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RESEARCH ARTICLE

Chemical composition of essential oils of *Anthemis secundiramea* Biv. subsp. *secundiramea* (Asteraceae) collected wild in Sicily and their activity on microorganisms affecting historical art craft

Simona Casiglia[a], Maurizio Bruno[a]*, Felice Senatore[b] and Sergio Rosselli[a]

[a.] *Department STEBICEF, University of Palermo, Viale delle Scienze, Parco d'Orleans II - 90128 Palermo, Italy*

[b.] *Department of Pharmacy, University of Naples "Federico II", Via D. Montesano, 49 - 80131 Naples, Italy.*

* Corresponding authors*:* Maurizio Bruno: E-mail: maurizio.bruno@unipa.it

simonacasiglia@libero.it

maurizio.bruno@unipa.it

fesenato@unina.it

sergio.rosselli@unipa.it

Chemical composition of essential oils of *Anthemis secundiramea* Biv. subsp. *secundiramea* (Asteraceae) collected wild in Sicily and their activity on microorganisms affecting historical art craft

In the present study the chemical composition of the essential oil from aerial parts of *Anthemis secundiramea* Biv. subsp. *secundiramea* L. collected in Sicily was evaluated by GC and GC-MS. The main components of *A. secundiramea* were (*Z*)-lyratyl acetate (14.6%), (*Z*)-chrysanthenyl acetate (9.9%), (*Z*)-chrysanthenol (8.7%) and (*E*)-chrysanthenyl acetate (7.7%). The comparing with other studied oils of genus *Anthemis* belonging to the same clade is discussed. Antibacterial and antifungal activities against some microorganisms infesting historical art craft, were also determined.

Keywords: *Anthemis secundiramea* Biv. subsp. *secundiramea*; Asteraceae; Volatile components; (*Z*)-Lyratyl acetate; Chrysanthenyl derivatives, Antibacterial and Antifungal activity

1. Introduction

The genus *Anthemis* belongs to the Asteraceae family and comprises of approximately 210 species (Fernandes 1976) distributed across Europe, Southwestern Asia, Northern and Northeastern Africa, Southern Arabia, and tropical East Africa (Bremer 1994). Since the Roman times, the species of this genus have been commonly used in traditional medicine therapies, in fact, *Anthemis nobilis* has been known from Roman times as an antispasmodic and sedative in treatment of digestive and rheumatic disorders (Der Marderosian 2001). The species of the *Anthemis* genus are widely used in the pharmaceutics, cosmetics and food industry and in Europe extracts, tinctures, and tisanes are widely used as anti-inflammatory, antibacterial, antispasmodic, and sedative agents. Extracts are used to allay pain and irritation, clean wounds and ulcers, and aid prevention as well as therapy of irradiated skin injuries, treatment of cystitis and dental afflictions (Mann & Staba 1986). Recently, numerous *Anthemis* species have shown potential antimicrobial activity that could be correlated to their pehnolics and flavanoids composition (Vaverkova et al. 2001). Additionally, digestive,

antispasmodic and anti-*Helicobacter pylori* activities were all linked to *Anthemis* species in several reports (Honda et al. 1996; Konstantinopoulou et al. 2003).

Several studies have been published on sesquiterpene lactones (Staneva et al. 2008), polyacetylenes (Bohlmann et al. 1963; Bohlmann, Kleine et al. 1965; Bohlmann, Bohm et al. 1965; Bohlmann & Kleine 1966), and flavonoids (Williams et al. 2001) isolated from taxa of the genus *Anthemis*, and, recently, a review on the chemotaxonomic markers of the essential oils was published (Maggio et al. 2014).

The essential oils from different *Anthemis* species are often used as preservatives and flavouring agents in pharmaceuticals and cosmetic products and several biological activities have been shown for them. In fact, the essential oil of *A. nobilis* has shown to possess anti-inflammatory and sedative properties (Rossi et al. 1988) and anti-candida activity (Teixeira et al. 2005) and it is commonly used for pharmaceuticals, food additives and cosmetic industries (Melegari et al. 1988). *A. cotula* showed antimicrobial activity against both Gram-negative and Gram-positive microorganisms (Quarenghi et al. 2000). The essential oil of *A. melampodina* had a moderate larvicidal activity against *Culex pipens* (Grace 2002). Antimicrobial and /or antioxidant activities were detected for the essential oils of *Anthemis mixta* and *A. tomentosa* (Formisano et al. 2102), *A. altissima* L. var. *altissima* (Samadi et al. 2012), *A. arvensis* (Boulanouar et al. 2013), *A. mauritiana* (Karim et al. 2011), *A. palestina* (Bardaweel et al. 2014) and *A. wiedemanniana* (Conforti et al. 2012), the last two showing also interesting anti-proliferative properties (Bardaweel et al. 2014; Conforti et al. 2012).

