Chemical composition, acetylcholine esterase and Anti-bacterial activities of essential oils of *Elaeosilenum thapsioides* (Desf.) Maire from Algeria.

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Abstract

Background: Traditionally, the Algerian medicinal plant *Elaeosilenum thapsioides* (Desf.) Maire have been used for many diseases. **Objective:** The present research work is aimed to explore the chemical and biological characterization of its essential oil. **Methods:** The essential oils were obtained by hydrodistillation of different *Elaeosilenum thapsioides* (Apiaceae) aerial parts samples collected from two different regions (Mahouane and Megres) Setif, Eastern Algeria. The chemical characterization of the obtained essential oils is here reported for the first time. Besides, they were evaluated for their *in vitro* acetylcholinesterase (AChE) inhibitory activity which is involved in Alzheimer's disease. Using Ellman's spectrophotometric method. Additionally, their *in vitro* antimicrobial activity was assessed by the disc diffusion method. Both activities were performed at various oil concentrations. **Results:** The GC/MS analysis of the aerial parts (leaves, stems, flowers, and seeds) essential oils of *E. thapsioides* permitted to identify 47 constituents. Monoterpene hydrocarbons were the main components, ranging from 72.78 to 99.13%. Oxygenated monoterpenes and oxygenated sesquiterpenes ranged between 1.37 and 17.25 % and 0.12 and 3.53% in leaves and stems essential oils. Sesquiterpene hydrocarbons were present in small to large quantities in the essential oils of both populations, ranging from 0.69 to 13.44%. For the isothiocyanates, their presence was recorded in leaves and stems essential oils from Mahouane and Merges (9.73 and 3.72%, respectively). The stems essential oil obtained from plants collected in Mahouane showed the highest AChE inhibitory activity. Whereas, the highest antibacterial activity was shown by the essential oil obtained from Megres leaves against *Bacillus cereus* ATCC 11778. **Conclusion:** The oils exhibited a moderate inhibitory activity in both tests.

Keywords: *Elaeosilenum thapsioides*, essential oils, GC-MS, anti-cholinesterase activity, anti-bacterial activity, Algeria.

1. Introduction

Plants of Apiaceae family have the characteristic of synthesizing secondary metabolites such as phenols, monoterpenes and sesquiterpenes [1]. Essential oils, also known as volatile odoriferous oils, are aromatic and natural compounds that can be extracted from different plant parts, such as flowers, peelings, bark, leaves, roots, stems, seeds, etc. They are very complex mixtures of different chemicals [2].

There are many factors that determine the variation in both the composition and the yield of essential oils. These factors are interdependent and influence each other, so they must considered as a whole [3]. Seasonal and maturity variation, geographical origin, genetic variation, growth stage, the part of the plant utilized, the post-harvest, the drying and storage are some of these variables [4].

Elaeosilenum is a genus of the Apiaceae family (Umbelliferae), Apioideae subfamily (Laserpitieae tribe), [5]. This genus includes two species: *E. asclepium* subsp. *meoides* (Koch.) (synonym: *Thapsia asclepium* L. or *Laserpitium asclepium* L.) Fiori, and *E. thapsioides* (Desf.) Maire (synonym: *E. fontanesii Boiss*); locally it is known as ¨Becibsa¨ [1].

E. thapsioides is a perennial plant, which grows on dry and rocky soils, up to 3 m high. Its leaves are characterized by short and acute linear divisions that are stalked, hairless and smooth, all arranged in one plane and the fruits are shorter than the umbels, which are pedunculated. This species is widespread in lawns and bushes throughout Algeria [6].

