibacterial Activity of *H. cheirifolia* L. essential oils (mg/mL).

| Microorganisms | | Flowers | | Leaves + stems | | Roots | | Thymol |
|---|---|---|---|---|---|---|---|---|
| | | MIC | MBC | MIC | MBC | MIC | MBC | MBC |
| Gram positive bacteria | *S. aureus* | 0.078 | 0.078 | 0.156 | 0.156 | 2.500 | 5.000 | 0.200 |
| | *E. faecalis* | 0.156 | 0.312 | 0.312 | 0.625 | 1.250 | 1.250 | 0.600 |
| Gram negative bacteria | *E. coli* | 1.250 | 1.250 | 2.500 | 5.000 | 2.500 | 2.500 | 0.250 |
| | *P. aeruginosa* | 2.500 | 5.000 | 5.000 | 5.000 | 5.000 | > 5.000 | 1.000 |
| Clinical strain | *A. baumanii* | 2.500 | 2.500 | 5.000 | > 5.000 | - | - | n.d |

n.d: Not determined; -: Not active. *E. faecalis*: *Enterococcus faecalis* ATCC 29212.

## 4. Discussion

It is known that Gram negative bacteria are more resistant to essential oils than Gram positive ones[17], this resistance could be due to the differences in the cell membrane of these microorganisms. Indeed, Gram negative bacteria have an outer membrane surrounding the cell wall, which limits the diffusion of hydrophobic compounds. However, Gram positive bacteria possess a cell wall without this outer membrane[18].

The significant antibacterial activity of the essential oils from aerial parts [flowers and the vegetative part (leaves + stems)] could be associated with the presence of high content of monoterpene hydrocarbons such as α-pinene[19] and some sesquiterpene hydrocarbons such as germacrene D[20] and β-caryophyllene[21]. The abundance of the oxygenated sesquiterpenes such as caryophyllene oxide and the low amount of the oxygenated monoterpenes in the roots essential oil could considerably reduce the activity[22]. However, in a mixture of several molecules of essential oils, it is difficult to differentiate the component responsible for the antibacterial activity. In fact, this activity can be attributed to major or trace compounds or both together. Thus, the synergistic and antagonistic effects of these molecules which may be responsible for biological activity should not be omitted.

This study investigated the *in vitro* antibacterial activity of the essential oils extracted from *H. cheirifolia* for the first time. In this study, the obtained results highlight a strong antibacterial activity against *S. aureus*, thus justifying the use of this plant in traditional medicine. Thereby, this oil can be used in pharmaceutical treatments. However, it is still necessary to investigate the activity of these oils against other bacterial and fungal strains.

## Conflict of interest statement

We declare that we have no conflict of interest.

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