Nutraceutical potential of monofloral honeys produced by the Sicilian black honeybees (Apis mellifera ssp. sicula)

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A B S T R A C T

In the light of the growing interest in food and food products obtained through organic and environmentally friendly techniques, the present work represents the first approach to the evaluation of the biological profile of some Sicilian honeys produced in purity by the local black honeybees. Samples exhibited up to 10 times more total phenolics and higher antioxidant capacity than what already reported for the same variety of honeys produced by other honeybee subspecies from Sicily, other Italian regions and abroad. Noteworthy, the gallic acid contents in medlar and almond honeys represented the highest level of single phenolic acid reported in honey so far. A broad antimicrobial spectrum was showed by all of the honey samples and a good correlation between their inhibition capacity and polyphenolic contents was measured. Experimental results highlighted samples among the honeys characterised by the highest nutraceutical added value and most excellent quality.

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1. Introduction

Antioxidant compounds are biosynthesised by a large number of plants that may be used by honeybees to collect nectar; consequently, a wide variety of free radical-scavenging phytochemicals can be transferred to honey (Alvarez-Suarez et al., 2010; Baltrusaityte et al., 2007). Among these components, polyphenols, mainly flavonoids and phenolic acids, are regarded as the major responsible for a wide range of biochemical activities, including the direct or indirect scavenging of free radicals by inhibiting the reactive oxygen species (ROS) generation or chelating metals, respectively (Chimi et al., 1991; Shahidi and Wanasundara, 1992). These natural antioxidants highlight the role of honey, along with fruits and vegetables, as a valuable nutritional source provided with protective and therapeutic potential on human health, such as cardioprotective, anti-carcinogenic, immune-stimulant and anti-inflammatory effects (Schramm et al., 2003; Blasa et al., 2007).

The major antimicrobial properties of honey are related to hydrogen peroxide whose concentration is determined by relative levels of glucose oxidase, synthesised by the honeybee, and catalase, originating by flower pollen (Weston, 2000). The non-peroxide factors contributing to honey antimicrobial activity are mainly lysozyme, phenolic acids and flavonoids (Snowdon and Cliver, 1996). All of these factors give honey unique properties as a wound dressing: it leads to rapid clearance of infections, rapid debridement of wounds, rapid suppression of inflammation, minimization of scarring, and stimulation of angiogenesis as well as tissue granulation and epithelium growth (Molan, 2002).

The Sicilian black honeybee (Apis mellifera ssp. sicula) is a subspecies of the more common honeybees (Apis mellifera ssp. ligustica) from which it differs by its darker color and smaller wings (Franck et al., 2000). The black honeybee, which had existed in Sicily for thousands of years, began to disappear in the 1970s and 1980s when Sicilian beekeepers ceased using their cane hives and began to import from northern Italy the subspecies ligustica, considered more docile and productive. At this time the black honeybee risked total extinction, which was avoided only thanks to the research of some Sicilian entomologists who took several hives of black honeybees to the Aeolian Islands of Vulcano, Alicudi and Filicudi, off the north east coast of Sicily, where they could breed in isolation without the risk of contamination by other honeybees. Today, the honey produced by these honeybees is the only Sicilian honey produced entirely by the black honeybee (www.slowfood.org). The black honeybee has African origins, but it differs from the African honeybees due to its high docility and productivity. It even tolerates temperatures above 40 °C, to which the other honeybee subspecies. In addition, the black honeybees stop producing, and it consumes less honey in the hive (Franck et al., 2000). The black honeybee, which had existed in Sicily for thousands of years, began to disappear in the 1970s and 1980s when Sicilian beekeepers ceased using their cane hives and began to import from northern Italy the subspecies ligustica, considered more docile and productive. At this time the black honeybee risked total extinction, which was avoided only thanks to the research of some Sicilian entomologists who took several hives of black honeybees to the Aeolian Islands of Vulcano, Alicudi and Filicudi, off the north east coast of Sicily, where they could breed in isolation without the risk of contamination by other honeybees. Today, the honey produced by these honeybees is the only Sicilian honey produced entirely by the black honeybee (www.slowfood.org). The black honeybee has African origins, but it differs from the African honeybees due to its high docility and productivity. It even tolerates temperatures above 40 °C, to which the other honeybee subspecies. In addition, the black

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Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; QE, quercetin equivalents; ROS, reactive oxygen species; TE, trolox equivalents; TPTZ, 2,4,6-tripyridyl-s-triazine.