Alzheimer's disease is represented, in the industrialized countries, the most frequent form of dementia in the elderly subjects [7]. Approximately 10 % of people in our planet older than 65 years are affected by this disease [8]. This illness causes reduced cognitive function, with deterioration of memory, as well as changes in mood and behavior [9,10]. Some of the most effective treatments for this disease are cholinesterase (ChE) inhibitors that have been shown to be effective in mild and moderate to severe forms of Alzheimer's disease [11]. The use of acetylcholine precursors, such as choline, lecithin and dimethylaminoethanol has been proposed as a treatment for Alzheimer's disease, Also, acetylcholinesterase inhibitors have been revealed to be useful because of their pro-memory effect, i.e. physostigmine. [12]. Galanthamine, the current newest anti-ChE drug in the market, is a plant-based compound [13]. On the other hand, donepezil, tacrine and rivastigmine can also be used as synthetic and semi-synthetic inhibitors However, these drugs have disadvantages, such as short half-lives and undesirable side effects, including hepatotoxicity and gastrointestinal irritation. Indeed, this has encouraged researchers to search for new naturally occurring AChE inhibitors, such as secondary metabolites obtained from essential oils [14]. The aims of this work were to perform: i) the analysis of the essential oil obtained from different *E. thapsioides* aerial parts from different geographical areas using gas chromatography-mass spectrometry (GC-MS) and, ii) the determination of its AChE inhibitory ability, together with the antimicrobial activity against four pathogenic bacteria strains.

2. Material and methods

2.1. Plant materials

The separated organs (leaves, stems, flowers) of *E. thapsioides* were collected in May 2018, while the seeds in July of the same year, from Mahouane population, while those of Megres population (leaves, stems, flowers) were collected in early July 2018, during the flowering season and at the end of July 2018. The two localities were situated at an altitude of 800 m (Mahouane) and 1730 m (Megres), in eastern Algeria. The taxonomic identification was carried out by Prof. Hocine Laouer, Department of Vegetable Biologie and Ecologie, Faculty of Science of Nature and Life, University Ferhat Abbas, Setif 1; a voucher specimen was deposited at the Herbarium of the Department of Biology and Plant Ecology, University of Setif 1, Algeria.

2.2. Spectral measurements and chemicals

The anti-ChE activity was assessed by a spectrophotometric method and the measurements were performed using a microplate reader, Perkin Elmer Multimode Plate

Reader EnSpire at National Center of Biotechnology Research (CRBt) Constantine, Algeria. The used chemicals were: AChE from electric (Type-VI-S, EC 3.1.1.7, 827,84 U/mg, Sigma), acetylthiocholine iodide, 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB), galantamine. They were all purchased from Sigma-Aldrich and BiochemChemopharma. Dimethylsulfoxyde (DMSO) was used as solvent for essential oil and negative control in antibacterial activity.

2.3 Extraction of the essential oil

A dried sample of the plant aerial parts (100 g) was hydro-distilled for 3h using a Clevenger-type apparatus. The *E. thapsioides* oils of Mahouane population had a transparent color and gave yields (w/w) of 0.26% for leaves, 0.26% for stems, 1.12% for flowers and 0.75% for seeds. In the case of *E. thapsioides* oil of Megres population, the yields were of 0.41, 0.33, 0.70 1.40, respectively. The obtained oils were collected and dried over anhydrous sodium sulfate, stored in opaque glass vials at +4°C until analysis and tests.

2.4 GC and GC-MS analysis

The gas chromatography-electron impact-mass spectrometry (GC/EI-MS) analyses were performed with a Varian CP-3800 apparatus equipped with a DB-5 capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm) and a Varian Saturn 2000 ion-trap mass detector. The oven temperature was programmed from 60 to 240 °C at 3 °C/min; injector temperature, 220 °C; transfer-line temperature, 240 °C; carrier gas, He (1 ml/min). The acquisition parameters were as follows: full scan; scan range: 35-300 m/z; scan time: 1.0 sec; threshold: 1 count. The identification of the constituents was based on the comparison of their retention times (Rt) with those of pure reference samples and their linear retention indices (LRIs) determined relatively to the Rt of a series of *n*-alkanes. The mass spectra were compared with those listed in the commercial libraries NIST 14 and ADAMS and in a homemade mass-spectral library, built up from pure substances and components of known oils, and MS literature data [15-20].