Anthemis secundiramea group (Asteraceae, Anthemideae) is made up of six closely related species: the coastal central (and western) Mediterranean *A. secundiramea* Biv., the Sicilian inland endemic *A. muricata* (DC.) Guss., the North African inland species *A. confusa* Pomel, *A. glareosa* E.A. Durand & Barratte and *A. ubensis* Pomel, and the coastal *A. urvilleana* (DC.) R. Fern. endemic to Malta (Oberprieler 1998). According to nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequence information (Lo Presti & Oberprieler 2009), all these species are part of a clade, which also includes the perennials *A. maritima*, *A. pedunculata* (both widespread in the west Mediterranean), *A. cupaniana* (endemic to the mountains of Sicily) and *A. abylaea* (endemic to the mountains of Morocco) (Lo Presti & Oberprieler 2011).

Anthemis secundiramea Biv. subsp. *secundiramea* ("Camomilla costiera") is a perennial herb native to the Mediterranean region, in northwest Africa (Lybia, northern Algeria, Tunisia), southern Europe (Baleares, South Italy, Sicily, Malta, Sardinia, Corse, southern France), and the eastern Mediterranean (Cyprus, Lebanon). It occurs primarily uncultivated sandy ground or grassy ground near the sea and it blooms between April and June (http://ww2.bgbm.org/euroPlusMed/).

No previous phytochemical research has been reported either on *Anthemis secundiramea* or on the other species belonging to its group. Among the taxa belonging to its clade only *A. maritima* and *A. cupaniana* have been previously studied. In fact, the chemical composition of the essential oils of both species have been investigated (Maggio et al. 2014; Darriet et al. 2009; Ciccarelli et al. 2013) and from the aerial parts of *A. cupaniana* several germacrane sesquiterpenoids have been isolated (Bruno et al. 1991).

The growing interest on natural products that can be used as alternative to synthetic chemicals in order to prevent and reduce the dangerous effects of microorganisms on historical artifacts (Mansour 2013, Rakotonirainy & Lavèdrine 2005; Stupar et al. 2014; Casiglia et al. 2014a; 2014b; 2015a; 2015b) induced us, in the frame of our previous reports on Mediterranean *Anthemis* ssp. (Bruno et al. 1991; Conforti et al. 2012; Formisano et al. 2102; Maggio et al. 2014), to investigate on the chemical composition and anti-microbial properties against several microorganism, including *Bacillus subtilis*, *Staphylococcus aureus*, *Fusarium oxysporum* and *Aspergillus niger*, species infesting historical material (Kamel et al. 2014; Gupta 2013), of the essential oil of *A. secundiramea*, collected on the northern coast of Sicily. Considering that in addition to microorganisms using biochemical processes of degradation, may be found on art craft materials microorganisms whose growth can be connected with the presence of substances added during the production process (adhesives, dyes, finiture, etc.), we have extended the study to other species potentially present and pathogenic e.g. *Staphylococcus epidermidis*, *Escherichia coli* and *Proteus vulgaris*.

In this study we report the chemical composition and the antimicrobial properties of the essential oils from aerial parts of *Anthemis secundiramea* Biv. subsp. *secundiramea*, growing wild in Sicily.