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honeybee has very marked ability of pollination ensuring the persistence of many species of fruits and vegetables, some of which are endangered of extinction, as the case of the Japanese medlar. Interestingly, the black honeybee shows, in common with African honeybees, a very high physical resistance for which it differs from the other subspecies, generally characterised by a typical immunological weakness (Franck et al., 2000). For this reason, the black honeybee is part of a project aimed to stop the current massive honeybee die-offs (www.epa.gov; www.slowfood.org).

The aim of this work was to evaluate the antioxidant profile and antimicrobial properties of some Sicilian honeys produced in purity by the local black honeybees in consideration of the total lack of scientific studies on these products.

2. Materials and methods

2.1. Reagents and standards

All chemicals and reagents used were either analytical-reagent or HPLC grade. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA) before use. The reagents employed for the antioxidant activity tests and the phenolic compounds used for the identification and quantification of phenolic acids and flavonoids in honey samples were purchased from Sigma Chemical Co., (St. Louis, MO, USA) and were: DPPH (1,1-diphenyl-2-picrilhydrazyl), 2,4,6-tris(2-pyridyl)-1,3,5-triazine, ferric chloride (III), tri-(potassium) tetrakis (4-chlorophenyl) borate, potassium permanganate, acetonitrile, acetic acid, trifluoroacetic acid, mannitol, quercetin, kaempferol, chrysin, pinocembrin. Methyl alcohol (RPE) was purchased from Carlo Erba (Milano, Italy).

2.2. Honey samples

Honey samples were harvested in 2011 by individual apiarists in the area of Termini Imerese (37°38' north, 13°70' east, Palermo, Italy). Samples were classified following the melissopalynological analysis (Louveaux et al., 1978) according to the floral source was confirmed if the pollen content in the honeys was not lower than 10% (this percentage ranged between 32% and 56%). During the experiments, samples were kept at 5°C in the dark in airtight containers for less than 15 months until the analysis.

2.3. Polyphenolic extracts

Fifty grams of Amberlite XAD-2 resin (pore size 9 mm; particle size 0.3–1.2 mm; Supelco, Bellefonte, PA, USA) were soaked in methanol, stirred for 10 min and then packed into a glass column (50 × 2 cm). Honey samples (100 g) were mixed with 500 mL of distilled water and adjusted to pH 2 with HCl. The solution was slowly filtered through the column packed as previously described. The column was washed with 500 mL of acidified water (pH 2) and 300 mL of deionised water for sugar and other honey polar compound removal. The absorbed phenolic compounds were extracted from the resin by elution with 500 mL of methanol, which was evaporated by reduced pressure (Rotavapor Ika, Staufen, Germany). The residues were dissolved in a little volume of water and extracted three times with 30 mL of diethyl ether. The extracts were combined and the solvent was removed by flushing with nitrogen. Part of the residues were re-dissolved either with methanol for antioxidant tests and HPLC analysis or with DMSO (dimethylsulfoxide) for antimicrobial activity assays. Samples were previously filtered through a 0.20 μm syringe PTFE filters (Anoto®; Whatman International Ltd., Kent, UK).

2.4. Total phenolic content

The concentration of total phenolics was measured by the method described by Singleton and Rossi (1965), with some modifications. Briefly, an aliquot (20 μL) of honey extracts and calibration solutions of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to a 25 volumetric flask containing 9 mL of ultrapure water (ddH2O). A reagent blank using ddH2O was prepared. One mL of Folin & Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 min, 1 mL of Na2CO3 aqueous solution (10 g/100 mL) was added. At zero time, 0.15 mL NaNO2 aqueous solution (5 g/100 mL) was added to the flask. After 5 min, 0.15 mL AlCl3 aqueous solution (10 g/100 mL) was added. At 6 min, 1 mL NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with 1.2 mL of ddH2O and thoroughly mixed. Absorbance of the mixture, in pink colour, was determined at 510 nm versus prepared water blank using a Jasco V-530 UV–vis spectrophotometer (Tokyo, Japan). Total flavonol content was expressed as mg quercetin equivalents (QE)/100 g honey.