2.5 Anticholinesterase activity

AChE inhibitory activity was evaluated according to the method developed by Ellman et al., (1961) [21]. The enzyme was AChE and acetylthiocholine iodide was employed as reaction substrate. 5,5'-Dithio-bis-(2-nitrobenzoic acid) (DTNB) was used for the measurement of the ChE activity. In this method, 150 μl of 100 mM sodium phosphate buffer (pH 8.0), 10 μl of extract, and 20 μl AChE (5.32×10^{-3} U) solution were mixed and incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 10 μl of 0.5 mM DTNB and 10 μl of AChE iodide (0.71 mM). The hydrolyses of these substrates were monitored spectrophotometrically at 412 nm by the formation of yellow 5-thio-2-nitrobenzoate anion (TNB^{2-}), as the result of the reaction of DTNB with thiocholine, released by the acetylthiocholine iodide enzymatic hydrolysis,. Percentage of inhibition of AChE = AChE Inhibition (%), was determined following this formula= (E-S)/E × 100. E: is the enzymatic activity without the test sample, and S is the enzymatic activity with the tested sample. (S is commonly used for substrate, I suggest to change the change the symbol). Galantamine was used as a reference compound. The results were given as 50% inhibitory concentration (IC_{50}).

2.6 Antimicrobial activity

The anti-microbial activity of the *E. thapsioides* essential oils was investigated against two gram-negative bacteria strains, *Escherichia coli* ATCC 25922 (*E. coli*), *Proteus mirabilis* ATCC 35659 (*P. mirabilis*) and two gram-positive bacteria, *Bacillus cereus* ATCC 117 (*B. cereus*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*); *S. aureus* was obtained from the Laboratory of Applied Microbiology, Faculty of Pharmacy, Setif, Algeria, *P. mirabilis* and *B. cereus* were obtained from Pasteur Institute of Msila, Algeria and *E. coli* from the Microbiology Laboratory of Setif hospital, Algeria. The bacterial inhibition zone diameter at different oil dilutions was assessed by the disc diffusion method. Different dilutions of essential oils were prepared in DMSO (1/2, 1/5 and 1/10 v/v). Bacteria were cultured on Mueller-Hinton agar (Bio-Rad, France). A volume (20 ml) of the medium was poured into 90 mm diameter Petri dishes. Sterilized 6 mm diameter Whatman discs were impregnated (10 μl) at each dilution; other discs were impregnated with the same volume of pure DMSO as a negative control and then placed on inoculated agars. Moreover, discs containing 10 μg of gentamycin (Bio-Rad, France) were used as a positive control. The bacteria used in this test were obtained from 24 h cultures and suspended in physiological saline solution (0.9% NaCl), to obtain a concentration of approximately 10^8 (colony-forming unit) CFU/ml by comparison with tube No. 1 of a sterile McFarland scale. The medium surfaces were spread with a sterile swab containing the microbial suspension, dried and left for 30 minutes at room temperature to allow diffusion of the oil, and then the prepared Petri dishes were incubated at 37°C for 24 h. All tests were performed in triplicate. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs.

2.7 Statistical analysis

Data were recorded as means ± standard deviation of three measurements. Analysis of variance (ANOVA) was performed by one-way analysis of variance. Significant differences between means were determined by Tukey test, p values < 0.05 were regarded as significant.

