SHORT COMMUNICATION

Composition, antibacterial, antioxidant and antiproliferative activities of essential oils from three Origanum species growing wild in Lebanon and Greece

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The essential oils from Origanum dictamnus, Origanum libanoticum and Origanum microphyllum were analysed by GC-MS, finding carvacrol, p-cymene, linalool, \( \gamma \)-terpinene and terpinen-4-ol as major components. The antioxidant activity by the DPPH and FRAP tests and the antiproliferative activity against two human cancer cell lines, LoVo and HepG2, were investigated, showing that the essential oil of \textit{O. dictamnus} was statistically the most inhibitory on both the cell lines, while all the oils exerted a weak antioxidant activity. Furthermore, the samples were tested against 10 Gram-negative and Gram-positive bacteria; all the oils were active on Gram-positive bacteria but \textit{O. dictamnus} essential oil was the most effective (MIC = 25–50 \( \mu \)g/mL), showing also a good activity against the Gram-negative Escherichia coli (MIC = 50 \( \mu \)g/mL). Data suggest that these essential oils and particularly \textit{O. dictamnus} oil could be used as valuable new flavours with functional properties for food or nutraceutical products.

Keywords: Origanum species; essential oil; antibacterial; antioxidant; antiproliferative

1. Introduction

The genus Origanum (Lamiaceae) is characterised by a large morphological and chemical diversity (Ietswaart 1980). Due to their several biological effects such as being antispasmodic, antimicrobial, expectorant, carminative and aromatic for whooping and convulsive coughs, Origanum species have been used as ethnopharmacological drugs in traditional medicine to treat various ailments (Barros et al. 2010). \textit{Origanum dictamnus} L. was considered as Panacea according to the ancient Greeks and was used in many cases for its healing effects (Strid & Tan...
A number of studies describe the biological activities of its extracts, such as antioxidant (Proestos et al. 2013), antimicrobial (Alexopoulos et al. 2011) and cytotoxic activity against different cell lines (Chinou et al. 2007). *Origanum microphyllum* (Bentham) T. Vogel is a dwarf shrub endemic to Lefka Ori and Dhikti Mountains in Crete. The shrub of *Origanum libanoticum* Boiss is instead endemic to Lebanon (Arnold et al. 2000). To the best of our knowledge, the studies on the essential oil of *O. microphyllum* species are limited, and there are no previous papers on the biological activity of the essential oil of *O. libanoticum*. Furthermore, there are no reports on the biological activity of *O. dictamnus* collected on Mavri summit, Mt. Ida (Crete). The aim of this work was to compare the chemical composition of the essential oils from these three *Origanum* sp. in order to characterise the species through the qualitative and quantitative evaluation of chemical compounds. In view of the potential pharmaceutical applications, the antioxidant activity by the DPPH and FRAP tests, the antiproliferative activity against the two human cancer cell lines LoVo and HepG2, and the antimicrobial activity against 10 Gram-negative and Gram-positive bacteria were evaluated taking into account the chemical composition of the oils.

2. Results and discussion

2.1. Chemical composition of the essential oils

Analytical gas chromatography and GC-MS analysis were performed to study the chemical composition of the essential oils from the three *Origanum* species (Table S1). Thirty compounds were identified in the oil from *O. dictamnus* (D), which accounted for 89.3% of the total oil. *p*-Cymene (32.7%) and *γ*-terpinene (12.4%) were the main constituents, which together accounted for almost half of the total oil (45.1%). Also carvacrol (14.7%) and linalool (7.8%) were quite abundant. On the whole, monoterpenes (60.6%) and particularly monoterpene hydrocarbons (50.6%) prevailed over all the other components. Phenols accounted for 15.5% of the oil and were represented almost entirely by carvacrol (14.7%). Our results are essentially in agreement with previous papers on the study of the essential oils of *O. dictamnus* collected in different countries of the Mediterranean area (Liolios et al. 2010). Sixty compounds were identified in the other oil from Greece, *O. microphyllum* (M), accounting for the 94.5% of the oil. Terpinen-4-ol (16.2%), carvacrol (13.3%), sabinene (7.5%) and trans-sabinene hydrate (7.1%) were the main compounds. These results are different in comparison with previous studies that found as main compounds cis-sabinene hydrate (30%), sabinene (20%), trans-sabinene hydrate (14%), linalool, *p*-cymene and *γ*–terpinene (Skoula et al. 1999; Gotsiou et al. 2002; Figuérèo et al. 2006). Our results are instead very similar to those of Aligiannis et al., who found the presence of terpinen-4-ol (24.86%) as the main compound together with *γ*–terpinene (13.83%), linalool (10.81%), *α*-terpinene (9.86%) and sabinene (7.70%) (Aligiannis et al. 2001). Sixty-five components were found in Lebanese *O. libanoticum* oil (L), which constituted 87.3% of the oil, in which prevailed linalool (6.5%), thymol methyl ether (9.8%), (E)-β-caryophyllene (7.7%) and hexadecanoic acid (11.3%). Differently from the Greek oils D and M that were both rich in monoterpenes, in this case sesquiterpenes prevailed (32%). These results differ from those found by Arnold et al. (2000).

