Food and Nutrition Sciences, 2014, 5, 2053-2062
Published Online November 2014 in SciRes. http://www.scirp.org/journal/fns
http://dx.doi.org/10.4236/fns.2014.521217

Polyphenol Composition, Antioxidant, Antimicrobial and Quorum Quenching Activity of the "Carciofo di Montoro" (*Cynara cardunculus* var. *scolymus*) Global Artichoke of the Campania Region, Southern Italy

Florinda Fratianni[1], Rosa Pepe[2], Filomena Nazzaro[1*]

[1]Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche (ISA-CNR), Roma, Italy
[2]Experimental Institute for Vegetable Crops—CRA, Pontecagnano, Italy
Email: fratianni@isa.cnr.it, rosa.pepe@entecra.it, *mena@isa.cnr.it

Received 26 August 2014; revised 25 September 2014; accepted 15 October 2014

Abstract

Biochemical characteristics, antimicrobial and quorum quenching activity of the extract of the "Carciofo di Montoro", a typical ecotype of *Cynara cardunculus* var. *scolymus* of the Campania region (Southern Italy) were studied, to consider it as potential reserve of bioactive constituents useful for food industry and beneficial for managing and preventing several chronic illnesses in humans. The extract exhibited a good polyphenol content (528 μg GAE/g) and antioxidant activity (EC_{50} less than 5 mg). Ultra pressure liquid chromatography (UPLC) revealed high amount of chlorogenic acid, cynarin and epicatechin. The extract showed antimicrobial activity against *Escherichia coli*, *Staphylocccus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Bacillus cereus* pathogen strains. Finally, quorum quenching activity was demonstrated. The variety Carciofo di Montoro could represent a good source of health-promoting polyphenols, encouraging a nutraceutical use of such ecotype, for several phyto-pharmaceutical applications.

Keywords

Artichoke, Polyphenols, Antioxidant, Antimicrobial, Quorum Sensing

*Corresponding author.

How to cite this paper: Fratianni, F., Pepe, R. and Nazzaro, F. (2014) Polyphenol Composition, Antioxidant, Antimicrobial and Quorum Quenching Activity of the "Carciofo di Montoro" (*Cynara cardunculus* var. *scolymus*) Global Artichoke of the Campania Region, Southern Italy. *Food and Nutrition Sciences*, **5**, 2053-2062. http://dx.doi.org/10.4236/fns.2014.521217

1. Introduction

Polyphenols and antioxidants, present in plant-based foods, offer several health benefits further than basic nutrition and are positively implicated in the prevention of chronic diseases. Many studies discovered several interesting biological properties, such as anti-inflammatory, antioxidant, antimutagenic, antiviral, antimicrobial and quorum quenching activities [1]-[3], and many of them are also used in the preservation of food [4]. Globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori), belonging to the family of *Asteraceae* (*Compositae*), is an herbaceous perennial crop, widely cultivated in the Mediterranean area. Heads, represented by the large immature inflorescences with edible fleshy leaves (bracts) and receptacle, are used worldwide and represent a basic element of the Mediterranean diet. Leaves are used as herbal medicine and are appreciated for their beneficial and therapeutic effects, including promotion of blood circulation, mobilization of energy reserves, induction of choleresis, inhibition of cholesterol biosynthesis, hepatoprotective effects and LDL oxidation, as well as antibacterial, antifungal and antioxidant activities [5] [6]. Many studies converged on the artichoke health and antioxidant properties, assuming that these actions could be strictly related to the polyphenolic fraction, mainly composed of mono- and dicaffeoylquinic acids, and flavonoids. Such properties are consistent with the well-known double role of phenolic compounds as antioxidants and as substrates for oxidative browning reactions, primarily in the presence of high iron concentrations [7]. Chemical activity of polyphenols in terms of their reducing properties, as hydrogen or electron-donating agents, predicts their potential effect as free-radical scavengers. In Italy, artichoke is considered the most important horticultural crop together with tomato and potato, with a surface of about 33,296 ha and a production of about 372,378 tons in the year 2013, mainly giving rise from Apulia, Sicily, Campania, Lazio and Tuscany [8]. Italy is also the richest source of artichoke genetic resource, with numerous local varieties; these last, in the course of the centuries, were capable to adapt themselves to the different environments, and which now can differ in chemical composition, especially of the polyphenolic fraction, hence revealing diverse nutraceutical and pharmacological properties. Many ecotypes of artichoke present in the Campania region of Southern Italy reveal excellent results in terms of polyphenols content and antioxidant activity [9]. The ecotype "Carciofo of Montoro", cultivated mainly in the province of Avellino, is a product with exceptional organoleptic characteristics. The techniques of cultivation for such species include the transplanting of the plant, recurrent irrigations and a very low use of synthetic chemicals. A particular aspect of the cultivation of this ecotype is the practice of covering its little heads with a terracotta cup to defend them from the damaging chill (**Figure 1**). The traditional Campania vegetal panorama is well recognized as

