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Chemical Composition and Cholinesterase Inhibitory Activity of Different Parts of *Daucus aristidis* Coss. Essential Oils from Two Locations in Algeria

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Abstract: The chemical composition of the essential oils obtained by hydrodistillation from the different parts of *Daucus aristidis* Coss. (syn. *Ammiopsis aristidis* Batt.) (Apiaceae) from two locations (Ghoufi and Bousaada) in East of Algeria, was investigated for the first time by GC and GC-MS and evaluated for their *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, the enzymes linked to Alzheimer's disease, by a spectrophotometric method of Ellman using ELISA microplate reader at 100 µg/mL. The main components of *D. aristidis* oils from Ghoufi and Bousaada were α-pinene (74.1%- 49%) and β-pinene (11.9% - 19.2%). This suggests that β-pinene was the main component from Bousaada. The oils exhibited a moderate inhibitory activity (over 50%) against both of the enzymes.

Keywords: *Daucus aristidis;* Apiaceae; essential oil; anticholinesterase; α-pinene; β-pinene.

1. Introduction

Since ancient times, the plants from Apiaceae family have been used as spices or crude drugs, particularly due to their essential oils. A dozen important herbal medicinal products from this botanical family are described in several pharmacopoeias, having antiseptic, expectorant, diuretic, carminative, vasodilator or spasmolytic actions [1]. *Daucus* is a genus belonging to this family, which comprises of about 300-455 genera and 3000-3750 species worldwide [2]. In Algeria, it is represented by 55 genera, 130 species and 27 subspecies. The species have a bipolar distribution (in temperate regions), but the majority live in the temperate northern hemisphere [3].

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Daucus aristidis Coss. (synonymous: *Ammiopsis aristidi*s Batt. [4]) is an endemic plant to Algeria and has been locally known as "noukhia", which is an annual plant with high and smooth erect stem. The leaves are glabrous and pinnatisect with capillary segments. It possesses bracts that have many divided involucel and involucral with white flowers which yellowish in a herbarium and large to very large umbels rays, contracted at the end. The ovoid fruit is small 2-2.5 mm, grayish and finely tuberculate over their entire surface [3]. Several investigations have reported the chemical composition of the essential oils from *D. carota* as well as its subspecies [5-10]. However, many species and subspecies of *Daucus* still remain to be examined for their essential oil components such as *D. aristidis*. In this paper, we investigated the chemical composition of *D. aristidis* essential oil from two locations in eastern Algeria (Ghoufi and Boussaada) for the first time. The volatile compounds, extracted using hydrodistillation, were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS), and evaluated for their *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, the key enzymes that are linked to Alzheimer's disease (AD),which is a progressive and degenerative neurologic disorder resulting in impaired memory and behavior. Most treatment strategies have been based on the cholinergic hypothesis which postulated that memory impairments in patients suffering from this disease result from a deficit of cholinergic function in brain [11]. One of the most promising approaches for treating this disease is the cholinesterase inhibitors, which show consistent efficacy towards mild and moderate to severe types of Alzheimer's disease. Amongst them, galanthamine, the latest anticholinesterase drug in the market, is a plant originated-compound [12], which reinforces the interest in finding better cholinesterase inhibitors from natural resources.

Therefore, in the present study, we aimed to investigate the cholinesterase inhibitory effects of *D. aristidis* essential oils tested by the spectrophotometric method of Ellman using ELISA microtiter assays at concentration of 100 µg/mL.

2. Materials and Methods

2.1. Plant material

The separated organs (stem, leaves and umbels) of *D. aristidis* were collected in May, 2014 and 2015 from Ghoufi (Algeria), while the aerial parts of *D. aristidis* from Boussaada region (Algeria) were collected in May, 2014 at the flowering stage at the altitude of 708 m and 854 m, respectively. After taxonomic identification by Dr. Boulachaab Nacira from Department of Pharmacy, Faculty of Medicine, University Ferhat Abbas, Setif 1, a voucher specimen was deposited at the Herbarium of Department of Biology and Plant Ecology, University of Setif 1, Algeria.

2.2. Chemicals

Electric eel AChE (Type-VI-S; EC 3.1.1.7, Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis,MO, USA) were employed as the substrates of the reaction. 5,5′-dithio-bis(2-nitrobenzoicacid) (DTNB; Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity.

