Essential oil components of orange peels and antimicrobial activity

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ABSTRACT
In this study, the orange peel of 12 cultivars of Citrus sinensis from central-eastern Sicily was employed to obtain essential oils and extracts. The ones were extracted through steam distillation, the others through extraction in hexane. Chemical constituents were evaluated in terms of qualitative and quantitative analyses by gas chromatography/mass spectrometry. Fifty-four components were identified in the steam essential oils and 44 in the extracts. In all the cultivars, the main component is Δ-limonene (73.9–97%); discrete percentages of linalool, geraniol and nerol were also found. Cluster analysis based on essential oils composition showed a certain degree of affinity between cultivars of the same type. The antimicrobial activity was investigated against three micro-organisms (Staphylococcus aureus, Listeria monocytogenes and Pseudomonas aeruginosa). ‘Sanguinello’ and ‘Solarino Moro’ essential oils are significantly active against L. monocytogenes, while ‘Valencia’ hexanic extract against all the tested micro-organisms.

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1. Introduction

Citrus is one of the world’s major fruit crops with global availability and popularity that contributes to human diets (FAO 2009; Kahn et al. 2001). It is widely grown in most areas with suitable climates: tropical, subtropical and borderline subtropical/temperate. Although many citrus fruits, such as oranges, tangerines and grapefruits, can be eaten fresh, approximately a third of citrus fruits worldwide are utilised after processing and orange juice production accounts for nearly 85% of total processed consumption. This food and agro-food processing industry yields a considerable amount of waste or by-products (peels, seeds and pulps), which represents 50% of the raw processed fruit (Anwar et al. 2008). These by-products are considered a valuable source of functional ingredients, such as flavonoids, dietary fibres and essential oils (Senevirathne et al. 2009). Several medical and epidemiological studies have shown an inverse relationship between fruit consumption and the incidence of coronary and heart diseases, as well as certain cancers (Rice-Evans et al. 1997; Rice-Evans 2001; Yang et al. 2001; Bub et al. 2003). Citrus fruits are very rich in minerals, vitamin C and polyphenolic compounds, which contribute to the antioxidant activity (Tripoli et al. 2007) and enhance the liver function through a reduction of the accumulation of fat (Gliozzi et al. 2014). Moreover, orange is the main fruit consumed during wintertime in the Mediterranean diet and it represents one of the most important sources of phytochemicals in the Sicilian population. Among cultivated citrus, sweet orange (Citrus sinensis L. Osbeck) originates as a natural hybrid between mandarin (C. reticulata Blanco) and pummelo (C. maxima (Burm. f.) Merr. (Swingle & Reece 1967). In spite of differences in morphological traits such as size and shape of canopy, colour, size, type and ripening season of the fruits and the number of seeds per fruit, the cultivars showed a low level of genetic diversity (Fang & Roose 1997; Novelli et al. 2006; Uzun et al. 2009). Orange cultivars are classified into four groups: common, low acidity, pigmented and navel oranges (Hodgson 1967).

Sicily is characterised by a natural vocation of the soil and the climate for the production of citrus fruits. Nowadays, the area cultivated with citrus is slightly more than 71,000 hectares, of which approximately 60% is invested in sweet orange (INEA 2014). The first place, both in terms of quantity and quality, is held by pigmented oranges (cultivar ‘Tarocco’, ‘Moro’ and ‘Sanguinello’), which are produced mainly in eastern Sicily and are appreciated on foreign markets for their organoleptic and nutritional properties (IGP as Red Orange of Sicily). In Sicily, navel oranges, acidless and blonde varieties are also largely cultivated.

The citrus essential oils, placed within the glands in the outer layer of the fruit skin, are the most important citrus by-products usually obtained from the peels. They are widely used in pharmaceutical preparations as agents for drinks, ice creams, cakes, air fresheners, household products and perfumes (Ferhat et al. 2006; Hosni et al. 2010); they are also largely employed in aromatherapy (Yavari Kia et al. 2014; Tadtong et al. 2015; Hoenen et al. 2016; Ragusa 2015) and it has been recently suggested that treatment with alcoholic orange peel extracts could prevent or ameliorate diabetic nephropathy (Parkar & Addepalli 2014). Volatile chemical compounds of citrus essential oils are among the most distinctive components for identification and evaluation of the varieties (Njoroge et al. 2005).

Several studies were performed both on the composition of the essential oils from the leaves and the peels of C. sinensis and its hybrids and on their biological activities, such as antifungal, antioxidant, anti-aflatoxigenic and antimicrobial activities (Njoroge et al. 2005, 2009; Celikel & Kavas 2008; Tao et al. 2009; Singh et al. 2010; Kamal et al. 2013; Hasija et al.
This oil is composed of many constituents, including hydrocarbons, alcohols, esters and aldehydes. Citrus oils contain large amounts of monoterpane hydrocarbons (70–95%) and d-limonene is dominant in all the reported sweet orange oils. Sesquiterpene hydrocarbons, which are responsible for a characteristic flavour, have been reported at minor concentrations in most sweet orange peel oils. Aldehydes contribute to the total content of oxygenated compounds and are an important indicator to represent a reference of quality of essential oils (Dugo 1994; Braddock 1995; Mitiku et al. 2000; Minh Tu et al. 2002; Njoroge et al. 2005; Ahmad et al. 2006).