Figure 1. A typical "Carciofo di Montoro" artichoke.

environment characterized by a rich genetic biodiversity. The protection of such vegetables, as well as the study of their biochemical and nutritional aspects is an essential instrument also to protect the local economy. Such aspects are of noticeable significance, taking into account that the actual trend of the global food market is devoted to its standardization, with a concurrent drastic decrease in the number of traditional species and varieties (often with high functional properties) and a dramatic decline of the genetic variability. Vegetables belonging to the family *Asteraceae* represent an important font of natural antioxidants with high capability to manage against oxidative stress and, thus, with high potential to act as strong anticancer as well as anti-degenerative foods. Furthermore, they have been recently also recognized as natural antimicrobial compounds capable to reduce outbreaks of food-borne and human pathogenic microorganisms [10] [11]. The well-known capability of these phytochemicals to contribute for the maintenance of health and for the protection against heart disease and cancer [12], as well as to act against microbial attacks, is also raising interest among scientists and food manufacturers to identify foods, also among autochthonous ecotypes, with specific health effects for consumers. Therefore, the aim of our work was to study the polyphenols composition, the antioxidant activity and antimicrobial potential of the extract from the "Carciofo di Montoro" globe artichoke, to evaluate the possibility to consider this ecotype as reserve of bioactive constituents and as a resource, due to its antimicrobial and quorum quenching properties, for food preservation and human health.

2. Materials and Methods

2.1. Standards and Reagents

Luteolin was provided from Extrasynthese (Genay, France). All other standards were obtained from Sigma (Milan, Italy). Acetoni-trile and trifluoroacetic acid were obtained from Carlo Erba Reagenti (Milan, Italy). Acetone, methanol, ethanol and ethyl acetate were purchased from Sigma (Milan, Italy). All reagents were of analytical grade.

2.2. Extraction of Polyphenols

Samples of artichoke "Carciofo di Montoro" were obtained from the experimental fields of CRA, located in Montoro (AV), Italy. Polyphenols were extracted following the method of Fratianni *et al.* [9] with some modifications. Briefly, bracts were subjected to a two-time extractive process, the first one to allow the extraction mainly of phenolic acids, the other to permit the extraction of flavonoids. Samples of frozen bracts were incubated for 1 h at 4˚C in 3 volumes of acetone:ethanol:meth-anol (70:15:15). After the recovery of the first supernatant, the residues of bracts were treated with ethyl acetate (1:3 w/vol) and kept at 4˚C for 1 hour. The two supernatants were separately dried under air flow, re-dissolved in ethanol, pooled and stored at −30˚C in the dark until analysis was performed.

2.3. Colorimetric Analysis of Polyphenols

Total polyphenols were determined following the method of Singleton and Rossi [13] using the Folin-Ciocalteu reagent. The absorbance was determined at room temperature at λ = 760 nm using a Cary Uv/Vis spectrophotometer (Varian, Palo Alto, CA, USA). Quantification was based on a standard curve generated with gallic acid. Results were expressed as micrograms gallic acid equivalent (GAE)/g of fresh weight product ± Standard Deviation (SD).

2.4. Free Radical Scavenging Capacity

The free radical scavenging capability of the extract was determined using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [14]. The analysis was performed in microplates by adding 7.5 µL of extract to 303 µL of a methanol DPPH solution (153 mM). Next, the absorbance at λ = 517 nm was measured (Cary 50 MPR, Varian, Palo Alto, USA). Absorbance of DPPH without antioxidant (control sample) was used for baseline measurement. Scavenging activity was expressed as the 50% effective concentration (EC_{50}), which is defined as the sample concentration (mg) necessary to inhibit the 1 mL DPPH radical activity by 50% during a 60 min incubation. Ascorbic acid (Fluka Buchs, Switzerland) was dissolved in methanol. The solution was used for a calibration curve of DPPH reduction and as a chemical reference in comparison to the antioxidant capacity of the extracts. These experiments were performed in triplicate, and the results are expressed as the mean values ±

standard.