3. Results

3.1. Phytochemical composition

The analysis by GC/MS of the aerial parts, leaves, stems, flowers, and seeds essential oils of *E. thapsioides*, permitted to identify 47 chemicals (Table 2). The essential oil yields ranged between 0.26 and 1.40%. Monoterpene hydrocarbons were the main components, ranging from 72.78 to 99.13%. Oxygenated monoterpenes and oxygenated sesquiterpenes followed (1.37 to 17.25% and 0.12 to 3.53% in leaves and stem essential oils, respectively). Sesquiterpene hydrocarbons were present in variable quantities in the essential oils of both populations, (0.69-13.44%), but they were scarcely represented in the essential oils of leaves and stem of the Megres population. The presence of isothiocyanates was recorded in the essential oil obtained from leaves and stem of both Mahouane and Megres regions (9.73 and 3.72%, respectively). Diterpene hydrocarbons and oxygenated diterpenes were present in small quantities only in the essential oil obtained from leaves of the Megres population (1.76 and 0.39%, respectively). Non-terpene derivatives were also present in small quantities in the in the essential oil of the leaves and the stems from Megres (0.3 and 0.47%). The main constituent of *E. thapsioides* aerial parts (leaves, stems, flowers, and seeds) from Mahouane and Megres regions was the monoterpene myrcene. Besides, the major components of the essential oils obtained from flowers and seeds from Mahouane and Megres populations were α-pinene (36.93, 56.67% and (43.67, 49.26%) and β-pinene (14.54, 22.93%and 16.17, 23.72%). However α-pinene was present in lesser percentages (1.41 and 5.26%) in the leaves and stems essential oils of both populations. The opposite trend was observed for β-pinene in leaves and stem oils, with 0.9 and 11.51 in Mahouane and 7.41 and 2.5% in Megres. Limonene (6.71 and 10.89%) characterized the seeds essential oil of Mahouane and Megres. In the essential oil of the leaves from Mahouane station was perillene and 4-terpineol were present in appreciable percentages (8.03 and 7.36%), but they were almost absent in the leaves of the Megres population. *m*-Methoxybenzyl isothiocyanate (9.37%) characterized the stems essential oil of the Mahouane population, whilst it was present in small amounts in Megres population oils; on the contrary, it was not present in the oils obtained from the other aerial plant parts of both populations.

Another important compounds, γ-muurolene, was identified in leaves and stems essential oil from Merges population (8.8 and 7.83%), whilst it reached smaller percentages in the oil obtained from the stems and it was completely absent in that of the leaves of Mahouane population. The essential oils yields averaged from 0.26 to 1.40%, in good agreement with other species of the same genus.

3.2 Anticholinesterase activity

The results of anti-cholinesterase activity are summarized in Table 3. The essential oils of leaves, stems, flowers and seeds from Mahouane population gave the following IC_{50}: 67.66, 52.71, 154.52, and 158.35 µg/ml, respectively. In the case of the oils from Megres population the IC_{50} were 93.86, 67.15, 62.65 and 78.15 µg/ml, respectively.

Thus, the most effective inhibitory activity was observed for the essential oil obtained from the plant stems of the Mahouane population (IC_{50}=52.71µg/ml), while the least effective was the one extracted from the plant seeds harvested in Megres (IC_{50}=158.35 µg/ml).

3.3 Antibacterial activity

To evaluate the anti-bacterial activity of *E. thapsioides*, the various essential oils were tested by the disc diffusion method. The diameters of inhibition of the bacterial strains (gram negative and positive) are expressed by measuring the diameter of the inhibition halos in mm after 24 h of incubation at 37°C (Tables 4, 5, 6 and 7). The essential oils had limited action on the growth of *B. cereus* ATCC 11778, especially the samples obtained from leaves and seeds of Megres population (15 and 12 mm, respectively); this strain resulted to be resistant to the essential oils obtained from flowers and stems of both Mahouane and Megres populations. Even smaller inhibition diameters were observed for *S. aureus* ATCC 25923 (7.5 to 11 mm), *E. coli* ATCC 25922 (8 to 11.5 mm) and *P. mirabilis* ATCC 35659 (6 to 9.5 mm). All these results were observed for oils at dilution of 1/2.

4. Discussion

A yield of 0.5% was previously reported for the aerial plant parts of *E. thapsioides* [22]. The fruits and flowers yields of *E. tenuifolium* were 4.93 and 4.63 %, respectively, while leaves yield was 0.66 % [23]. On the other hand, the study of Bouchekrit et al. [1] showed that *E. asclepium* yielded was 1.2% of essential oil, a different value from that obtained for the seeds from Megres population. Nevertheless, the seeds of the same species also showed a yield of 4.66% [23], and 3.8% [5]. However, an alternative study performed by the Evergetis research group, in 2009, reported that the *E. asclepium* aerial plant parts yield was about 0.02 % [24], the lowest value until now.