The aim of the present investigation was, therefore, to analyse the chemical composition of both essential oils obtained through steam distillation from the peels of 12 selected C. sinensis cultivars and hexanic extracts from 5 representatives of different groups (we chose a cultivar of navel oranges, two of blonde pulp group, and two of pigmented pulp). Moreover, an assessment of the degree of similarity among the cultivars was conducted on the basis of their essential oils composition. Such information would be fundamental to promote the citrus processing industry in Sicily and the selection of the most valuable source of fragrant compounds, as well as to extend our knowledge on volatile constituents of the Sicilian orange citrus germplasm. Furthermore, we carried out a preliminary evaluation of the antimicrobial activity of selected C. sinensis cultivars against two important bacterial strains which often cause food-transmitted disease and food spoilage (Staphylococcus aureus and Pseudomonas aeruginosa) (Schillaci et al. 2013) and against Listeria monocytogenes that is among the leading causes of death from food-borne illness in developed countries (Vitale & Schillaci 2015).

2. Results and discussion

In the 12 varieties (Table S1) of peel orange essential oils analysed, a total of 54 compounds were identified, including 20 hydrocarbons (11 monoterpenes; 9 sesquiterpenes), 10 aldehydes (4 monoterpenes, 2 sesquiterpenes and 4 aliphatic), 2 ketones (1 monoterpene; 1 sesquiterpene), 15 alcohols (8 monoterpenes, 4 sesquiterpenes and 3 aliphatics), 4 monoterpenic esters, 2 oxides (1 monoterpene; 1 sesquiterpene) and one other compound. In Table S2, the percentages of the chromatographic peaks of the substances identified in the 12 analysed varieties of oranges are reported. The obtained data are largely in agreement with the studies in the literature on oranges’ essential oils composition (Dugo & Mondello 2011). In all the essential oil samples from the orange cultivars, it was found that limonene is the most abundant component, whose peak area percentages ranged between 73.9 and 97.6%. Discrete percentages of monoterpenic alcohols such as linalool, geraniol and nerol as well as α-terpineol were found, while the remaining part of the components is present in smaller quantities. Among sweet orange groups, some differences were found in terms of composition. In the Navel or umbilicate oranges group, ‘Navelina’ showed the lowest percentage of limonene and a discrete presence of either hydrocarbons sesquiterpenes (as also ‘Thomson Navel’ belonging to the same group) or of monoterpenic alcohols (Table S2). In the blonde pulp oranges group, the ‘Salustiana’ cultivar presents high prevalence of monoterpenic hydrocarbons as well as ‘Valentia’, according to the literature data (Mitiku et al. 2000; Njoroge et al. 2005; Hosni et al. 2010). In ‘Valentia’ itself, valencene (1.84%) and linalool (4.11%) were well represented as well. All the essential oils obtained from varieties belonging to the group pigmented pulp oranges show high prevalence of monoterpenic hydrocarbons such
as d-limonene (90.1 to 97.6%), α-pinene, sabinene, β-myrcene, α-phellandrene and δ-3-carene. In these varieties, we observed that monoterpenic aldehydes such as citronellal, Z-citral and E-citral and aliphatic aldehydes such as octanal, nonanal, decanal and dodecanal occurred as well. Carvone, carveol and cis-p-menthen-1-ol were found only in some of these pigmented cultivars (Table S2).

In the hexanic extracts of the five analysed varieties of oranges, a total of 44 compounds were identified, including 18 hydrocarbons (9 monoterpenes, 8 sesquiterpenes and 1 aliphatic); 11 aldehydes (4 monoterpenes, 2 sesquiterpenes and 5 aliphatic); 1 sesquiterpene ketone; 9 alcohols (4 monoterpenes, 3 sesquiterpenes and 2 aliphatic); 2 monoterpenic esters; 1 oxide; and 2 other compounds (Table S3). Similar to the composition of the essential oils, in the sweet orange hexanic extracts, the main component was the monoterpane limonene, with values of peak area percentages comprised between 89.5 and 97.1%. Discrete percentages of monoterpenic alcohol, linalool (from 0.48 to 3.18%), monoterpenic hydrocarbon, β-myrcene (0.59 to 0.99%), sesquiterpenic hydrocarbon and valencene (from 0.06 to 1.04) were found too; the remaining part of the components occurs in small quantities. As regards the comparison between essential oils and hexanic extracts in terms of chemical composition, some small differences were found within samples obtained from the same cultivar.