2.3. *Isolation of the essential oils*

The oils were isolated by hydrodistillation (100 g of plant *per* sample) for 3 h using a Clevenger-type apparatus. The oils have a light green color with a yield of 0.68% (w/w), 0.85% (w/w), 0.95% (w/w) and 0.26% (w/w) for aerial parts, stems, leaves and umbels of *D.aristidis* from Ghoufi and 0.72% (w/w) for *D.aristidis* from Bousaada The obtained essential oils were dried over anhydrous sodium sulfate, stored at +4°C until tested, and analyzed.

2.4. Determination of AChE and BChE inhibitory activities

The AChE and BChE inhibitory activity was measured by slightly modified spectrophotometric method of Ellman [13]. In this method, 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of the test solution and 20 µL of AChE/BChE solution were added by multichannel automatic pipette (Gilson pipetman, Paris) in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of 10 µL of acetylthiocholine iodide/butyrylthiocholine chloride. Hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (VersaMax Molecular Devices, Sunnyvale, CA, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software (Sunnyvale, CA, USA). The percentage of inhibition of AChE/BChE was determined by comparison of the rates of reaction of samples relative to the blank sample (ethanol in phosphate buffer pH=8) using the formula (E−S)/E×100, where E is the activity of the enzyme without test sample and S is the activity of the enzyme with the test sample. The experiments were performed in triplicate. Galanthamine (Sigma, St. Louis, MO, USA), the anticholinesterase alkaloid-type of drug obtained from the bulbs of snowdrop (*Galanthus* sp.), was used as the reference.

2.5. GC and GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium as a carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

The GC analysis was carried out using an Agilent 6890N GC system. The FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder3 Library), and in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data was used for the identification.

3. Results and Discussion

GC–MS analysis of *D. aristidis* essential oils (from Ghoufi) obtained from the aerial parts, stems, leaves and umbels accounted for 99.7%, 93.6%, 94.4% and 99.0% respectively, and the essential oil obtained from the aerial parts of *D. aristidis* originated from Bousaada represented 99.5%, these analyses allowed the identification of 61 and 32 compounds in essential oils from the separated parts of *D. aristidis* from Ghoufi and the whole aerial parts from Bousaada, respectively (Tables 1 and 2).

The constituents were characterized by a high hydrocarbon fraction ([97.1%-57%-70.4%-74.5%] and 95.7%), respectively, and represented mainly by monoterpene hydrocarbons ([95.8% - 56.5%- 70%- 71.6%] and 94.3%). Sesquiterpenes presented only ([2.1%-0.2%-0.2%-2.9%] and 2.2%) of the essential oils, whereas stems, leaves and umbels of *D. aristidis* from Ghoufi were characterized by a high oxygenated fraction (36.6% - 24% - 24.5%), respectively.

Table 1. Chemical composition of the different parts of *Daucus aristidis* essential oil from Ghoufi

Constituents	**RI**[a]	**Ghoufi (%)**	**S**[b]	**L**[c]	**U**[d]
α-Pinene	1032	74.1	43.5	53.5	55.5
α-Thujene	1035	-	0.1	0.1	0.1
Camphene	1076	0.5	0.5	0.5	0.5
Hexanal	1093	-	0.2	-	-
β-Pinene	1118	11.9	7.5	7.8	8.1
Sabinene	1132	2.2	1.1	1.4	1.6
Thuja-2,4(10)-diene	1135	0.1	0.9	1.3	0.6
δ-3-Carene	1159	t[e]	-	-	-
Myrcene	1174	3.9	0.6	0.9	1.3
α-Terpinene	1188	0.1	-	-	-
Limonene	1203	2.4	1.5	3.6	3.1
β-Phellandrene	1218	0.3	-	0.2	0.2
(*Z*)-β-Ocimene	1246	t	-	-	-
γ-Terpinene	1255	0.2	0.1	0.1	0.2
(*E*)-β-Ocimene	1266	t	-	-	-
p-Cymene	1280	0.1	0.6	0.6	0.4
Terpinolene	1290	0.1	0.1	-	-
Perillene	1429	-	0.1	0.1	-
γ-Campholene aldehyde	1439	-	0.2	0.2	t
p-Cymenene	1452	-	0.3	0.2	-
α-Copaene	1497	0.1	-	-	-
α-Campholene aldehyde	1499	0.1	2.3	1.4	1.0
Linalool	1553	0.1	0.3	0.2	0.5
Pinocarvone	1586	tr	1.5	1.0	0.8
Bornyl acetate	1591	0.1	0.2	0.1	0.2
Terpinen-4-ol	1611	0.4	0.5	0.5	t
β-Caryophyllene	1612	0.4	0.2	0.2	0.6
β-Cedrene	1613	-	-	-	0.4
Thuj-3-en-10-al	1642	0.1	-	-	-
Myrtenal	1648	-	2.9	1.8	1.0
cis-Verbenol	1663	0.1	2.5	1.4	0.6
trans-Pinocarveol	1670	0.1	4.4	2.6	1.1
α-Humulene	1687	0.1	-	-	0.4
trans-Verbenol	1683	0.2	10.6	6.5	3.1
α-Terpineol	1706	0.2	0.5	0.3	0.3
Verbenone	1725	-	3.1	2.6	0.8
Germacrene D	1726	0.4	-	-	-

