NOTE

Composition of the Essential Oil of *Allium neapolitanum* Cirillo Growing Wild in Sicily and its Activity on Microorganisms Affecting Historical Art Crafts

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Abstract: Essential oil of the aerial parts of *Allium neapolitanum* Cirillo collected in Sicily were analyzed by gas-chromatography-flame-ionization detection and gas-chromatography-mass spectrometry. Nineteen compounds were identified in the oil and the main components were found to be (*E*)-chrysanthenyl acetate (28.1%), (*Z*)-chrysanthenyl acetate (23.8%), (*E*)- β -farnesene (9.6%), dimethyl trisulfide (9.6%), camphor (7.4%), methyl allyl disulfide (6.8%) and 1-methyl-3-allyl trisulfide (5.8%). The essential oil showed good antimicrobial activity against 11 strains of test microorganisms, including several species infesting historical material.

Key words: *Allium neapolitanum*, essential oil, (*E*)-chrysanthenyl acetate, (*Z*)-chrysanthenyl acetate, antimicrobial activity

1 INTRODUCTION

The genus *Allium* is the largest genus of the family Alliaceae and comprises more than 700 species distributed all over Europe, North America, Northern Africa and Asia, each differing in taste, form and colour, but close in biochemical, phytochemicals and nutraceutical content¹. The *Allium* genus is rich of flavonoids, saponins, sapogenins and volatile sulphur compounds and their characteristic organoleptic properties derive from the presence of non-volatile flavour precursors, alk(en)yl-L-cysteine sulfoxides². Many sulphur compounds found in *Allium* species are responsible for several biological properties such as antimicrobial³, antiprotozoal, antioxidant, antihypertensive, hypolipidaemic, hepatoprotective and antithrombotic activities⁴.

Allium neapolitanum Cirillo is a perennial bulbous plant, native to the Mediterranean Region and the Mid-East from Spain to Pakistan⁵. In Sicily it is a non common plant, growing below 1000 m s/l in pastures, cultivated grounds (vineyards and olive-grove) and dry, open habitats⁶.

Previous phytochemical studies on the composition of *A. neapolitanum* led to the isolation of thirteen flavonoid glycosides, based on kaempferol, quercetin and isorhamne-

tin⁷ and two canthin-6-one alkaloids with antibacterial activity⁸. Furthermore, the antioxidative properties of the aqueous extracts of bulbs, bulblets, leaves and flowers of *A. neapolitanum* were investigated showing a good antiradical power⁹. It was also showed that the good antihypertensive effects of the aqueous extract of bulbs of *A. neapolitanum* could to be associated to interactions with adrenergic receptor β_2 antagonist, involved in blood pressure regulation¹⁰. Finally, the chloroform extract of this species was found to have antimicrobial activity on several bacteria and on *C. albicans*, in some cases better than streptomycin sulphate and nystatin, respectively, used as standard antibiotics¹¹. On the hand no report has been published on the composition of the essential oil of this species.

Several heterotrophic microorganisms (bacteria and fungi) have the ability to interact with historical organic materials such as textile, leather, paper, paintings, wood, papyri, incunabula and books, all consisting essentially of natural fibres. Microbial growth causes loss of strength and elongation, oxidation state, discoloration, changes in appearance, degree of polymerization and breakdown of molecular structure. Species of genus *Bacillus*, frequent in

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archives, libraries and on museum cellulosic objects can cause deep deteriorations of the items but the most dangerous microorganisms both for cellulose fibers and fibers of animal origin (wool, silk) belong to some genera of fungi: *Chaetomium*, *Myrothecium*, *Trichoderma*, *Fusarium*, *Penicillium* and *Aspergillus*.

Also the surfaces of stone monuments can be altered by fungal activity via hyphal penetration through the porous stone matrix and by the production of organic acids and pigments¹². The growing interest on natural products that can be used as an alternative to synthetic chemicals in order to prevent and reduce the dangerous effects of microorganisms on historical artifacts^{13–15} prompted us to investigate on the chemical composition and anti-microbial properties against several microorganism, including *Bacillus subtilis*, *Fusarium oxysporum* and *Aspergillus niger*, species infesting historical material¹³, of the essential oil from aerial parts of *A. neapolitanum*, growing wild in Sicily.

