The chemical composition of the aerial parts essential oil of *Acinos alpinus* subsp. *nebrodensis* (Lamiaceae) growing in Sicily (Italy)

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\textbf{ABSTRACT}

*Acinos* is a small genus of economically important plants belonging to Lamiaceae family whose botanical collocation is quite problematic due to the disagreement among the botanists and the presence in literature of several names and synonyms from different sources. In the present study the chemical composition of the essential oil from aerial parts of *Acinos alpinus* subsp. *nebrodensis* (Strobl) Brullo & Brullo collected in Central Sicily was analyzed by GC-MS. The result showed the presence of large quantity of sesquiterpene hydrocarbons with germacrene D (37.9%) as the most abundant component followed by (E)-\-\beta\-caryophyllene (5.1%). Among the oxygenated monoterpenes thymol (8.3%) was the most abundant; good quantity of hexadecanoic acid (6.8%) was also observed. Chemotaxonomic considerations with respect all the other oils of *Acinos* taxa, studied so far, were carried out.

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1. Introduction

Acinos Miller is a small genus of annual and short-lived evergreen perennial woody plants, belonging to Lamiaceae family, distributed in Europe, Mediterranean, central Asia, North Africa and North America. Its name takes origin from the Greek word ἂκινος ‘akinos’ that means ‘small aromatic plant’ (Bonnier 1927; Bown 1995). According to The International Organization for Plant Information (IOPI), genus Acinos Miller comprises 11 species, although some botanists do not agree and several names and synonyms from different sources are present in literature. Even being a small genus, Acinos is an important one within the Lamiaceae family from the economic point of view. In fact, several Acinos species, sold as the herbs of ‘mint’ group, are available in the markets of some Mediterranean countries (Stojanović et al. 2009).

Several traditional applications of Acinos species, depending on the area and on the species, have been reported in folk medicine although they are not justified enough through biochemical researches (Kaya et al. 1999; Mulas 2006; Kültür 2007; Karousou et al. 2007).

Biological information about Acinos species mainly regard the anti-microbial, anti-oxidant and anti-inflammatory properties of their essential oil (Jovanović et al. 2005; Stojanović et al. 2009; Jennan et al. 2018) and extracts (Stojanović et al. 2009; Ulukanli et al. 2005).

Several investigations have been carried on the essential oils of Acinos taxa (Table S1) and their composition will be discussed below.

Acinos alpinus subsp. nebrodensis (Strobl) Brullo & Brullo (syn. Thymus alpinus L., Satureja alpina Scheele, Calamintha alpina Lam., Clinopodium alpinum L., Calamintha pseudoacinos, Calamintha nebrodensis) is a native Sicilian taxon with camphritic habitus, of critical taxonomic attribution, as can be deduced from the large variety of genera to which the Sicilian populations have been referred in recent and past literature. Two different taxa have been identified by Pignatti (1982, 2018) in Sicily, referring to Acinos Miller: A. alpinus subsp. nebrodensis (Strobl) Brullo & Brullo and A. granatensis (Boiss. et Reuter) Pignatti subsp. aethnensis (Strobl, Kerner 1874) Pignatti. The last one is exclusive of Calabria and Sicily, in particular limited to Etna lava substrates, while A. granatensis subsp. granatensis is not part of the Italian flora, but is limited to North Africa and the Iberian Peninsula. Recently, other authors (Guarino and La Rosa 2019) merge the Acinos Miller genus in Clinopodium L. and refer to the two Sicilian taxa as a single subspecies, Clinopodium alpinum subsp. meridionale (Nyman) Govaerts.

Studies about the chemotaxonomy of these plants could be crucial to help and facilitate a clear partition of the genus and species, and also provide important information about the effects of the geographic isolation of populations, related to the processes of speciation and differentiation, as well as the specific variability in geographical areas with different edaphic and weather conditions.

The analysis carried out in the present work concern to plant samples picked up on Monte Sant’Otiero, a quarzerenitic mountainous relief of Madonie (Sicily), Italy.