2.5. Total flavonol content

The total flavonol content was measured by a colorimetric assay developed by Zhishen et al. (1999). A 50 μL aliquot of honey extracts and calibration solutions of quercetin (20, 40, 60, 80 and 100 mg/L) was added to a five volumetric flask containing 2 mL of ddH2O. At zero time, 0.15 mL NaNO2 aqueous solution (5 g/100 mL) was added to the flask. After 5 min, 0.15 mL AlCl3 aqueous solution (10 g/100 mL) was added. At 6 min, 1 mL NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 1.2 mL of ddH2O and thoroughly mixed. Absorbance of the mixture, in pink colour, was determined at 510 nm versus prepared water blank using a Jasco V-530 UV–vis spectrophotometer (Tokyo, Japan).

2.6. Antioxidant activity

For each antioxidant assay, a trolox aliquot was used to develop a 50–500 μM L standard curve. All data were then expressed as Trolox Equivalents (μmol TE/100 g honey).

2.6.1. DPPH radical-scavenging assay

The ability of the samples to scavenge the DPPH radical was measured using the method of Brand-Williams et al. (1995). Aliquots (20 μL) of honey extracts were added to 3 mL of DPPH solution (6 × 10−5 mol/L) and the absorbance was determined at 515 nm every 5 min until the steady state using a Jasco V-530 UV–vis spectrophotometer (Tokyo, Japan).

2.6.2. Reducing potential assay

The antioxidant potential of the samples was determined using the ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996). A solution of 10 mmol/L TPTZ in 40 mmol/L HCl and 12 mmol/L ferric chloride was diluted in 300 mL sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Aliquots (20 μL) of honey extracts were added to 3 mL of the FRAP solution and the absorbance was determined at 593 nm every 5 min until the steady state using a Jasco V-530 UV–vis spectrophotometer (Tokyo, Japan).

2.7. HPLC quantification of phenolic acids and flavonoids

HPLC separation of phenolic acids and flavonoids from extracts was performed according to earlier studies with some modifications (Biesaga and Pyrzynska, 2009). Identification was possible by recording chromatograms at 280 and 350 nm and by comparing spectra and retention times with those of commercial standards and with those reported in previous works (Biesaga and Pyrzynska, 2009). Elution conditions consisted in 0.5% formic acid in water (Solvent A) and methanol (Solvent B) gradient at a flow rate of 0.6 mL/min. The column selected was a C-18 Zorbax (150 mm × 4.6 mm, 5 μm packing; Agilent, USA) coupled to a PE-Sciex API-2000 triple-quadrupole mass spectrometer (Warrington, Cheshire, UK) equipped with a Turbospray (TSI) source. MS detection was carried out in negative ion mode at unit resolution using a mass range of 150–1500 m/z and a mass peak width of 0.7 ± 0.1. Selected ion monitoring (SIM) experiments were carried out using the following operational parameters: vapouriser, 350 °C; heated capillary, 150–200 °C; carrier gas, nitrogen, at a sheath pressure of 70 psi; auxiliary gas, nitrogen, to assist in nebulization, at a pressure of 30 psi; declustering potential, 44.0 eV; focusing potential, 340.0 eV; entrance potential, 10.0 eV; collision energy, 33.0 eV for ion decomposition in the collision cell at 0.8 mTorr.

2.8. Antimicrobial activity

2.8.1. Microbial strains

The in vitro antimicrobial activity of honey samples and their polyphenolic extracts was evaluated against yeasts, moulds and bacterial species known to be bone pathogens, to cause respiratory, gastrointestinal, skin and urinary disorders.