Thirty-nine common components were detected through the two methods, but some differences must be focused. First of all, among hydrocarbons, γ-terpinene, α-farnesene and α-humulene were found in essential oils while germacrene and decane were found in hexanic extract; among aldehydes and ketones, carvone occurred in essential oils and 9,17-octadecadienal in hexanic extract. Six more alcohols were detected among essential oils (carveol, terpinen-4-ol, cis-p-menthen-1-ol, citronellol, nerolidol and nonanol). Among the esters, α-terpinil acetate, neryl acetate and geranyl acetate were present only in essential oils and linalyl acetate was present in hexanic extracts; among other compounds, cis-p-mentha-2,8-dienol was in essential oils and azulene in hexanic extracts.

The clustering obtained in the dendrogram (Figure S1), constructed from Jaccard’s similarity coefficient on the basis of the concentration of 54 volatile compounds (see Table S2), reflects partly the differences between the various cultivars, typically recognised under a morphological point of view. ‘Thomson Navel’ (ID 2) and ‘Valentia’ (ID 3) formed two single clusters; among pigmented oranges, the following different groups clustered together: three cultivars of ‘Tarocco’ (ID 5, ID 6 and ID 7); two cultivars of ‘Sanguinello moscato’ with ‘Sanguinello’ (ID 8, ID 9 and ID 10); and two cultivars of ‘Moro’ (ID 11; ID 12). ‘Salustiana’ (ID 4) grouped with these latter and ‘Navelina’ (ID 1) with ‘Sanguinello’ cultivar groups.

All samples were tested at concentrations ranging from 100 to 2.5 mg/mL against a group of Gram-positive and Gram-negative strains (Table 1). The antibacterial activity of essential oils and hexanic extracts was expressed as minimum inhibitory concentrations (MICs). In particular, the essential oil ‘Sanguinello’ from Paternò showed a MIC value of 15 mg/mL against the two strains of L. monocytogenes, whereas the essential oil ‘Moro Solarino’ was less active, showing a MIC value of 92 mg/mL against the two above-mentioned strains. All tested bacterial strains (S. aureus ATCC 25,923, S. aureus ATCC 6538, P. aeruginosa and L. monocytogenes) showed susceptibility to the antibacterial action of ‘Valencia’ hexanic extract at a concentration of 10 mg/mL. The detected antimicrobial activity confirms the efficacy of the orange peel essential oils against food-borne pathogens, but the essential oils of ‘Sanguinello’ and ‘Moro’ cultivars seem less active against Gram-negative bacteria such
as *S. aureus* and *P. aeruginosa*. These data agree with some previous studies showing that Gram-negative bacteria were usually more resistant to citrus essential oils than Gram-positive (Burt 2004), and in particular, *S. aureus* was more resistant than *L. monocytogenes* when treated with citrus essential oils (Fisher & Phillips 2006).

### 3. Experimental

Please refer to the Supplementary Material online for more on the “Experimental” section.

### 4. Conclusion

For the benefit of human health and the environment, new frontiers of chemistry are looking for products with a low environmental impact. The innovations in the field of ‘sustainable green chemistry’ aim to reuse waste industrial products for environmentally friendly products. In this regard, the preparation, chemical analysis and assays of antimicrobial activity of essential oils and extracts of Sicilian oranges were carried out concurrently in this study. The hexanic extracts showed, in some cases, chemical composition and activities comparable to essential oils with the advantages of lower cost and higher yield (0.3–2.5%). However, they can retain traces of solvent and since they might cause toxicity problems, they are not suitable in health care. The steam distillation of essential oils, on the contrary, although presenting a higher cost and a lower yield (0.2–1.2%), is preferred because only natural and non-toxic substances are involved. Consequently, these oils are suitable in ‘sustainable green chemistry’ and they can be used to preserve the quality of food (shelf life extension) and to combat food-borne diseases. The challenge is to maximise essential oils as natural substances with biological activity, replacing chemical additives. The use of bioactive packaging will probably increase in the world due to the consumer’s preference for naturally preserved food and because industry wishes to increase the shelf life extension of packaged food, preserving at the same time product quality and safety (Tongnuanchan & Benjakul 2014). On this point, the European Commission too has defined a line of strategic importance of packaging for a healthy and safe feeding within the specific programme implementing the Framework Programme for Research and Innovation (2014–2020).

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### Table 1. Antibacterial screening of some essential oils and hexanic extract. Activity expressed as MIC in mg/mL.

|                      | *S. aureus* ATCC 25923 | *S. aureus* ATCC6538 | *L. monocytogenes* ATCC 7644 | *L. monocytogenes* B2 | *P. aeruginosa* ATCC 15442 |
|----------------------|------------------------|-----------------------|-----------------------------|-----------------------|-----------------------------|
| ‘Sanguinello’ Paternò (Essential oil) | > 100                  | > 100                 | 15                          | 15                    | > 100                       |
| ‘Moro’ Solarino (Essential oil) | > 100                  | > 100                 | 92                          | 92                    | > 100                       |
| ‘Valencia’ (Hexanic extract) | 10                     | 10                    | 10                          | 10                    | 10                          |
Disclosure statement

No potential conflict of interest was reported by the authors.

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