2.5. Chromatographic Analysis

All standards utilized in the experiments were accurately weighed, dissolved in methanol, treated with ultrasonics for 10 min and filtered (0.45 μm, Waters, Milford, MA, USA). The calibration curves were generated with concentrations ranging from 0.001 to 0.5 mM of chlorogenic acid (5-O-caffeoyl-D-quinic acid), p-coumaric acid (p-hydroxycinnamic acid), ferulic acid, gallic acid, epicatechin, apigenin, luteolin, cynarin (1-3-dicaffeolylquinic acid), and rutin. The extracts of the globe artichoke Carciofo di Montoro (previously dissolved in methanol) were filtered (0.45 μm, Waters, Milford, MA, USA) before analysis. The analysis of polyphenols was performed by using an ACQUITY^{TM} ultra performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) linked to a PDA 2996 Photodiode Array Detector (Waters). Empower software (Waters) was used to control the instruments and for data acquisition and processing. Analysis was carried out at 30˚C using a reversed phase column (BEH C18, 1.7 μm, 2.1 × 100 mm, Waters) [15]. Mobile phase consisted of solvent A (7.5 mM acetic acid) and solvent B (acetonitrile) at a flow rate of 250 $\mu L \cdot min^{-1}$. Gradient elution was employed, starting with 5% B for 0.8 min, then 5% - 20% B over 5.2 min, isocratic 20% B for 0.5 min, 20% - 30% B for 1 min, isocratic 30% B for 0.2 min, 30% - 50% B over 2.3 min, 50% - 100% B over 1 min, isocratic 100% B for 1 min, and finally 100% - 5% B over 0.5 min. At the end of this sequence, the column was equilibrated under the initial conditions for 2.5 min. Pressure ranged from 6000 to 8000 psi during the chromatographic run. The effluent was introduced into an LC detector (scanning range: 210 - 400 nm, resolution: 1.2 nm). The injection volume was 5 μL.

2.6. Antimicrobial Assays

To screen the antimicrobial activity, a filter paper disc method was used [16]. The bacteria used in this study included Gram-positive *Bacillus cereus* (strains DSM 4313 and DSM 4384), *Enterococcus faecalis*, and *Staphylococcus aureus* DSM 25923 and Gram-negative *Escherichia coli* DSM 8579 and *Pseudomonas aeruginosa* ATCC 50071 strains. All strains were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). The strains were incubated in a Nutrient broth (Oxoid) at 37˚C for 18 h. The optical densities of all cultures were adjusted to match a 0.5 McFarland standard of 1×10^8 colony forming units (cfu)/mL; then 2.625 mg - 5.25 mg and 10.5 mg of the extracts were added to sterile filter paper discs (5 mm) previously placed in Nutrient agar Petri dishes inoculated with the above mentioned pathogen strains. A disc treated with Dimethyl sulfoxide (DMSO, Sigma) alone was used as negative control; tetracycline (7 μg/disc; Sigma) served as positive control. Plates were left for 30 min at room temperature under sterile conditions and then incubated at 37˚C for 24 h, and the inhibition halo around the disc was measured. The experiments were performed in triplicate and averaged. All of the experiments were carried out in triplicate. The results are expressed as means ± standard deviation. Means followed by different letters in each column differ significantly to Dunnett's multiple comparisons test, at the significance level of $p < 0.05$.

2.7. Quorum Sensing Activity

The *Chromobacterium violaceum* quorum sensing system was used for this assay. Quorum sensing (QS) in this wild-type strain of bacterium controls the production of the purple pigment violacein [3] in response to autoinducer molecules, such as C6-acyl and C4-acyl homoserine lactones. The disc diffusion method was employed to detect the anti-QS activity of the extract. In this test, bacterial growth inhibition would result in a clear halo around the disc, whereas a positive result of quorum sensing inhibition (quorum quenching activity) would result in a turbid halo harbouring the pigmentless bacterial cells of *C. violaceum* DSM 30191 (purchased from DSMZ). The test strain was incubated in Lab Lemco broth (Oxoid, Milano, Italy) for 16 - 18 h at 26˚C. The culture was adjusted to the 0.5 McFarland standard (1×10^8 CFU /mL). Different doses of the extract, prepared as described above, were added to *C. violaceum* inoculated Lab Lemco agar plates (0.1 mL/plate), followed by incubation at 26˚C for 24 - 48 h.

2.8. Statistical Analysis

All of the experiments were carried out in triplicate. The results are expressed as means ± standard deviation.