Other *Elaeosilenum* species showed higher values, such as the aerial plant parts of *E. gummiferum* that gave a yield of 2.6 6% [25]. The yields reported for *E. foetidum* yield were 0.55% for both leaves and stems and 5% for fruits [23].

In the essential oil of *E. fontanisii* collected in different regions of Morocco the major components were α-pinene, sabinene, myrcene, limonene, terpinen-4-ol, α-terpinene and γ-terpinene, in good agreement with our findings [26]. The major constituents of the essential oil extracted from the aerial plant parts of *E. gummiferum* and harvested from the Toledo Mountains in Spain were α-pinene (37.4%), myrcene (22.8%), β-pinene (14%), α-fenchene (10.4%), β-phellandrene (7.1%) and α-terpeneol (2.5%) [25]; these values ere very similar to the composition reported for the oils obtained from flower and seed of *E. thapsoides*. *Distichoselinum tenuifolium*(=*Elaeoselinum tenuifolium*), in the province of Algarve, South Portugal, is considered as the unique species of the genus containing a high percentage of myrcene (48-85%) that normally is not detected in the essential oil of other *Elaeoselinum* species [27]. It was like essential oil extracted from leaves and flowers of *E. thapsioides* (54. 86 % - 74.09 %). So, *E. thapsioides* can be ordered in the second place of Elaeoselinum species after *E. tenuifolium* containing of myrcene. The different percentages of 4-terpineol in the essential oil of different species of the genus Elaeosilenum were mentioned in the (Table1) varying from (1 % - 4 %), while it was 7.36 % in the essential oil extracted from the leaves of the Mahouane population, and this latter one has contained perillene (8.03 %), while the perillene compound is totally absent in all the species listed in (Table1).

On the other hand, the essential oil obtained from stems of the Mahoane population and those extracted from leaves and stems of the Megres population contain 4.61 %, 8.8 %, 7.83 % of γ-muurolene, respectively, and the essential oils extracted from stems of the Megres population contain 9.73 % as m-methoxybenzyl isothiocyanate while they are absent in the chemical composition of different species of the (Table1).

In AChE inhibitory tests, the IC_{50} (6.27µg/ mL) of the essential oils of different plant parts was lower than that of galantamine, the reference compound (Table 3). In detail, the essential oil of the stems of the Mahouane population (IC_{50}=52.71±0.75 µg/mL) exhibited the best inhibitory activity, similar to that of acorenone B (IC_{50}=40.8 µg/ml) a component extracted from *Niphogeton dissecta* (Apiaceae) in Ecuador [28], while it is better agreement for essential oils of *Eryngium campestre* and *Eryngium amethystine* (Apiaceae) with (10.5 %, 19.5%), respectively [29]. A previous study revealed a weak inhibitory activity of myrcene against AChE (Karolina A et al.2017) [31]. Being myrcene the most abundant constituent in the stems oil, it may contribute to the total activity of the oil, probably with a synergistic effect.