Consequently, in the frame of our on-going researches on Sicilian plant essential oil (Gagliano Candela et al. 2020; Rigano et al. 2020; Badalamenti et al. 2021; Ilardi et al. 2021) and on chemotaxonomic differences (Bancheva et al. 2021; Catinella et al. 2021)
we decided to investigate the composition of the essential oil of *Acinos alpinus* subsp. *nebrodensis*, collected in Sicily.

### 2. Results and discussion

#### 2.1. Chemical composition of essential oil

Hydrodistillation of *Acinos alpinus* subsp. *nebrodensis* aerial parts gave a pale-yellow oil ([Aan](#)). Overall, forty-eight compounds were identified, representing 96.3% of total components, listed in Table S2 according to their retention indices on a HP-SMS column and classified into five classes on the basis of their chemical structures. Sesquiterpene hydrocarbons formed the main class, representing 50.5% of the oil, with germacrene D (37.9%) as the most abundant component. In the same class, (E)-β-caryophyllene (5.1%) was also present in good amounts. Oxygenated monoterpenes were the second most abundant class (18.3%), with thymol (8.3%) as the main component of this class; instead, monoterpane hydrocarbons (9.0%) and the oxygenated sesquiterpenes (7.0%) were present in minor amount.

Comparison with the essential oils of other taxa belonging to *Acinos* genus (Table S1) allowed us to carry out some interesting comments. In fact, germacrene D, the main compound of our oil, except for *A. majoranifolius*, *A. suaveolens* and for the Greek accession of *A. alpinus* and *A. arvensis*, is among the main metabolites of all the other oils. Also (E)-β-caryophyllene is quite common in *A. alpinus*, *A. alpinus* ssp. *meridionalis*, *A. arvensis*, *A. graveolens* and *A. troodi* ssp. *grandiflorus*. It is noteworthy the presence in [Aan](#) of thymol occurring only in the Turkish accession of *A. alpinus* (Kaya et al. 1999a). Among the other metabolites (9.7% in [Aan](#)) it is worthy of mention the presence of hexadecanoic acid (6.8%), also found in *A. alpinus* (Kaya et al. 1999a), *A. arvensis*, *A. rotundifolius*, *A. suaveolens* (Kaya et al. 1999) and *A. troodi* ssp. (Kaya et al. 1999b). An aspect that emerges, concerning the main compounds present in the different essential oils of *Acinos* taxa studied and analyzed (Table S1), is the possibility of distinguishing two chemotaxonomic groups: the first one includes all the species rich in germacrene D (*A. troodi* ssp. *grandifloras*, *A. rotundifolius*, *A. hungaricus*, *A. graveolens*, *A. alpinus*, *A. alpinus* ssp. *meridionalis*, *A. arvensis*); the second one, on the other hand, could contain all those *Acinos* characterized by a large content of pulegone (*A. suaveolens*, *A. majoranifolius*). Only one sample, *A. arvensis* from Greece, is disconnected from these conclusions as it is rich in pulegone and not in germacrene D.

### 3. Experimental (see supplementary material)

### 4. Conclusions

The comparison of the chemical compositions of the essential oils shows that in many cases they can be used as chemotaxonomic markers to support taxonomic decisions related to the establishment of separate taxa. In our study, the gas chromatographic analysis of the essential oil of *Acinos alpinus* subsp. *nebrodensis* showed that this is rich in hydrocarbon sesquiterpenes, representing 50.5% of the oil, with germacrene D
(37.9%) as the most abundant component. Thymol, an oxygenated monoterpane, was found to be the most abundant compound in this class (8.3%). Monoterpene hydrocarbons and the oxygenated sesquiterpenes were present, on the other hand, in a minor amount (9 and 7%, respectively). An aspect that emerges from the chemotaxonomic analysis of the main compounds present in the various essential oils of *Acinos* taxa (Table S1), is the distinction of the taxa into two different groups: the first one includes species rich in germacrene D; the second one, in contrast, groups the species with a high content of pulegone.

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