2. Results and discussion

Hydrodistillation of the aerial parts of *Anthemis secundiramea* Biv. subsp. *secundiramea*, collected at Fondo Orsa (**A.s.a.**), gave a yellow oil. Overall, 53 compounds were identified, representing 90.1% of the total oil composition. The components, listed in Table 1 according to their retention indices (*RI*) on a HP 5 MS column, were divided into seven classes on the basis of their chemical structures. (*Z*)-Lyratyl acetate (14.6%) was recognized as the main constituent of the essential oil, together with (*Z*)-chrysanthenyl acetate (9.9%), (*Z*)-chrysanthenol (8.7%) and (*E*)-chrysanthenyl acetate (7.7%). Generally, the oil consisted principally of oxygenated monoterpenes (48.5%), mainly with irregular skeletons (42.4%). Sesquiterpene hydrocarbons (19.8%) was represented by 13 compounds with (*E*)-β-bergamotene (5.1%), α-cedrene (3.8%) and δ-cadinene (3.4%) as principal ones whereas among the monoterpene hydrocarbons (12.6%) only α-pinene (3.0%), *p*-

cymene (3.0%), and β-pinene (2.3%) are worth of mention. Hydrocarbons, carbonylic compounds and oxygenated sesquiterpenes were present in very low amount.

The comparison of our data with those reported in literature for the other two only studied species, belonging to the same clade (*A. maritima*, *A. cupaniana*), allows same interesting remarks.

With the aid of principal component analysis on the essential oils of 6 populations from Corsica and 12 populations from Sardinia of *A. maritima* they were divided in two groups. The first one, that includes the populations of Corsica and of west Sardinia was characterized by the presence of 6-methylhept-5-en-2-one, as main component, and of several chrysanthenyl derivatives but devoid of (*E*)-chrysanthenyl acetate. The second group, that includes populations from east Sardinia, was characterized, on the other hand, by high amount of (*E*)-chrysanthenyl acetate together with other chrysanthenyl derivatives and by lesser quantity of 6-methylhept-5-en-2-one (Darriet et al. 2009). A subsequent investigation on 6 populations of *A. maritima* from Tuscany confirmed the high presence in the oils of 6-methylhept-5-en-2-one and chrysanthenyl derivatives with (*E*)-chrysanthenyl acetate ranging among 0% and 34.0% (Ciccarelli et al. 2013).

In the oil isolated from the aerial parts of *A. cupaniana*, for which 70 compounds were identified, the main class was represented by oxygenated monoterpenes (41.1%) with artemisyl acetate (12.7%), β-thujone (11.8%), and yomogi alcohol (8.2%) as the main components. The most abundant compound of the oil was α-pinene (18.4%), which, together with sabinene (5.0%), was also the principal component of the monoterpene-hydrocarbon class (26.8%) (Maggio et al. 2014).

These literature data indicate a completely different profile of our oil (**A.s.a.**) with respect to the oil of *A. cupaniana*, in fact, artemisyl acetate, β-thujone, and yomogi alcohol were not present in (**A.s.a.**) whereas the oil of *A. cupaniana* was completely devoid of chrysanthenyl derivatives. On the other hand, as the oils of *A. maritima*, **A.s.a.** is rich of chrysanthenyl derivatives (27.8%), but, differently from the latter, it is completely devoid of 6-methylhept-5-en-2-one.

The antimicrobial and antifungal activities of essential oil were tested against a panel which included eight bacteria species, selected as representative of the class of Gram positive and Gram negative, one yeast and two moulds. The oil shows a good activity especially against *Bacillus subtilis*, *Staphylococcus aureus*, *Fusarium oxysporum* and *Aspergillus niger* (Table 2). The antibacterial properties of the oil can be attributed to the presence of (*E*)-chrysanthenyl acetate, (*Z*)-chrysanthenol and (*E*)-chrysanthenol, compounds recently proved to be very active against *B. subtilis* (Móricz et al. 2015), and to the presence of bornyl acetate, that was shown to be moderately active against *S. aureus* (Runyoro et al. 2010). Furthermore, the good antifungal activity of the essential oil of *Chrysanthemum coronarium* against *Aspergillus* and *Fusarium* ssp. was also attributed to (*Z*)-lyratyl acetate (Alvarez-Castellanos et al. 2001), the main component of our oil.