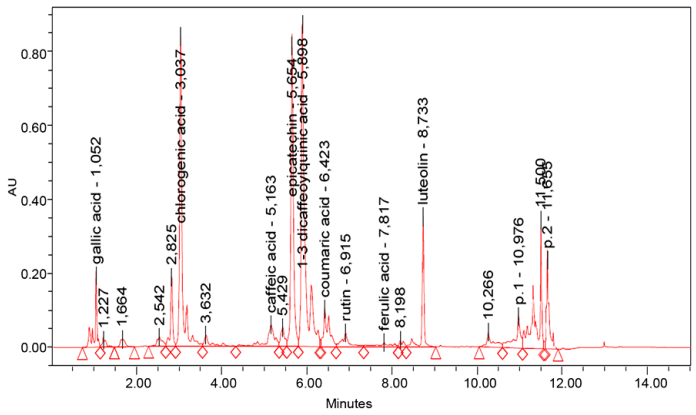
3. Results and Discussion

3.1. Total Polyphenols and Antioxidant Activity

Total polyphenols and the antioxidant activity of the globe artichoke variety Montoro are shown in **Table 1**. The amount of polyphenols was 525 GAEμg/g of product. This result are in agreement with the range observed for other cultivars, such as Green Globe or Violetto di Toscana [7], and higher than other typical Italian artichokes, such as Violetto di Sicilia, Violetto di Provenza, and Tema [17] indicating that "Carciofo di Montoro" could represent another important source of phytochemicals, capable to exhibit therapeutic activity [18]. The content of polyphenols present in such ecotype affected, with all probability, also its antioxidant activity: in fact, the amount of extract needed to inhibit 1 ml of DPPH at 50% was just 4.24 mg, more effective than other artichokes studied, for example, by Velez *et al.* (EC_{50} = 9.16 mg) [19] or Menghini (EC_{50} of about 15.95 mg) [20], Ferracane *et al.* [21] observed that the cooking process of artichokes in boiling water increased their antioxidant content and activity with respect to raw artichokes, independently of the assay used, stating that the spatial arrangement of the phenolic groups can deeply affect the antioxidant activity of the molecules. Thus, we could hypothesize that the antioxidant activity of the Carciofo di Montoro might increase as cooking process is improving their extraction out of the plant tissue. Thus, a wider utilization of this typical variety Montoro should be strongly encouraged both for the fresh market and for the food industry, in view of its capability to supply a high level of these important biomolecules.

3.2. Chromatographic Analysis

Chromatographic analysis, herein performed for the first time on this typical Italian globe artichoke through UPLC, is shown in **Figure 2**. Panel A of the **Figure 2** reports the amount (as μg GAE/g) of the polyphenols found in the extract of the Carciofo di Montoro globe artichoke. We identified different polyphenols, basically gallic, chlorogenic, caffeic, p-coumaric, ferulic acid, 1 - 3 dicaffeoylquinic acid (cynarin), as well as epicatechin, rutin and luteolin. These results are consistent with those obtained on other *Cynara cardunculus* var. *scolymus* [7] [8]. Epicatechin, cynarin and chlorogenic acid were very abundant in the Carciofo di Montoro globe artichoke, representing the 33.1%, 32.7% and 26%, respectively. The presence of epicatechin as the most abundant polyphenol (173.79 μg GAE/g) in this ecotype is noteworthy. Epicatechin can be easily absorbed [22] and reach



Polyphenols (μgGAE/g)	
gallic acid	2,80
chlorogenic acid	136,75
caffeic acid	4,18
epicatechin	173,79
1-3 dicaffeoylquinic acid	171,58
p-coumaric acid	3,40
rutin	30,48
ferulic acid	0,50
luteolin	2,19

Panel A

Figure 2. UPLC profile of polyphenols present in Carciofo di Montoro artichoke. On the right (Panel A) is shown (as μg GAE/g of sample ± SD) the amount of each polyphenol known detecte.

Table 1. Phenolic content and antioxidant activity of the artichoke Carciofo di Montoro. Concentration of phenolic compounds are expressed as μg of gallic acid equivalents (GAE)/g sample (dry matter basis). The scavenging activity was expressed as the 50% effective concentration (EC_{50}), which was defined as the sample concentration (mg) necessary to inhibit activity of the radical DPPH by 50% after a 60-min incubation.