2 MATERIALS AND METHODS

2.1 Plant material

Aerial parts of *Allium neapolitanum* were collected 2 km west of Portella della Ginestra, Piana degli Albanesi, Palermo (Sicily, Italy) (37° 58' 33" N; 13° 14' 11" E; 790 m s.l.), at the end of May 2014, from plants at the full flowering stage. Typical specimens (PAL 14/84), identified by Mr. Emanuele Schimmenti, have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

2.2 Isolation of the essential oil

The air-dried sample was ground in a Waring blender and then subjected to hydrodistillation for 3 h using *n*-hexane as solvent, according to the standard procedure previously described¹⁶. 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 0.22% of oil (w/w) (A.n.) with a typical garlic smell.

2.3 Qualitative and quantitative analyses

The essential oil was analyzed to determine the chemical components by GC and GC-MS as previously described and identification of constituents was made by comparison of their retention indices (*R*_i) with either those of the literature or with those of authentic compounds available in our laboratories¹⁷.

2.4 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 and Gram negative (Table 2). 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 Saboureaud dextrose broth (SDB) for yeasts and fungal strains were used.

2.5 Antimicrobial screening

The antimicrobial activity was evaluated, as previously reported¹⁴, by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC), which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), using the broth dilution method¹⁸.

3 RESULTS AND DISCUSSION

3.1 Chemical composition of the essential oil

Hydrodistillation of the aerial parts of *Allium neapolitanum* gave a yellow oil (A.n.). Overall, nineteen compounds were identified in the oil, representing 96.8% of the total components. The components are listed in Table 1 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into five classes.

The main class was represented by oxygenated monoterpenes with (*E*)-chrysanthenyl acetate (28.1%), (*Z*)-chrysanthenyl acetate (23.8%) and camphor (7.4%) as main compounds. Among the sulphur-containing compounds (23.6%), dimethyl trisulfide (9.6%), methyl allyl disulfide (6.8%) and 1-methyl-3-allyl trisulfide (5.8%) were the most abundant products. It is worthy of mention the good quantity of (*E*)- β -farnesene (9.6%), the only sesquiterpene hydrocarbon detected in the oil.

The comparison with the compositions of the essential oil from aerial parts of *Allium* sp. studied so far shows some interesting points. In fact, it is noteworthy that the percentage of sulphur-containing compounds varies drastically: *A. ampeloprasum*¹⁹ and *A. nigrum*²⁰ are devoid of these compounds whereas other species such as *A. cepa* (100%)²¹, *A. fistulosum* (95.8%)²², *A. chinense* from Cuba (94.0%)²³ and *A. tuberosum* from China (92.9%)²⁴ are extremely rich of this class of compounds. The oil of *Allium neapolitanum* shows a moderate content of sulphur-containing compounds (23.6%) and it has to be pointed out that (*E*)-chrysanthenyl acetate, (*Z*)-chrysanthenyl acetate and (*E*)- β -farnesene have never been identified in significant amount in any *Allium* oil. Among the oxygenated monoterpenes present in *A. neapolitanum* only camphor has been identified in good amount (13.4%) in *A. roseum* var. *odoratissimum* from Tunisia²⁵.

Table 1 Percent composition of the essential oils of *Allium neapolitanum* Cirillo (Alliaceae).