The panel included laboratory control strains obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA): four Gram-positive bacteria, Bacillus cereus (ATCC 11778), Staphylococcus aureus (ATCC 13709), Enterococcus faecalis (ATCC 14428), Listeria monocytogenes (ATCC 15313); nine Gram-negative bacteria, Escherichia coli (ATCC 25922), Proteus mirabilis (ATCC 7002), Proteus vulgaris (ATCC 12454), Pseudomonas aeruginosa (ATCC 27853), Salmonella typhi Ty2 (ATCC 19430), Yersinia enterocolitica (ATCC 23715), Klebsiella pneumoniae (ATCC 27736), Enterobacter cloacae (ATCC 10509), Enterobacter aerogenes (ATCC 13048); two yeasts, Candida albicans (ATCC 10231), Rhizoctonia solani (ATCC 13048); four moulds: Fusarium oxysporum...
The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) using the broth dilution method (Barry, 1976). Each strain was tested with sample that was serially diluted in broth to obtain concentrations ranging from 300 to 0.8 μg/mL. The sample, previously sterilised with Millipore filter of 0.20 μm, was inoculated with 50 μL of suspension of the tested microorganisms, containing 2.0 × 10^6 CFU/mL for bacteria and 2.0 × 10^4 CFU/mL for fungal strains, and incubated for 24 h at 37 °C for bacteria, 48 h at 30 °C for yeasts and 10 days at room temperature for moulds. The MIC value was determined as the lowest concentration of the sample at which the tested microorganisms did not demonstrate any visible growth after incubation. As positive control cultures containing only sterile physiologic solution Tris buffer were used. Cefotaxime, Pentamidine, Tetracycline, Amphotericin B and Econasol were used as standard antimicrobial agents.

### 3. Results and discussion

#### 3.1. Polyphenolic composition and antioxidant capacity

The results obtained for the polyphenolic composition of orange and lemon honeys (Fig. 1) were generally higher than those reported elsewhere for the same variety of honeys produced by other honeybee subspecies from Sicily (Pichichero et al., 2009), other Italian regions (Truchado et al., 2009) and abroad (Escrice et al., 2011; Isla et al., 2011). It is established that honey polyphenolic composition and antioxidant capacity mostly depend on their floral sources that predominantly are affected by environmental and climatic conditions (Al-Mamary et al., 2002). Particularly, regions characterised by a hot, humid climate, with very high levels of exposure to sunlight, are known to exert a marked influence on the polyphenolic content of plants, so that sun-exposed plants can contain much more total phenolics than the same varieties growing in the shade (Spayd et al., 2002). Nevertheless, our orange and lemon honeys showed (Fig. 1) not only a higher antioxidant concentration than the corresponding varieties from regions characterised by comparable climatic conditions to those of Sicily (Escrice et al., 2011; Isla et al., 2011), but, interestingly, almost 10 times more total phenolics than the same Sicilian varieties (Pichichero et al., 2009). No studies are currently available on the chemical composition and antioxidant profile of prickly pear, medial and almond honeys in general. Our results highlighted for these samples a considerable polyphenolic content that was about 27%, 70% and 56% higher, respectively, than that of orange and lemon honey samples (Fig. 1). Interestingly, comparing our data with those reported for different types of fruits and vegetables widely recognised as a dietary source of antioxidants (Marinova et al., 2005), we found that the polyphenolic contents in the honey samples were approximately from 2 to 10 times lower than those in blueberry (phenols, 670.9 mg/100 g; flavonoids, 190.3 mg/100 g) and black grape (phenols, 213.4 mg/100 g; flavonoids, 77.1 mg/100 g), respectively, and from 1 to 9 times higher than those in leek (phenols, 27.7 mg/100 g; flavonoids, 2.6 mg/100 g) and green bean (phenols, 35.5 mg/100 g; flavonoids, 4.1 mg/100 g), respectively. These results highlight the role that honey could play in providing dietary antioxidants in a highly palatable form. The averages of total phenol and flavonoid contents of honey samples were significantly different at a level of P = 0.001.

Owing to the complex reactivity of phytochemicals, the antioxidant activities of food and food extracts cannot be evaluated by only a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (Schlesier et al., 2002). For this reason, the antioxidant activity of honey samples was determined by two spectrophotometric methods, DPPH and FRAP tests, and expressed as trolox equivalents (TEs). The reduction of DPPH absorption is indicative of the capacity of the samples to scavenge free radicals, while the FRAP method is used to determine the capacity of reducing agents in a sample. Antioxidant activity at the steady state resulted slightly higher in FRAP test (ranging from 0.021 to 0.058 mmol TE/100 g) than in DPPH test (ranging from 0.013 to 0.046 mmol TE/100 g) for all of the honey samples (Table 1). Medlar honey showed the highest antioxidant capacity in both assays, while lemon honey demonstrated to be the least active. Polyphenolic compounds are reported to have a high radical scavenging capacity, particularly those showing O-dihydruxy structures that confer great stability to the radical form and participate in the electron delocalization (Francisco et al., 2009). Thus, our result was of great interest because it highlighted that polyphenols occurring in the honey samples were preferentially involved in electron-transfer reactions rather than hydrogen atom-transfer mechanisms. Results revealed for the honey samples a good antioxidant activity when compared with that of authentic standards chosen as widely employed food preservatives and strong hydrophilic or lipophilic antioxidants (Table 1). It is accepted that flavonoids and their metabolites, thanks to their both hydrophilic and relatively lipophilic properties, may interact with plasma proteins as well as the polar surface region of phospholipid bilayers in lipoproteins and cell membranes (Alvarez-Suarez et al., 2012; Blasa et al., 2007). Because of the nature of these interactions, flavonoids may have the ability to protect against free radical attack in both aqueous and lipid environments, thus providing an effective antioxidant defense in biological systems. The averages of total antioxidant activities of honey samples were different at a significance level of P < 0.05.