Polyphenol content (gGAE/g of product) ± SD	Antioxidant activity (mg EC_{50})
525.49 ± 0.11	4.24 ± 0.87

different tissues, plasma, and the gut, where it not only inhibits the growth of some pathogens, as observed by an in vitro study by Parkar *et al.* [23], but also can act as potent metal chelator and free radical scavenger, thereby significantly influencing the function of various mammalian cellular systems [24]. Flavanols like epicatechin represent an important part of the human diet and have different biological activities including antioxidant and anti-inflammatory properties. Like the other polyphenols, flavanols concur to the beneficial effects of a diet rich in fruits and vegetables and play a beneficial impact against a wide range of diseases from cardiovascular pathologies to cancer and degenerative conditions. The intake of epicatechin has been demonstrated inversely associated with coronary heart disease [25]. Carciofo di Montoro exhibited high amount of cynarin, suggesting that such ecotype could be of particular relevance from a health point of view. Cynarin, isolated from artichoke and characterized for the first time by Panizzi and Scarpati [26], has a strong effect in stimulating bile secretion and cholesterol metabolism, as well as in protecting liver [27], although its effect can be certainly reinforced (as in all fruit and vegetables provided by nature) by the concurrent presence of other active components present in the artichoke extract. Preziosi *et al.* [28] observed that a dose of 15 - 30 mg/kg of body weight of cynarin could increase the secretion of bile similarly to equimolar doses of Na-dehydrocholate (>130%), and stimulate the elimination of the biliary cholesterol; in addition, the diuretic activity could be enhanced by increasing the doses of cynarin until 100 mg/kg. Artichoke is a rich source of polyphenolic compounds, with mono- and dicaffeoylquinic acids as the major chemical components [7]. It accumulates various hydroxycinnamic acids, for which formation different mechanisms are described [29], allowing also to the production of chlorogenic acid. These biomolecules may act as an acyl donor molecule for caffeoyltransferase [30] and constitute the sckeleton of other biomolecules. The content of chlorogenic acid in the artichoke Carciofo di Montoro is also in accordance with the range of such biomolecules (0.013 mg - 1.3 mg GAE/g) observed in the unique other study performed on artichoke through UPLC [31], even if this study was performed in different chromatografic conditions. *In vivo* studies demonstrated the antioxidant and anticarcinogenic properties of chlorogenic acid [32]: it is usually poorly absorbed in the small intestine; however, the absorbed fraction of such bio-molecules enters into the blood system and has posi-tive effects on the cardiovascular system; the not absorbed fraction arrives to the colon and has biological effect on microbiome, which provides high levels of important microbial metabolites, active compounds responsible for the biological properties attributed to dietary polyphenols, such as an indirect action of cancer colon prevention [33] [34]. Therefore, both chlorogenic acid and cynarin have important effects, for instance acting in beneficial manner on the cardiovascular system and on the colon, and diminishing the risk of type II diabetes [35]. The presence of rutin, (30.48 µg GAE/g corresponding to 6% of total polyphenols, Panel A **Figure 1**) is of particular importance. Rutin is a well known flavonoid which can be metabolized to quercetin, another powerful antioxidant. The team rutin/quercetin can decrease allergic and inflammatory reactions in different parts of the body, affecting cytokine pattern, decreasing the level of Th2 cytokine, and down regulating neutrophilic inflammation; it is also capable to affect the IgE mediated mast-cell activation [36]. Rutin/quercetin may inhibit the oxidation of high-density lipo-protein (HDL) and cholesterol [37] [38], with an indirect effect in the prevention of the risk for arteriosclerosis. Rutin has also dilating effect on insulated rat aorta, and may affect the nitric oxide (NO) synthesis [39]. Flavonoids can be absorbed in the gastrointestinal tract and excreted as whole form or like metabolites. At intestinal level, microbiome splits the heterocyclic ring of flavonoids which can be subsequently be decomposed to phenolic acids; these, on the other hand, can be absorbed, conjugated, excreted or further metabolized by bacteria, and supply the body with other defense mechanisms against inflammation, allergic reactions or in the prevention of cancer. From a quantitative viewpoint, luteolin confirmed to be a minor constituent of the total polyphenols present in the extract of Carciofo di Montoro artichoke (2.19 µg GAE/g). Nevertheless, the presence of luteolin is very important, taking into account that such flavonoid has a strong antioxidant activity and, in this role, it has the capability to protect low density lipoproteins against oxidation. Screening of individual phenolic constituents of artichoke extracts revealed that luteolin is mainly responsible for the inhibition of cholesterol biosynthesis, and high luteolin concentrations are capable to efficiently block the insulin effect on cholesterol biosynthesis, as well as to improve aortic relaxation [40].