β-Bisabolene	1741	-	-	-	1.3
Carvone	1751	-	0.3	0.3	0.3
Bicyclogermacrene	1755	0.1	-	-	-
δ-Cadinene	1773	0.1	-	-	-
(*E*)-α-Bisabolene	1784	0.1	-	-	0.2
Myrtenol	1804	0.2	2.2	1.2	0.6
trans-Carveol	1845	0.1	1.2	0.9	0.5
Germacrene-B	1854	t	-	-	-
p-Cymen-8-ol	1864	t	1.1	0.9	0.2
p-Cymen-9-ol	1912	-	0.2	0.2	-
Isocaryophyllene oxide	2001	-	-	-	0.2
Caryophyllene oxide	2008	0.1	0.7	0.7	3.0
Carotol	2045	0.1	0.1	-	0.5
Humulene epoxide-II	2071	-	0.2	0.2	1.2
Humulene epoxide-III	2081	-	-	-	0.2
Cedrol	2143	0.1	-	-	3.7
Spathulenol	2144	-	0.4	0.3	-
τ-Cadinol	2187	0.1	0.2	-	0.2
epi-α-Bisabolol	2256	0.1	0.2	0.2	0.5
β-Eudesmol	2257	0.1	0.3	0.2	0.3
Alismol	2264	0.1	-	-	-
Guaia-6,10(14)-dien-4β-ol	2269	-	0.2	0.2	0.9
Juniper camphor	2320	0.1	-	-	2.0
β-Asarone	2361	t	-	-	0.8
Total		99.7	93.6	94.4	99.0

[a]RI: retention indices [b]S: Stems L[c]: Leaves U[d]: Umbels t[e:] trace

The major constituents of the aerial parts, stems, leaves and umbels of *D. aristidis* oils from Ghoufi were established as α-pinene (74.1%- 43.5% - 53.5% - 55.5%) and β-pinene (11.9%- 7.5%-7.8%-8.1%), respectively. In addition the stems and leaves oils had another major component, *trans*-verbenol (10.6% -6.5%), respectively. However, α-pinene (49%), β-pinene (19.2%), limonene (7.5%), myrcene (6.7%) and sabinene (4.3%) were the major components of *D. aristidis* oil native to Boussaada region.

The essential oils were screened for their AChE and BChE inhibitory activity *in vitro* by Ellman method at concentration of 100 µg/mL. As illustrated in Table 3, the essential oils appeared to have a moderate level of inhibitory effect against both enzymes as compared to that of galanthamine (reference compound). The essential oil of *D. aristidis* from Boussaada region displayed a modest inhibition against AChE and BChE (61.75 % and 56.79%, respectively), while the aerial parts, stems, leaves and umbels of *D. aristidis* essential oils from Ghoufi displayed low to moderate inhibition against AChE (51.0, 34.69, 13.44, and 33.07%, respectively),and BChE (41.46,22.32, 23.70, and 30.00%, respectively).