In addition, the contribution given by each class of polyphenolic compounds to the antioxidant activities of honeys was estimated. Our results revealed a good linear correlation (R) between antioxidant capacity determined by both assays, DPPH and FRAP, and total phenols (R ranging from 0.9726 to 0.9826) and flavonoids (R ranging from 0.9396 to 0.9700) of all of the tested samples.

#### 3.2. Polyphenolic profiles

All of the honey samples showed very similar but quantitatively different polyphenolic patterns (Table 2). Although more than 16 chromatographic distinct peaks were detected for each honey sample, some were present only in trace amounts, thus making their identification and quantification difficult. Their identification was based on MS experiments, UV–Vis absorption spectra, and chromatographic retention times, which were compared with reference compounds and data from other studies (Biesaga and Pyrzynska, 2009).

Phenolic acids represented approximately 83% of total phenolic content in all of the honey samples. Gallic acid was found as the most abundant antioxidant (ranging from 46.8% in lemon to 70.7% in medlar) with the exception of prickly pear whose main representative was ferulic acid (37.5%), followed by sinapic acid (19.5%). These compounds may be regarded as potential markers of the origin of honey. In the strawberry tree honey, Cabras et al. (1999) found that homogentisic acid varied 19.7–54.0 mg/100 g honey, with an average of 37.8 mg/100 g. This was the high-
lowest level of single phenolic acid reported in honey so far, and this level was higher than the level of total phenolic acids found for any floral type of honey examined by Cabras et al. (1999). In the light of our results, current knowledge is to be updated as regards the gallic acid contents in the Sicilian medlar and almond honeys produced by the local black honeybees (Table 2).

The highest amount of flavonoids was detected in medlar, with quercetin (24.1%) dominating such profile. Generally, quercetin and kaempferol are the most widespread flavonoids in food and in natural honeys (Socha et al., 2011). Nevertheless, our data showed, except for medlar, moderate concentrations of these two compounds in the tested honeys (quercetin, ranging from 1.08 mg/100 g in orange to 2.35 mg/100 g in almond; kaempferol, ranging from 0.32 mg/100 g in lemon to 2.00 mg/100 g in almond) (Table 2). Our results confirmed that naringenin and hesperetin are specific markers for the floral origin of citrus honeys, especially hesperetin that has not been detected in honey of any other floral origin and is a constitutive phenolic compound of citrus nectar (Escriche et al., 2011). Rutin, on the contrary, was revealed as a minor component in lemon and orange honeys, while it appeared significantly in the rest of the analysed samples (representing 59.5%, 17.2% and 10.5% of the flavonoid profile in prickly pear, medlar and almond, respectively) (Table 2). Knekt et al. (2002) carried out a clinical study on how some chronic diseases may be lower at higher dietary flavonoid intakes. Particularly, men with higher myricetin intakes and 12 times, respectively, the quantity of myricetin correlated by Knekt et al. (2002) to a significantly lower cancer risk in the human subjects. The differences between the means of polyphenolic compounds were considered significantly different at a level of $P < 0.05$.

The correlation of the individual polyphenolic contents with the antioxidant properties was also measured. In agreement with literature (Socha et al., 2011), gallic acid seemed to largely influence both assays, DPPH and FRAP ($R$ ranging from 0.8686 to 0.8709), as expected for the predominant phenolic acid and the one with the highest number of hydroxy groups. Ferulic and sinapic acids, in fact, although their significant amount in most samples, exhibited quite low correlations ($R$ mean value 0.0571). Interestingly, syringic acid revealed to poorly influence FRAP assay ($R = 0.0718$), while its good correlation with DPPH test ($R = 0.8779$) highlighted a higher radical-scavenging ability than reducing capacity. Among flavonoids, mainly quercetin, luteolin, kaempferol and chrisin seemed to contribute to total antioxidant activity ($R$ mean value 0.9151) while the other compounds gave lower correlations ($R$ mean value 0.5333).