3.3. Antimicrobial Activity

The inhibition halo test, as shown in **Table 2**, indicated that the extract was capable to exhibit antimicrobial activities against all tested microorganisms. Generally, the degree of the extract activity is revealed by the size of inhibition zone that is expressed by the diameter of the referred inhibition zone. Due to the simple nature of thisassay and the reduced amount of extract required, use of such technique is generally recommended. The test,

Table 2. Antimicrobial activity exhibited by the extract of Carciofo di Montoro globe artichoke against different Gram + and Gram-pathogen strains used as tester. Tetracycline (7 µg) and DMSO were used as positive and negative control, respectively. Results are shown as mean (in mm) (±standard deviation) (n = 3). Means followed by different letters in each column differ significantly to Dunnett's multiple comparisons test, at the significance level of $p < 0.05$. *PA = Pseudomonas aeruginosa*; *EC = Escherichia coli*; *BC= Bacillus cereus*; *EF = Enterococcus faecalis*; *SA = Staphylococcus aureus.*

	Gram (+) tester strains		Gram (−) tester strains			
	PA	EC	BC DSM 4313	BC DSM 4384	EF	SA
2.625 mg	10 (0^{a})	13.3 (2.9^{a})	10.4 (0.6^{a})	10 (0^{a})	0	13 (1.7^{a})
5.25 mg	10.7 (1.1^{a})	15.7 (2.9^{a})	11.3 (2.1^{a})	16.7 (2.9^{e})	2.2 (0^{e})	14.3 (2.0^{b})
10.5 mg	12.1 (2.9^{a})	16.8 (2.9^{a})	15.3 (2.8^{b})	16.5 (1.1^{e})	3.7 (1.4^{e})	15.5 (2.9^{c})
Tetracycline	9.8 (1.6^{a})	12.6 (1.1^{a})	9.6 (1.1^{a})	8.4 (1.2^{a})	9.7 (1.3^{a})	11.5 (0.5^{a})
DMSO	0	0	0	0	0	0

although less suitable for more precise quantification purposes, such as the determination of the MIC values, is also used to determine the susceptibility of a range of microbial species to a particular compound or mixture [41]. On the whole, the most resistant strain was *E. faecalis*, (3.7 mm of zone of inhibition using 10.5 mg of extract). The other strains showed higher susceptibility against the artichoke extract, with zones of inhibition ranging from 10 mm (with 2.625 mg GAE/g of polyphenols, against *P. aeruginosa*, and *B. cereus*) to 16.8 mm (observed using 10.5 mg against the Gram negative *E. coli*); interestingly, *E. coli* was the most sensitive strain, and a zone of inhibition of 13.3 mm was observed using just 2.625 mg GAE/g of polyphenols. Such results are consistent to Zhu and others [10] [11] but are in contrast with Ionescu and others [42], which observed a resistance of *S. aureus* against the antimicrobial activity of artichoke extracts, confirming the capability of the extracts of *Cynara cardunculus* var. *scolymus* to act as antimicrobial agents, but in different manner, probably due to a different method of extraction as well as to the different ecotype and strains used in the experiments. In our study, we used two different strains (DSM 4313 and DSM 4384) of the *B. cereus*, which exhibited a different sensitivity/resistance against the extract, mainly when they were exposed to 5.25 mg GAE/g of polyphenols, confirming that natural extract may act in different way also within the same species [16]. The antimicrobial activity exhibited by the extract against both Gram positive and negative bacteria may indicate the presence of a broad spectrum of compounds with antibiotic activity [43]. Usually, natural extracts have antimicrobial activity which supplies a natural barrier against the invasion of microorganisms and, when possible, block the systems of communication among pathogens. The antimicrobial activity exhibited by this extract might be ascribable to the presence of high amounts of chlorogenic acid, cynarin and epicatechin, which, with all probability, affected in synergistic way its antimicrobial activity.

3.4. Quorum Sensing Activity

Phenolic compounds bond strongly to bacterial cell walls and are generally classified as surface-active compounds that provoke leakage of cytoplasmic constituents, disruption of cell peptidoglycan, and injure to the cell membrane. Phenolics can also act as strong protein cross-linkers and protein-denaturing agents. The presence of different polyphenols, such as epicatechin, and cynarin present in high concentrations in Carciofo di Montoro artichoke probably affected in synergistic way the quorum sensing activity of *C. violaceum*. Due to their acidic side chains, phenolic acids, such as chlorogenic acid, the most abundant in our extract, can be easily transported across the cell membrane; this property may explain their stronger inhibitory effect. Alternatively, they can interact with membrane lipids to neutralize the membrane's electric potential subsequent to the penetration of the molecule. A similar effect may occur in the bacterial cell membrane to affect energy metabolism and perhaps also the production of some molecules, such as violacein, expressed by *C. violaceum* in response to the QS inducers C6-acyl- and C4-acyl-homoserine lactones [3]. In our experiment, quorum quenching activity, the capability to inhibit the quorum sensing action of the strain, was observed with 5.25 mg GAE of extract. Such dose did not cause the block of the growth of *C. violaceum*; however it was sufficient to negatively change the bacterial metabolism, and to stop the production of violacein (**Figure 3**). Castillo and others [44] reported an effect

