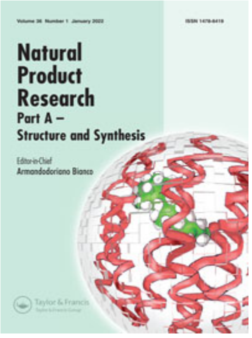

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SHORT COMMUNICATION

Daucus carota subsp. *maximus* (Desf.) Ball from Pantelleria, Sicily (Italy): isolation of essential oils and evaluation of their bioactivity

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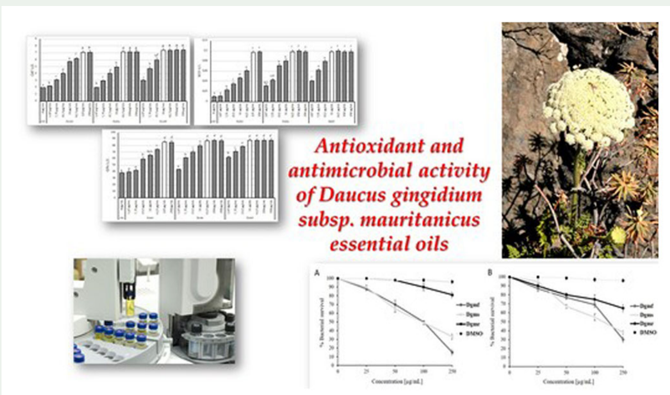
ABSTRACT

Daucus is a genus of economically important plants belonging to Apiaceae family spread in temperate regions. Species of this genus are used as food and several biological properties have reported. The chemical composition of the essential oils from different organs (roots, stems and flowers) of *Daucus carota* subsp. *maximus*, a species not previously investigated, was analyzed by GC-MS. Our results showed the presence of β-phellandrene as the most abundant component of stems and flowers and of γ-terpinene as a major compound of the oil from the roots. Flower essential oil caused a greater increase in the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in polymorphonuclear leukocytes (PMN) cells compared to stem and root essential oils. The antimicrobial activity of the flower and stem oil were more effective, compared to root oil, against *Escherichia coli* and *Staphylococcus aureus*.



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1. Introduction

The genus *Daucus* (Apiaceae Family) that includes more than 30 accepted species and subspecies, is distributed mainly in Europe, North Africa, West Asia although some species are present also in North America and Australia (The Plant List; Sáenz Laín 1981). Several taxa of this genus are economically important as food and among these the best-known member is certainly *Daucus carota* L., whose fruits are called "nanheshi" in China, and traditionally used for their diuretic, antibacterial, anthelmintic, antifungal and cytotoxic properties. Carrot has been spread in the Northern Mediterranean regions by the Arab invaders, and they also introduced several cultivars, such as yellow, purple and red carrot, and several reviews concerning both the phytochemistry and pharmacological properties of this species have been published (Akhtar et al. 2017; Ahmad et al. 2019; Que et al. 2019; Kartika et al. 2021). In the frame of our on-going researches on Mediterranean plant (Badalamenti et al. 2020; Di Napoli et al. 2020; Ilardi et al. 2020; Badalamenti et al. 2021; D'Agostino et al. 2021; Gagliano Candela et al. 2021) and on chemotaxonomic differences (Bancheva et al. 2021; Catinella et al. 2021) the composition of the essential oils of different organs of *D. carota* subsp. *maximus* (Desf.) Ball (**Dcm**), picked up near Punta Spadillo in the island of Pantelleria, Sicily (Italy), as well as their antioxidant and antimicrobial properties have been investigated.

The phytochemistry of genus *Daucus* as well as the botanical description of the species investigated is reported in **Supplemantary Material**.

2. Results and discussion

2.1. Chemical composition of essential oil

Hydrodistillation of *D. carota* subsp. *maximus* roots (**Dcmr**) gave a pale-yellow oil. Overall, six compounds were identified, representing 96.7% of total components, listed in Table S1, according to their linear retention indices on a HP-5 MS apolar column. The predominant chemical class was that of monoterpene hydrocarbons, with γ-terpinene (35.9%) as the most abundant component. Other monoterpenes present in good amounts were β-phellandrene (16.3%), *p*-cymene (15.7%) and terpinolene (14.2%). Also, the oil from the *D. carota* subsp. *maximus* stems (**Dcms**) (14 compounds, 90.5%) was characterized by large amount of monoterpene hydrocarbons, with β-phellandrene (70.1%), by far, the most abundant one. Among the oxygenated monoterpenes only 4-terpineol (3.6%) was present and small quantity of the oxygenated sesquiterpenes carotol (0.8%) and 6-*epi*-shyobunone (0.6%) and of the phenylpropanoid β-asarone (2.6%) were also detected. The profile of the essential oil from the *D. carota* subsp. *maximus* flowers (**Dcmf**) was more complex. In fact, although, also in this case, the main class was represented by monoterpene hydrocarbons (38.4%) with β-phellandrene (19.8%) as main constituent of the class and of the oil, the occurrence of other classes of compounds was remarkable. Oxygenated sesquiterpenes were present in good amount (20.5%) with carotol (10.0%) and 4-terpineol (5.7%) as principal metabolites and among the phenylpropanoid (18.7%) is noteworthy the good quantity of β-asarone (15.8%).