3. Experimental

3.1. *Plant material*

Aerial parts of *Anthemis secundiramea* Biv. subsp. *secundiramea* were collected at Fondo Orsa (**A.s.a.**), 20 Km west of Palermo (Sicily, Italy) on the rocky sea-coast (38°11'27" N; 13°07'25" E; 3 m s/l), in the middle of May 2014, from plants at the full flowering stage. Typical specimens (PAL 14/61), identified by Mr. Emanuele Schimmenti, have been deposited have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

3.2. *Isolation of the essential oil*

The air-dried samples were ground in a Waring blender and then subjected to hydrodistillation for 3 h using *n*-hexane as solvent, according to the standard procedure previously described (Ben Jemia et al. 2013). The oil was dried over anhydrous sodium sulphate and then stored in sealed vials, at -20°C, ready for the GC and GC-MS analyses. The sample yielded 0.21% (**A.s.a.**), of oil (w/w) with a pleasant smell.

3.3. *Qualitative and quantitative analyses*

The essential oil and the four extract samples were analyzed to determine the chemical components at the "Department of Pharmacy" of the University of Naples "Federico II" by GC and GC-MS. The GC analyses were carried out with a Perkin-Elmer Sigma 115 gas chromatograph equipped with a flame ionization detector (FID) while Gas chromatography-Mass spectrometry (GC-MS) was recorded on an Agilent 6850 Ser. II apparatus coupled to an Agilent Mass Selective Detector MSD 5973 as previously described (Loizzo et al. 2013). Identification of constituents was made as elsewhere reported (Zito et al. 2013).

3.4. Antimicrobial assay

3.4.1. Microbial strains

The antimicrobial and antifungal activities of essential oil were tested against a panel which included eight bacteria species, selected as representative of the class of Gram positive: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATTC 29212); and Gram negative: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853); one yeast, *Candida albicans* (ATCC 10231); two moulds, *Fusarium*

oxysporum (ATCC 695) and *Aspergillus niger (*ATCC 16401). The strains were grown on Tryptone Soya Agar (Oxoid, Milan, Italy) for the bacteria, Saboureaud Dextrose Agar (SDA) with chloramphenicol for yeasts and SDA for moulds. For the antimicrobial tests, Tryptone Soya broth (Oxoid, Milan, Italy) for bacteria and Sabouraud dextrose broth (SDB) for yeasts and fungal strains were used.

3.4.2. Antimicrobial screening

The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC), which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), as previously described (Rigano et al. 2011), using the broth dilution method (Barry, 1976). Oil samples were tested in triplicate.

4. Conclusion

In conclusion, with regard to *A. secundiramea* essential oil, the results presented herein indicate a quite different chemical profile with respect to the other Sicilian taxa of the same clade studied so far. In fact, it shows the presence of chrysanthenyl derivatives completely absent in *A. cupaniana*, and although a close relationship with the oils of *A. maritima*, differently from it, **A.s.a.** is completely devoid of 6-methylhept-5-en-2-one.

The good antimicrobial activity detected for the essential oil against especially against *Bacillus subtilis*, *Staphylococcus aureus*, *Fusarium oxysporum* and *Aspergillus niger*, species infesting archives, libraries and historical cellulosic textiles objects quite frequently, makes this plant interesting for possible applications in the protection and disinfestation of museum objects.

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Table 1 Percent composition of the essential oil of *Anthemis secundiramea* Biv. (Asteraceae).