Figure 3. Quorum quenching activity expressed on plate by the extract of Carciofo di Montoro global artichoke, using 10.3 mg GAE of polyphenols.

tive activity of *Cynara scolymus* to inhibit adherence and cytotoxic activity of *Campylobacter* to host mucosal surfaces, which are well known critical steps in pathogenesis. Our results strengthen case for the "functional" significance of the artichoke, in this case Carciofo di Montoro, which can constitute a precious source of antimicrobial and quorum quenching molecules that might be applied in many fields, including medicine or food technology, safety and food preservation. In summary, our data suggest that the variety Carciofo di Montoro globe artichoke may represent a good source of health-promoting polyphenols; they encourage a nutraceutical use of this ecotype, in addition to the other *Cynara cardunculus* var. *scolymus* species, as an alternative to the more traditional phyto-pharmaceutical applications.

Acknowledgements

This work was funded by the Projects SALVE and AGRIGENET, PSR 2007-2013, mis. 214, action f2, of the Campania Regional Council, Italy.

References

[1] Dillard, C.J. and German, J.B. (2000) Phytochemicals: Nutraceuticals and Human Health. *Journal of Science of Food and Agriculture*, **80**, 1744-1756. http://dx.doi.org/10.1002/1097-0010(20000915)80:12<1744::AID-JSFA725>3.0.CO;2-W

[2] Fratianni, F., Di Luccia, A., Coppola, R. and Nazzaro, F. (2007) Mutagenic and Antimutagenic Properties of Aqueous and Ethanolic Extracts from Fresh and Irradiated Tuber Aestivum Black Truffle: A Preliminary Study. *Food Chemistry*, **102**, 471-474. http://dx.doi.org/10.1016/j.foodchem.2006.04.014

[3] Nazzaro, F., Fratianni, F. and Coppola, R. (2013) Quorum Sensing and Phytochemicals. *International Journal of Molecular Sciences*, **14**, 12607-12619. http://dx.doi.org/10.3390/ijms140612607

[4] Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R. and Nazzaro, F. (2010) Preservation of Chicken Breast Meat Treated with Thyme And Balm Essential Oils. *Journal of Food Science*, **75**, M528-M535. http://dx.doi.org/10.1111/j.1750-3841.2010.01791.x

[5] Adzet, T., Camarasa, J. and Carlos Laguna, J. (1987) Hepatoprotective Activity of Polyphenolic Compounds from *Cynara scolymus* against CCl4 Toxicity in Isolated Rat Hepatocytes. *Journal of Natural Products*, **50**, 612-617. http://dx.doi.org/10.1021/np50052a004

[6] Gebhardt, R. (1997) Antioxidative and Protective Properties of Extracts from Leaves of the Artichoke (*Cynara scolymus* L.) against Hydroperoxide-Induced Oxidative Stress in Cultured Rat Hepatocytes. *Toxicology and Applied Phar-*

macology, **144**, 279-286. http://dx.doi.org/10.1006/taap.1997.8130

[7] Lattanzio, V., Kroon, P.A., Linsalata, V. and Cardinali, A. (2009) Globe Artichoke: A Functional Food and Source of Nutraceutical Ingredients. *Journal of Functional Foods*, **1**, 131-144. http://dx.doi.org/10.1016/j.jff.2009.01.002

[8] Fratianni, F., Tucci, M., De Palma, M., Pepe, R. and Nazzaro, F. (2007) Polyphenolic Composition in Different Parts of Some Cultivars of Globe Artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). *Food Chemistry*, **104**, 1282-1286. http://dx.doi.org/10.1016/j.foodchem.2007.01.044

[9] ISTAT (2013). http://agri.istat.it

[10] Zhu, X.F., Zhang, H.X. and Lo, R. (2004) Phenolic Compounds from the Leaf Extract of Artichoke (*Cynara scolymus* L.) and Their Antimicrobial Activities. *Journal of Agricultural and Food Chemistry*, **52**, 7272-7278. http://dx.doi.org/10.1021/jf0490192

[11] Zhu, X.F., Zhang, H.X., Lo, R. and Lu, Y. (2005) Antimicrobial Activities of *Cynara scolymus* L. Leaf, Head and Stem Extracts. *Journal of Food Science*, **70**, M149-M152. http://dx.doi.org/10.1111/j.1365-2621.2005.tb07106.x

[12] Scalbert, A. and Williamson, G. (2000) Dietary Intake and Bioavailability of Polyphenols. *Journal of Nutrition*, **130**, 2073S-2085S.