Zarrad et. al., (2015) showed that pure limonene has anti-ChE activity [32]. Similarly, α-pinene and β-pinene, together with limonene, have been described to have a strong AChE inhibition activity on the adult rice weevil, *Sitophilus oryzae* [33]. In another study, α-pinene, β-pinene, limonene, 1,8-cineole, camphor, borneol, α-terpineol, β-caryophyllene and caryophyllene oxide were the main responsible for the anti-AChE activity of the oil obtained from flowers of *Artemisia annua*. Moreover, the different levels of the anti-AChE effect of the flower oil of *A. annua* at three flowering stages may be the result both from the different content of those terpenoids [34]. There is a correlation and a positive relationship between the essential oils of the different aerial parts (leaves, stems, flowers, seeds) of *H. cheirifolia* and its anti-AChE capacity due to the presence of sesquiterpene lactones. Essential oils extracted from the leaves of 14 populations of *Pistacia lentiscus* L. from Tunisia were found to have anti-AChE activity with an IC_{50} of 55.3-483.7 µg/ml, while Donepezil, used as positive control, IC_{50} was 18 µg/ml. Among the possible components responsible for this activity, β-myrcene, 2-undecanone, α-copaene, and α-muurolene have been proposed [36]. It is not mandatory that a single compound can affect a biological activity, also synergistic interaction should be taken into account [37]. ~~However, the inhibitory activity of the essential oil generally results from a complex interaction between its constituents, which produce both synergistic and antagonistic responses [38]. Early, Bonesi et. al., (2010) reveled that IC_{50} of the species Pinus as *P. heldreichii* subsp. leucodermis, *P. nigra* var. calabrica and *P. nigra* subsp. nigra were (51.1 ± 1.8, 101.5 ± 2.4 and 94.4 ± 1.8 µg/ml), respectively; furthermore, the mainly constituents of essential oils were α-pinene (24.6 %), β-pinene (10.9 %), γ-cadinene (9.9 %), β-phellandrene (6.3 %), manoyl oxide (6.2 %), and *trans*-caryophyllene (4.2 %), for *P.nigra* var. calabrica; α-pinene (25.3 %), limonene (22.6 %), sabinene (12.8 %), α-terpineol (8.3 %), β-pinene (4.8 %), and terpinolene (4.5 %) for *P. nigra* subsp. nigra; and α-Pinene (24.2 %), β-pinene (8.4 %), and limonene (7.8 %), α-cubebene (7.6 %), terpinolene (5.9 %), and *trans*-caryophyllene (4.5 %) for *P. heldreichii* subsp. leucodermis [39~~].

~~The results of anti-bacterial activity obtained by different aerial parts of *E. thapsioides* were in good agreement with those obtained by Lamamra et. al., (2016) for the essential oil of *Daucus aristidis*, an umbelliferous plant, showing that the diameters of inhibition were 13 mm against *E. coli* and *B. cereus* and 10 mm against *S. aureus* with the 1/2 di, whereas *P. mirabilis* showed resistance against this oil. However, the activity of the essential oil of *D. aristidis* could be attributed to the presence of α-pinene (20.14%) as a major component~~

~~known for its significant antimicrobial activity [40].~~ In a previous study, *E. thapsioides* essential oil show a high activity on *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, and no effect against *P. aeruginos*a ATCC 2785 and *K. pneumonia* ATCC 700603 [23]. Additionally, the essential oil of *E. asclepium* is able to inhibit the growth of the yeast *C. albicans* and both Gram-negative (*E. coli*) and Gram-positive (*S. aureus, M. luteus, B. cereus*) bacteria. The observed moderate antibacterial activity of this essential oil can be attributed to its high amount of monoterpenes, such as α-pinene (43.9%). This characteristic is in common with flower and seed essential oils, in which α-pinene varies from 36.93 to 56.67% [1]. ~~*E. campestre* essential oil was active against five of the seven Gram positive tested strains, while it was inactive against the five Gram-negative investigated strains. The antibacterial activity of *E. campestre* essential oil could directly be connected to the essential oil chemical composition [41]. Leaves essential oil of *Rosmarinus officinalis* from narrow phenotype (NP) had a (31,1%) of Myrcene as the mainly component, this oil gave a lower efficacy as antibacterial and it was unable to induce a total inhibition of the growth of *S. aureus*, *E. faecalis* and *K. pneumoniae*that α-pinene was the only compound that was able to inhibit all the tested microorganisms with MIC values ranging from 0.8 to 8 mL/ mL [42].~~

5. Conclusion

This is the first report on volatile constituents, anti-cholinesterase and antimicrobial activity of different aerial parts of *E. thapsioides*. The essential oil studied from these different parts showed a moderate anti-cholinesterase activity and gave an ordinary effect against the tested strains, except for Mahouane leaves oil against *Bacillus cereus* and Megres stem oil against *Proteus mirabilis*.

Ethics approval and consent to participate

Not applicable.

Human and animal rights

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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