Table 2. Chemical composition of *Daucus aristidis* essential oil from Bousaada

Constituents	**RI**[a]	**Boussaada (%)**
α-Pinene	1032	49.0
α-Thujene	1035	0.4
Camphene	1076	1.8
β-Pinene	1118	19.2
Sabinene	1132	4.3
Thuja-2,4(10)-diene	1135	0.1
δ-3-Carene	1159	0.2
Myrcene	1174	6.7
α-Terpinene	1188	0.1
Limonene	1203	7.5
β-Phellandrene	1218	3.3
(*Z*)-β-Ocimene	1246	0.3
γ-Terpinene	1255	0.4
(*E*)-β-Ocimene	1266	0.2
p-Cymene	1280	0.7
Terpinolene	1290	0.2
α-Campholene aldehyde	1499	0.1
Linalool	1553	0.3
Bornyl acetate	1591	0.4
Terpinen-4-ol	1611	0.9
β-Caryophyllene	1612	0.6
Thuj-3-en-10-al	1642	0.2
trans-Pinocarveol	1670	0.2
α-Humulene	1687	0.1
trans-Verbenol	1683	0.3
α-Terpineol	1706	0.3
Germacrene D	1726	0.6
Bicyclogermacrene	1755	0.1
Myrtenol	1804	0.2
Caryophyllene oxide	2008	0.2
Spathulenol	2144	0.2
epi-α-Bisabolol	2256	0.4
Total		99.5

[a]RI: retention indices

Table3. AChE and BChE inhibitory activities of the essential oils at 100 μg/mL

Essential oils tested	AChE inhibition	BChE inhibition
D. aritidis (aerial parts,Ghoufi)	51.00 ± 0.58	41.46± 2.33
D. aristidis (leaves,Ghoufi)	13.44± 0.87	23.70± 2.64
D. aristidis (umbels, Ghoufi)	33.07± 3.67	30.00± 1.09
D. aristidis (stems, Ghoufi)	34.69± 0.99	22.32± 2.83
D. aritidis (Boussaada)	61.75± 5.08	56.79± 2.95
Galanthamine (Reference)	94.48± 3.81	92.25± 0.79

The chemical analysis of *D. aristidis* essential oils from Ghoufi and Bousaada corroborate that α-pinene is the core constituent of the most essential oils of the genus species. In fact, it was reported that the major proportion of the essential oil of *D. carota* subsp. *carota* from Poland was represented by α-pinene with a percentage of 41% [7]. Similarly, the major constituents of the oil from the flowers of *D. carota* subsp. *carota* from Morocco were α-pinene (22.25%), β-asarone (15.13%), sabinene (12.46%), and α-himachalène (10.14%)[14]. Interestingly, in our study, the latter component was completely absent in both essential oils of *D. aristidis*, while β-asarone was found only in the umbels of *D. aristidis* oil from Ghoufi (0.8%). In addition, the essential oil of the aerial parts of *D. reboudii* from El Kala (Algeria) contained α-pinene (39.7%) followed by sabinene (21.2%) as the main constituents [15]. Nevertheless, the essential oil of the leaves and fruits of *D. sahariensis* revealed the presence of a significant amount of myristicin (34.3% and 43.9%, respectively) [16], which was absent in the oil of *D. aristidis* studied herein. α-Pinene was found to be also present in small amounts (5.4 to 13.1%) in the oils of *D. sahariensis* [16]. Similarly, the comparative study of essential oils of *D. guttatus* subsp. *zahariadii* and the wild sample of *D. carota* from Balkans demonstrated that the oil of *D. guttatus* subsp. *zahariadii* contained the following main constituents as apiole (43.3%) and β-bisabolene (34.2%) (this latter component was present in the umbels oils of *D. aristidis* from Ghoufi with small amount 1.3%), and contained α-pinene in a minor amount (0.3%), whereas, the oil of the aerial parts of wild *D. carota* contained 29.3% of α-pinene in major amount[17]. On the other hand, the seeds of the essential oil from the wild *D. carota* sample has been shown to possess a minor amount of α-pinene (3.3%) and the geranyl acetate (53.2%) as the major component [17], which is completely absent in the oils analyzed in the current study. The oils of the aerial part of *D. carota* subsp. *carota* and *D. carota* subsp. *gummifer* from Algeria were also characterized by the presence of α-pinene (26% - 34.1%, respectively), sabinene (1.5% -14%, respectively), limonene (0.5% -13%, respectively) and β-pinene (0.6% -11.2%, respectively) [18]. The latter component, β-pinene, is one of the dominating components of *D. aristidis* oil, which is known to be generally present in most oils of the genus *Daucus* in varying proportions. In fact, the essential oil of the flowers and umbels of *D. carota* subsp. *carota* from Portugal consisted of geranyl acetate (5.2% and 65%) and α-pinene (3.5% and 37.9%) as the major components, while β-pinene (3.5% and 2.3%) as the minor constituent [19]. Similarly, the essential oil of the aerial parts of *D. muricatus* was reported to contain 18.9% of sabinene, 16.7% of α-pinene, and 14.2% of limonene which were determined as the major constituents along with a minor amount of 2.5% of β-pinene[20].