### 3.3. Antimicrobial activity of honeys and their polyphenolic extracts

According to the MIC values reported in Table 3, honey samples exerted a broad antimicrobial spectrum by inhibiting the growth of all of the tested human pathogenic and/or food spoilage bacteria, moulds and yeasts. As regards citrus honeys, our results were in agreement with those of Isla et al. (2011), while nothing is reported on the other samples that exhibited higher values than those of lemon and orange ones. It is accepted that hydrogen peroxide, among the many honey constituents, is one of the major responsible for the honey antimicrobial activity (Weston, 2000). Actually, our results indicated the polyphenolic content as another important factor contributing to the honey antimicrobial properties. Infact, a significant correlation coefficient was observed between the antimicrobial activities and polyphenolic content of all of the tested samples ($R$ mean value 0.8544). In addition, almond and medlar honeys, characterised by the highest polyphenolic contents among the tested samples (Fig. 1), showed up to 2 and 3 times higher antimicrobial effects, respectively, than the other samples. The means of honey MIC values were different at a significant level of $P < 0.05$.

The antimicrobial capacity of phenolic compounds, in a general way, is well known (Pereira et al., 2006; Rauha et al., 2000). As previously described, individual phenolic compounds occurring in honey extracts were identified and quantified, but we chose to

### Table 1

Near equilibrium steady state antioxidant capacity of honey polyphenolic extracts.

<table>
<thead>
<tr>
<th>Honey sample</th>
<th>Assay method</th>
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<tbody>
<tr>
<td></td>
<td>FRAP</td>
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<tr>
<td>Lemon</td>
<td>0.026 ± 0.4</td>
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<tr>
<td>Orange</td>
<td>0.021 ± 0.3</td>
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<tr>
<td>Prickly pear</td>
<td>0.032 ± 0.3</td>
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<tr>
<td>Medlar</td>
<td>0.058 ± 0.4</td>
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<tr>
<td>Almond</td>
<td>0.037 ± 0.9</td>
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<tr>
<td>Vit. E</td>
<td>0.92 ± 0.3</td>
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<tr>
<td>Vit. C</td>
<td>0.32 ± 0.4</td>
</tr>
<tr>
<td>BHT</td>
<td>0.74 ± 0.0</td>
</tr>
<tr>
<td>Na2S2O5</td>
<td>0.53 ± 0.8</td>
</tr>
</tbody>
</table>

*Values are expressed as mmol TE ± SD per 100 g honey samples and per 100 mL standard solutions (1 mg/mL) at the steady state (DPPH, 45 min; FRAP, 55 min). The differences between the means were considered significant at a level of $P < 0.05$. 

submit the entire extracts to the antimicrobial activity studies. In fact, total food extracts may be more beneficial than isolated constituents, since a bioactive individual component can change its properties in the presence of other compounds occurring in the extract (Borchers et al., 2004), corresponding to a synergistic effect. The polyphenolic extracts from lemon and almond honeys exhibited a slightly higher antimicrobial effect than the other samples (Tables 3 and 4), corroborating what already reported on the antibacterial properties of food and food polyphenolic extracts (Kossah et al., 2011; Estevinho et al., 2008). It is well known that phenolic acids, representing most of the antioxidants occurring in our honey samples, are too polar compounds to penetrate the semipermeable bacterial membrane and react with the cytoplasm or cellular proteins (Corrales et al., 2008). 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common than the antibacterial one generally expressed by food and food polyphenolic extracts. In addition, it must be pointed out that honeys and their fractions were tested on several microbial strains among which some bacteria, moulds and yeasts, were taken into account for the first time in this work.

### 4. Conclusions

In conclusion, all the analysed Sicilian honeys produced by the local black honeybees demonstrated valuable biological profiles that, in comparison with the same or different honey varieties from Sicily, other Italian regions and the rest of the world, make them products with high therapeutic potential as antioxidants and anti-microbial agents against multi-resistant strains.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

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### References