2.2. Antibacterial activity

The in vitro antimicrobial activity of the essential oils D, M and L against 10 bacteria species was evaluated by determining the MIC and the MBC using the broth dilution method. The oils showed an interesting activity mainly against the Gram (+) pathogens, while among Gram (−) bacteria only *Escherichia coli* was affected by the two oils D and M from Greece but not by the Lebanese oil L (Table S2). On the whole, the most active essential oil was D, which was very effective
(MIC = 25 μg/mL, MBC = 50 μg/mL) against the Gram-positive Bacillus cereus, Bacillus subtilis and Staphylococcus epidermidis. The oil also showed a very good activity against Staphylococcus aureus, Streptococcus faecalis and the Gram (−) E. coli (MIC = 50 μg/mL). Also the other Greek oil M showed good MIC and MBC values against all the Gram (+) pathogens except S. faecalis and against the Gram (−) E. coli (MIC = 50 μg/mL for all). The activity of the oils can be very likely attributed to the existence mostly of carvacrol (Ipek et al. 2005). As regards L, whose antimicrobial activity was never shown before, its activity is probably due to the presence of linalool, thymol methyl ether and(\(E\))-\(\beta\)-caryophyllene (Burt 2004).

2.3. Antiproliferative effects against human tumour cell lines

The antiproliferative activity of the essential oils was determined after 24 and 48 h of treatment. Effects on proliferation of LoVo and HepG2 cell lines are shown in Figure 1, which report the percentages of inhibition of cell viability after 24 and 48 h of treatment, respectively. All samples reduced cell viability in a dose-dependent manner compared with control (0.5% dimethyl sulfoxide-treated control cells). On colon carcinoma cell line, the essential oil of \(O.\) dictamnus (D), at the highest concentration (100 μg/mL), was statistically the most inhibitory causing a 58.39% of inhibition after 24 h of incubation. At the same concentration (100 μg/mL), the essential oils of \(O.\) microphyllum (M) and \(O.\) libanoticum (L) caused 47.12% and 48.15% of inhibition, respectively. A similar trend was observed on HepG2 cell viability after 24 h of treatment. In order to verify possible changes in antiproliferative activity of \(Origanum\) essential

![Figure 1. Percentage of inhibition of LoVo cells (A) and HepG2 cells (B) viability after 24 h of incubation (C) and 48 h of incubation (D). ●: \(O.\) dictamnus; ○: \(O.\) microphyllum; ▼: \(O.\) libanoticum. Data represent mean ± SE (n = 4). Different letters indicate statistically significant differences at P < 0.05 (Tukey’s test).](image-url)
oils, effects on cell viability were also assessed after 48 h of incubation. At the highest concentration, 100 μg/mL D was still the most active sample on colon carcinoma LoVo cancer cell, inducing an inhibition of 61.96%. Inhibition induced by essential oils M (47.13%) and L (48.60%) was significantly lower. A different trend was observed on hepatocarcinoma cell line HepG2 cells: at the highest concentration (100 μg/mL), both D and L induced the highest antiproliferative activity (49.83% and 48.50% inhibition, respectively) in comparison to M (37.68%). The raw data were fitted through nonlinear regression in order to obtain the IC$_{50}$ parameter, which was detectable only on LoVo cell line treated with sample D. On the whole, O. dictamnus essential oil showed the best antiproliferative activity with IC$_{50}$ values of 84.76 ± 1.03 μg/mL after 24 h and 72.26 ± 1.05 μg/mL after 48 h of treatment.

2.4. Antioxidant activity

The essential oils of the three Origanum species were subjected to screening for their possible antioxidant activity by means of two spectrophotometric methods (DPPH and FRAP tests) and expressed as Trolox equivalents (TEs). Table S3 shows that according to the DPPH test, the essential oils revealed poor antiradical activity, and only the oil from O. dictamnus exerted a weak capacity to scavenge free radicals (0.15 ± 0.04 mmol TE/L), very scarce if compared to artificial antioxidants such as butylhydroxytoluene and known antioxidant compounds common in essential oils such as thymol and carvacrol used at the same dose (Table S3). Using the FRAP test, all the samples showed significant activity, demonstrating that they are not active in catching free radicals but they have a major ferric reducing/antioxidant power (Table S3). Our results are in accordance with previous findings that show that, in most cases, the antioxidant activity of Origanum species is due to their polar extracts rather than other extracts or essential oils, and this is true also for O. dictamnus (Liolios et al. 2009; Liolios et al. 2010; Proestos et al. 2013). No previous studies were done on the antioxidant activity of O. libanoticum and O. microphyllum essential oils.

3. Conclusion

The results described in this paper may suggest that the three Origanum species analysed, O. dictamnus and O. microphyllum both endemic in Greece and O. libanoticum endemic in Lebanon, possess compounds with antimicrobial and cytotoxic properties. This is particularly true for O. dictamnus that exerted the best activity in all the assays. In addition, the data in this study are supporting the use of Origanum plants as tea or additive in foods, and traditional remedies for the treatment of infectious diseases and for the prevention of cancer and gastrointestinal system diseases.

Supplementary material

Experimental details relating to this paper are available online at http://dx.doi.org/10.1080/14786419.2015.1040993, alongside Tables S1–S3.

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