K_i [a]	K_i [b]	Component	A.s.a [c]	Ident. [d]
930	1014	α-Thujene	0.2	1, 2
938	1032	α-Pinene	3.0	1, 2, 3
953	1076	Camphene	0.4	1, 2, 3
973	1132	Sabinene	0.5	1, 2
980	1118	β-Pinene	2.3	1, 2, 3
993	1174	Myrcene	t[e]	1, 2, 3
1025	1278	*p*-Cymene	3.0	1, 2, 3
1038	1045	(*Z*)-β-Ocimene	1.2	1, 2, 3
1049	1265	(*E*)-β-Ocimene	1.8	1, 2
1086	1265	Terpinolene	0.2	1, 2, 3
		Monterpene hydrocarbons	**12.6**	
1034	1213	1,8-Cineole	t	1, 2, 3
1114	1684	(*Z*)-Chrysanthenol **	8.7	1, 2
1128	1487	α-Campholenal	t	1, 2
1137	1383	Isocyclocitral	0.3	1, 2
1163	1764	(*E*)-Chrysanthenol **	1.5	1, 2
1236	1585	(*E*)-Chrysanthenyl acetate **	7.7	1, 2
1263	1535	(*Z*)-Chrysanthenyl acetate **	9.9	1, 2
1284	1597	Bornyl acetate	5.8	1, 2, 3
1482	1674	(*Z*)-Lyratyl acetate **	14.6	1,2
		Oxygenated monoterpenes	**48.5**	
1356	1579	α-Longipinene	1.6	1, 2
1382	1502	1,7-di-*epi*-α-Cedrene	2.7	1, 2
1411	1568	α-Cedrene	3.8	1, 2
1433	1620	Widdrene	0.6	1, 2
1437	1530	α-Guaiene	0.5	1, 2
1452	1672	(*E*)-β-Farnesene	t	1, 2
1463	1661	*allo*-Aromadendrene	0.3	1, 2, 3
1475	1715	β-Selinene	0.1	1, 2
1478	1704	γ-Muurolene	0.7	1, 2, 3
1482	1607	(*E*)-β-Bergamotene	5.1	1, 2
1505	1730	δ-Guaiene (α-Bulnesene)	0.5	1, 2
1515	1776	γ-Cadinene	0.5	1, 2
1526	1173	δ-Cadinene	3.4	1, 2
		Sesquiterpene hydrocarbons	**19.8**	
1553	2076	*cis*-α-Copaen-8-ol	0.3	1, 2
1570	2097	β-Atlantone	1.0	1, 2
1580	2008	Caryophyllene oxide	0.3	1, 2, 3
1602	2037	Salvial-4(14)-en-1-one (Mintketone)	t	1, 2
1622	2182	*nor*-Copaanone	1.0	1, 2
1642	2225	α-Muurolol	0.2	1, 2
1650	2258	β-Eudesmol	0.1	1, 2
1652	2255	α-Cadinol	0.2	1, 2
1678	2122	(*Z*)-γ-Atlantone	0.4	1, 2
1685	2143	(*E*)-γ-Atlantone	0.6	1, 2
1687	2143	Valeranone	0.4	1, 2
1689	2172	(*Z*)-α-Atlantone	0.3	1, 2
1774	2248	(*E*)-α-Atlantone	0.5	1, 2
		Oxygenated sesquiterpenes	**5.3**	
1619	1934	Tetradecanal	0.2	1,2
1845	2131	Hexahydrofarnesyl acetone	1.0	1, 2
1920	2387	(*E,E*)-Farnesyl acetone	t	1, 2
		Carbonylic compounds	**1.2**	
2300	2300	Tricosane	0.5	1, 2, 3
2500	2500	Pentacosane	0.8	1, 2, 3
2700	2700	Heptacosane	0.6	1, 2, 3
2900	2900	Nonacosane	0.8	1, 2
		Hydrocarbons	**2.7**	
2135	2625	(*E*)-Phytol	t	1, 2
		Others	**t**	
		TOTAL	**90.1**	

[a]: HP-5 MS column; [b]: HP Innowax column [c]: A.s.a.1 = *Anthemis secundiramea* Biv. aerial parts collected at Fondo Orsa; [d]: 1, retention index, 2: mass spectrum, 3: co-injection with authentic compound; [e]: t: trace, <0.05%; **: irregular terpenes.

Table 2 MIC (µg/mL) and MMC* (µg/mL) of essential oil from *Anthemis secundiramea* Biv. (Asteraceae)

Strain	A.s.a.	Ch	Am	Ke
Bacillus subtilis ATCC 6633	12.5(25)	12.5	NT	NT
Staphylococcus aureus ATCC 25923	25(50)	25	NT	NT
Staphylococcus epidermidis ATCC 12228	12.5(25)	3.12	NT	NT
Streptococcus faecalis ATCC 29212	50	25	NT	NT
Escherichia coli ATCC 25922	50	12.5	NT	NT
Klebsiella pneumoniae ATCC 10031	100	50	NT	NT
Proteus vulgaris ATCC 13315	100	25	NT	NT
Pseudomonas aeruginosa ATCC 27853	100(>100)	100	NT	NT
Candida albicans ATCC 10231	50	NT	1.56	NT
Fusarium oxysporum ATCC 695	12.5	NT	NT	3.12
Aspergillus niger ATCC 16401	6.25	NT	NT	3.12

* MBC are reported in brackets when different from MIC; NT: not tested; **Ch**: Chloramphenicol; **Am**: Amphotericin B; **Ke**: Ketoconazole