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Phytochemical screening of *Artemisia arborescens* L. by means of advanced chromatographic techniques for identification of health-promoting compounds

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ABSTRACT

Artemisia arborescens, also known as tree wormwood, is a typical species of the Mediterranean flora. It has been used in folk medicine for its antispasmodic, anti-pyretic, anti-inflammatory, and abortifacient properties. In the current study, the application of multidimensional comprehensive gas chromatography (GC × GC), allowed to obtain a detailed fingerprint of the essential oil from *A. arborescens* aerial parts, highlighting an abundant presence of chamazulene followed by camphor, β-thujone, myrcene, and α-pinene. Moreover, flavonoids in the dichloromethane extract were analyzed by means of liquid chromatography with photodiode array and atmospheric pressure chemical ionization-mass spectrometry detections (HPLC-PDA and HPLC-APCI-MS). Six polymethoxyflavones were identified and three of them, including chrysosplenetin, eupatin, and cirsilineol, were described in this species for the first time. The anti-angiogenic activity was investigated in the dichloromethane extract by two *in vivo* models, chick chorioallantoic membrane (CAM) and zebrafish embryos. Results showed that this extract produced a strong reduction on vessel formation, both on zebrafish (57% of inhibition, 0.1 mg/mL) and chick chorioallantoic membrane (58% of inhibition, 0.8 mg/mL).

The high separation power and sensitivity of the analytical methodology applied confirmed the safety of *A. arborescens* essential oil for human consumption, due to the very low level of the psychotrope α-thujone determined. Moreover, the knowledge of the flavonoidic profile holds a great significance for the use of *A. arborescens* as a valuable source of anti-angiogenic compounds that might contribute to the valorization of the phytotherapeutic potential of this plant.

1. Introduction

Artemisia arborescens L. (Asteraceae) is an upright shrub, one meter tall, with gray-white hairy branches, finely divided leaves (3–5 cm long), and yellow flowerheads grouped in cobs. Also known as tree wormwood, this species is typical of the Mediterranean flora, growing commonly along the coasts, on calcareous rocks or close to old walls [1]. The essential oil has been reported to possess antiherpesvirus, chemopreventive, antioxidant and antibacterial effects [2–5]. The plant is also used for pests control in the agricultural practices and in veterinary medicine [6,7]. In general, tree wormwood is listed among traditional medicinal plants: for instance, leaves decoction is used in tea, usually with mint, as tonic and sedative [8]. From an overview of the last ten years, it can be found that, although differing quantitatively depending upon the site of collection, common and predominant components of tree wormwood are: camphor, chamazulene, β-thujone and germacrene D. Some studies have been published on the essential oil composition of *A. arborescens* growing in different countries, leading to the identification of several chemotypes characterized by different amounts of