K _i ^a	K _i ^b	Component	A.n.	Ident. ^c
1145	1532	Camphor	7.4	1, 2, 3
1235	1583	(<i>E</i>)-Chrysanthenyl acetate **	28.1	1, 2
1257	1585	(<i>Z</i>)-Chrysanthenyl acetate **	23.8	1, 2
1284	1597	Bornyl acetate	1.6	1, 2, 3
		Oxygenated monoterpenes	60.9	
1452	1672	(<i>E</i>)- β -Farnesene	9.6	1, 2
		Sesquiterpene hydrocarbons	9.6	
908		2,5-Dimethylthiophene	t ^d	1, 2
917	1308	Methyl allyl disulfide ; Methyl 2-propenyl disulfide	6.8	1, 2
926	2285	Diethyl disulfide	t ^d	1, 2
952	1145	Methyl 1-propenyl disulfide	0.9	1, 2
973	1403	Dimethyl trisulfide; DMTS	9.6	1, 2
1083	1434	Di-2-propenyl disulfide; Diallyl disulfide	0.2	1, 2
1087	1330	Dipropyl disulfide	0.2	1, 2
1096	1436	Dipropenyl disulfide	0.1	1, 2
1137	1506	1-Methyl-3-allyl trisulfide	5.8	1, 2
		Sulphur-containing compounds	23.6	
802	1168	Hexanal; Caproaldehyde	0.3	1, 2, 3
		Carbonylic compounds	0.3	
2100	2100	Heneicosane	0.1	1, 2, 3
2300	2300	Tricosane	0.4	1, 2, 3
2500	2500	Pentacosane	0.7	1, 2, 3
2700	2700	Heptacosane	1.2	1, 2, 3
		Hydrocarbons	2.4	1, 2, 3
		TOTAL	96.8	

^a: HP-5 MS column; ^b: HP Innowax column ^c: 1, retention index, 2: mass spectrum, 3: co-injection with authentic compound; ^d: t: trace, <0.05%; **: irregular terpene.

3.2 Biological activity

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). Although for the two main compounds of the oil, (*E*)-chrysanthenyl acetate and (*Z*)-chrysanthenyl acetate, no biological properties have been reported, the good antimicrobial activity of the oil can be attributed to the presence of camphor, dimethyl trisulfide, methyl allyl disulfide and 1-methyl-3-allyl trisulfide, compounds already proved to be very active^{26–29}.

Previous studies on the structure-activity relationship of garlic metabolites showed antimicrobial activity for allyldisulfide, but not for allylsulfide³⁰ suggesting that the disul-

fide bond may be important for the antimicrobial effect. Furthermore, it has been reported²⁷ that the presence of the trisulfide bond, or of an extra sulphur in the molecule, may reduce the biological properties of the allylsulfide constituents of garlic oils. Consequently, the high antimicrobial activity of *A. neapolitanum* essential oil can be attributed to the presence both of allyldisulfides and camphor; the antibacterial and antifungal properties of the last one have been largely demonstrated^{26, 31–33}.

The antimicrobial activity of other essential oils from *Allium* species have been also demonstrated. The essential oils of different *Allium nigrum* L. organs²⁰ and *Allium roseum* var. *grandiflorum* subvar. *typicum* Regel.³⁴, both collected in Tunisia, exhibited antimicrobial activity, especially against *Enterococcus faecalis* and *Staphylococcus aureus*²⁰ and antifungal growth effect on *Fusarium solani* f. sp. *cucurbitae* and *Botrytis cinerea*³⁴. The es-

Table 2 MIC ($\mu\text{g/mL}$) and MMC* ($\mu\text{g/mL}$) of essential oil from *Allium neapolitanum*.

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

essential oil of the flowers of *A. roseum* var. *odoratissimum* from Tunisia showed good activity against *Micrococcus luteus*, *Staphylococcus epidermidis* and *S. aureus*²⁵⁾. Also the oil of the flowers of *A. rotundum* from Iran indicates a good activity against *Proteus mirabilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis*³⁵⁾. A good activity against Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) was reported for the essential oil of *A. schoenoprasum*, collected in Turkey³⁶⁾. On the other hand the oil obtained from the flowers of *A. sphaerocephalon* subsp. *sphaerocephalon* was proved to be quite active against *Pseudomonas aeruginosa* and *Aspergillus niger*³⁷⁾. Shallot (*Allium ascalonicum* L.) oil was studied for its major diallyl sulfide content and its antimicrobial activity against food-borne pathogenic bacteria including *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*, and *Vibrio cholerae*. Among them, *E. coli* O157:H7 and *B. cereus* were the most and

the least sensitive strains, respectively. The oil had a bactericidal effect on *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *V. cholerae* but had a bacteriostatic effect on *B. cereus* and *S. enterica*³⁸⁾.

The good antimicrobial activity detected for the essential oil of *A. neapolitanum* 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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