Comparison with the composition of essential oils of all the other taxa belonging to *Daucus* genus reported in literature (Table S2) allowed us to carry out some interesting comments. Among all the essential oils from *Daucus* species studied so far in the world (more than 100, Table S1), just two of them [*D. glaber* from Egypt (6.3%) (Mansour et al. 2004) and *D. aristidis* from Algeria (3.3%) (Lamamra et al. 2017)] contain small amount of β-phellandrene, the main component of **Dcms** and **Dcmf**. On the other hand, asarone isomers (15.8% in **Dcmf**) were present in good quantity in several studied taxa: *D. aristidis* from Algeria (18.5%) (Lamamra et al. 2016), *D. carota* subsp. *carota* from Algeria (20.8-9.4%) (Mohammedi et al. 2015), *D. carota* subsp. *carota* from Morocco (46.5%) (Elhourri et al. 2013), *D. carota* subsp. *maximus* from Portugal (9.8%) (Valente et al. 2015). Furthermore, it should be outlined that the oil of another accession of *D. carota* subsp. *maximus*, collected in Corsica (France), and indicated in the original paper as *D. carota* ssp. *commutatus* (Reduron et al. 2019), showed limonene (36.1%) and sabinene (19.8%) as main component of the roots, metabolites totally absent in **Dcmr**. Additionally, the oil from the roots of *D. carota* subsp. *maximus* var. *mauritanicus* collected in Corsica (Reduron et al. 2019), that according to some authors (The Plant List) is a synonymous of *D. carota* subsp. *maximus*, showed as main metabolites (*E*)-γ-bisabolene (34.5%), tetradecanal (30.5%) and dodecanal (27.8%) that were completely absent in **Dcmr**.

2.2. Antioxidant enzymes activity inducer properties

We assessed the antioxidant enzymes activity inducer properties inducer of essentials oil of roots (**Dcmr**), stems (**Dcms**) and flowers (**Dcmf**) of *D. carota* subsp. *maximus* in PMNs. The antioxidative enzymes status was measured by SOD, CAT, and GPx activities in PMN cells treated with essentials oil.

The activity of antioxidant enzymes in PMN cells increased after treatment with of essentials oil of roots (**Dcmr**), stems (**Dcms**) and flowers (**Dcmf**) compared to control (untreated samples) (Figure S1). In particular, the flower essential oil (**Dcmf**) already at relatively low concentrations (31 μg/mL) has induced an increase in the activity of antioxidant enzymes. While as regards stem (**Dcms**) and roots (**Dcmr**), the increase in the enzymes SOD, CAT and GPx is observed at higher concentrations, respectively 62.5 and 125 μg/mL.

The antioxidant properties of essential oils cannot be assessed simply by observing the increased activity of antioxidant enzymes. On the other hand, the increase in their activity is linked to oxidative stress. In fact, the activity of CAT, SOD and GST enzymes increases following an increase in ROS production, in order to counteract the negative effects induced by stress as reported in Haydari et al. (2019). In this case we are not dealing with stressful conditions but we suggest that an increase in these enzymes may indicate an increase in their antioxidant properties.

Our results are in agreement with those obtained by Di Napoli et. (2020), where an increase in PMN activity of antioxidant enzymes (SOD, CAT and GPx) is reported after treatment with *A. secundiramea* flower essential oil.

2.3. Antimicrobial activities

The antimicrobial activity of the flower (**Dcmf**), stem (**Dcms**) and root (**Dcmr**) oils of *D. carota* subsp. *maximus* were tested against a Gram negative *Escherichia coli* DH5α and a Gram positive *Staphylococcus aureus* ATCC6538*P* bacterial strains. We evaluated the activity by calculating the percentage of bacterial survival. A negative control was carried out using the solvent (DMSO 80%) in which the oil extracts were dissolved which was totally inactive when tested under the same conditions and against the same strains. The different oils show different antimicrobial activity, flower oil (**Dcmf**) and the stem oil (**Dcms**) were more effective, than root oil (**Dcmr**), against *E. coli* by promoting approximately 55% of bacterial mortality at a concentration of 100 μg/mL and between 70% and 90% mortality at a concentration of 250 μg/mL (Figure S2 panel A). For the Gram positive strain *Staphylococcus aureus* the activity of oils are more similar at low concentrations with a prevalence of **Dcmf** and **Dcms** at concentrations higher than 100 μg/mL (Figure S2 panel B). Based on our the results, on literature data and on the chemical composition of flower, stem and root oils it can be hypothesized that the antibacterial nature of flower and stem oil is related to a high content of oxygenated monoterpenes and monoterpenic hydrocarbons (*β*-phellandrene present at 70% in the stem), which destroy cell integrity and inhibit respiration and ion transport processes (Al-Burtamani et al. 2005; Kamatou et al. 2007; Deba et al. 2008); in agreement, it has previously been reported that the essential oils or extracts of some members of the *Apiaceae* family, including *Ferula gummusa, Prangos nechtritzii* and *Ferula persica* (Özcan 1999; Eftekhar et al. 2004; Mirjani et al. 2005), had antimicrobial activity.

3. Experimental (see supplemental material)

4. Conclusions

The analysis of the chemical compositions of the essential oils from roots (**Dcmr**), stems (**Dcms**) and flowers (**Dcmf**) of *D. carota* subsp. *maximus* showed a peculiar profile, quite different from all the other oils of species of *Daucus* studied so far. In fact, the occurrence of *β*-phellandrene, main metabolite of our oils, has never been previously reported in other taxa. This result suggests the need of further investigation on the essential oils composition of other accessions of this species, with the aim to improve the chemotaxonomic knowledge. Furthermore, the different oils have been tested for their ability to induce an increase in the activity of antioxidant enzymes and for their antimicrobial activity and among them the best one resulted the oil from flowers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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