It should be noted that the proportions of α-pinene and β-pinene found in *D. aristidis* oils from Ghoufi and Boussaada possess the highest percentage ever in comparison with the values found in the oils of the genus *Daucus* reported in the literature.

In addition to α-pinene and β-pinene, the major constituents present in the oils of *D. aristidis* native to Ghoufi and Boussaada were revealed as limonene (7.5%), myrcene (6.7%), and sabinene (4.3%). It was found the presence of these abundant components in the essential oil of *D. carota* subsp. *sativus* during ontogenesis: limonene (4.4% to 12.7%), sabinene (0.2% to 5.3%), myrcene (6.4% to 14.1%), α-pinene (21.2% to 41.2%) and carotol (10.2% -58.5%)[21]. In contrary, carotol was present only in a minor amount (0.1%, 0.5%, and 0.1%) in the umbels, leaves and aerial parts of *D. aristidis* oils from Ghoufi but totally absent in the oil from Bousaada. Similarly, carotol and sabinene were reported to be the major constituents of the oil of *D. carota* fruit from the northern Serbia with proportions of 20.3% and 18.7%, respectively [22]. Besides, the study of *D. carota* subsp. *major* fruit essential oil has proved the presence of geranyl acetate (34.2%), α-pinene (12.9%), geraniol (6.9%), myrcene (4.7%), *epi*-α-bisabolol (4.5%), sabinene (3.3%), and limonene (3%)[23]. In a study conducted on the effect of different vegetative stages on the chemical composition of the oil of *D. sahariensis*, this oil was found to consist of myristicine (29.8% -51.7%), myrcene (6.7% - 31.1%), α-pinene (11.6% -14.8%), and limonene (5.3% -11.5%)[24].

As also commented from findings, variations in chemical composition of the essential oil might be due to several factors, *i.e.* environmental (temperature, humidity, soil texture, altitude, etc.), geographical origin, plant organ, stage of growth, the time of picking, storage of plant material, individual genetic variability, and extraction method[25-30].

Regarding the moderate AChE inhibitory activity of *Daucus aristidis* essential oil, it could be explained by a notable AChE inhibitory activity reported for its individual components, α- and β-pinene. According to the study conducted on the *in vitro* inhibition of human erythrocyte AChE by *Salvia lavandulaefolia* essential oil and its major monoterpenes including camphor, 1,8-cineole, α-pinene, β-pinene, bornyl acetate, as well as some minor components (1% or less) geraniol, linalool, and γ-terpinene. Consequently, α-pinene, 1,8-cineole and camphor were found to be the competitive reversible inhibitors of AChE and it was suggested that the inhibitory activity of this essential oil was primarily due to main inhibitory terpenoids, which resulted from the major synergistic effect[31]. Besides, the anti-AChE activity of the oil of *Artemisia annua* flower has been mainly attributed to α-pinene, β-pinene, limonene, 1,8-cineole, camphor, borneol, *α*-terpineol, *α*-caryophyllene, and *α*-caryophyllene oxide, furthermore, the different level of the anti-AChE effect of the flower oil of *A. annua* at three flowering stages may have resulted from the different content of those terpenoids and their different interactions with anti-AChE activity [11]. In another study, worked on anti-AChE activity of bicyclic monoterpenoids commonly encountered in essential oils, the results pointed out that the bicyclic monoterpenoids containing allylic methyl group showed a strong inhibition. Moreover, (+) and (-)-α-pinene, and (+)-3-carene were observed to be the potent inhibitors of AChE as well[32]. Similarly, it was described that *α*-pinene had the strongest AChE inhibition activity followed by β-pinene and limonene on the adult rice weevil, *Sitophilus oryzae* [33]. Relevantly, the relatively high AChE inhibitory activity of *Pinus nigra* subsp. *dalmatica* essential oil was explained by marked AChE inhibitory activity of its main components, α- and β-pinene [34], which is in accordance with our data.

On the other hand, it was suggested for plant extracts or essential oils that is not always necessarily to be only one compound responsible for these effects, which may as well be depend on several compounds that act in a synergistic manner or on compounds which regulate one another because essential oils are complex mixtures of components that show usually higher activities than their isolated components; their final activities are due to the combine effects of several minor components [35].

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