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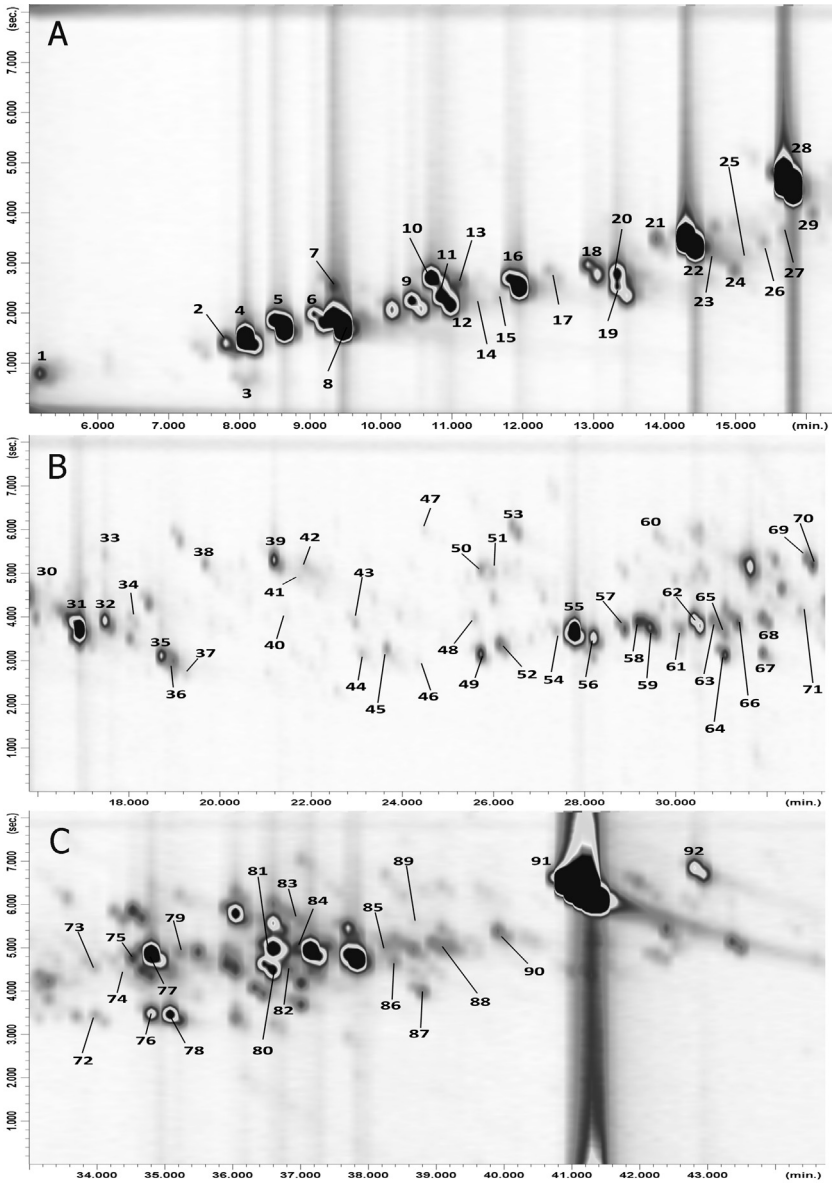

Fig. 1. GC × GC–MS chromatogram of *Artemisia arborescens* L. leaf essential oil. Capital letters define three expansions of the bidimensional plot. Peak top numbers refer to Table 1.

β-thujone and chamazulene [4,5,9–13]. Chamazulene, an azulenic compound, was proven to possess *in vivo* anti-inflammatory activity, by inhibiting the leukotriene synthesis and lipid peroxidation [14]. It has also been reported as one of the main constituents of the essential oils of other species of the genus *Artemisia*, such as *A. absinthium, A. gallica* ssp., *densiflora, A. rehan, A. santonicum, A. copa,* and *A. feddei* [5].

However, it seems quite reasonable that the chemical variability of *A. arborescens* essential oil, basically addressed to the different geographical origin of plants, may also be due to erroneous identifications of the plant individuals. The genus *Artemisia* comprises over 20,000 species, but sometimes different names are attributed to the same species. For this reason, one of the scopes of the present study was to give a substantial support to the knowledge of the chemical composition of *A. arborescens* essential oil. To this aim, comprehensive gas chromatography (GC × GC), which falls within the newly introduced muldimensional approaches, was applied in order to obtain a complete screening of the volatile constituents. The instrumental and theoretical features of GC × GC, as ahead explained in this report, have been attractive also to the plant scientist. Several are the applications of this technique to the investigation of plant extracts [15]. Recent applications are: the determination of volatiles and fatty acids from *Ruta chalepensis* aerial parts [16]; the assessment of aging markers, causing important allergies, in tea tree essential oils [17]; true quantitation of volatiles in mentha and lavandula species by means of a dual detection GC × GC system [18].

Also, focusing on polyphenolic/flavonoidic composition of the tree wormwood, liquid chromatography, with both UV and MS detection systems, was applied to plant extracts. In this respect, a detailed work was presented by Carvalho et al. [3] on six *Artemisia* species, including *A. arborescens*, based on the use of HPLC-PDA. Recently, 15 flavonoids have been isolated from *A. arborescens* extracts, obtained from plants harvested in Algeria [19]. The present study aims to integrate the information so far reported in literature about polyphenolic and flavonoidic fractions in *A. arborescens*.

Although the chemopreventive activity of tree worwood essential oil has been previously reported by Ornano et al. [5], the biological potential of other phytoconstituents like flavonoids has