[13] Singleton, V.L. and Rossi Jr., J.A. (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, **16**, 144-158.

[14] Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food and Science Technology*, **28**, 25-30.

[15] Fratianni, F., Coppola, R. and Nazzaro, F. (2011) Phenolic Composition and Antimicrobial and Antiquorum Sensing Activity of an Ethanolic Extract of Peels from the Apple Cultivar Annurca. *Journal of Medicinal Food*, **14**, 957-963. http://dx.doi.org/10.1089/jmf.2010.0170

[16] De Martino, L., De Feo, V. and Nazzaro, F. (2009) Chemical Composition and *in Vitro* Antimicrobial and Mutagenic Activities of Seven Lamiaceae Essential Oils. *Molecules*, **14**, 4213-4230. http://dx.doi.org/10.3390/molecules14104213

[17] Todaro, A., Peluso, O., Catalano, A.E., Mauromicale, G. and Spagna, G. (2010) Polyphenol Oxidase Activity from Three Sicilian Artichoke [*Cynara cardunculus* L. var. *scolymus* L. (Fiori)] Cultivars: Studies and Technological Application on Minimally Processed Production. *Journal of Agricultural and Food Chemistry*, **58**, 1714-1718. http://dx.doi.org/10.1021/jf903399b

[18] Llorach, R., Espin, J.C., Tomas-Barberan, F.A. and Ferreres, F. (2002) Artichoke (*Cynara scolymus* L.) Byproducts as a Potential Source of Health-Promoting Antioxidant Phenolics. *Journal of Agricultural and Food Chemistry*, **50**, 3458-3464. http://dx.doi.org/10.1021/jf0200570

[19] Velez, Z., Campinho, M.A., Guerra, A.R., Garcia, L., Ramos, P., Guerreiro, O., Felicio, L., Schmitt, F. and Duarte, M. (2012) Biological Characterization of *Cynara cardunculus* L. Methanolic Extracts: Antioxidant, Anti-Proliferative, Anti-Migratory and Anti-Angiogenic Activities. *Agriculture*, **2**, 472-492. http://dx.doi.org/10.3390/agriculture2040472

[20] Menghini, L., Genovese, S., Epifano, F., Tirillini, B., Ferrante, C. and Leporini, L. (2010) Antiproliferative, Protective and Antioxidant Effects of Artichoke, Dandelion, Turmeric and Rosemary Extracts and Their Formulation. *International Journal of Immunopathology and Pharmacology*, **23**, 601-610.

[21] Ferracane, R., Pellegrini, N., Visconti, A., Graziani, G., Chiavaro, E., Miglio, C. and Fogliano, V. (2008) Effects of Different Cooking Methods on Antioxidant Profile, Antioxidant Capacity and Physical Characteristics of Artichoke. *Journal of Agricultural and Food Chemistry*, **56**, 8601-8608. http://dx.doi.org/10.1021/jf800408w

[22] Yao, L.H., Jiang, Y.M., Shi, J., Tomás-Barberán, F.A., Datta, N., Singanusong, R. and Chen, S.S. (2004) Flavonoids in Food and Their Health Benefits. *Plant Foods in Human Nutrition*, **59**, 113-122. http://dx.doi.org/10.1007/s11130-004-0049-7

[23] Parkar, S.G., Stevenson, D.E. and Skinner, M.A. (2008) The Potential Influence of Fruit Polyphenols on Colonic Microflora and Human Gut Health. *International Journal of Food Microbiology*, **124**, 295-298. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.03.017

[24] Hollman, P.C.H. and Katan, M.B. (1999) Dietary Flavonoids: Intake, Health Effects and Bioavailability. *Food and Chemical Toxicology*, **37**, 937-942. http://dx.doi.org/10.1016/S0278-6915(99)00079-4

[25] Boyer, J. and Liu, R.H. (2004) Apple Phytochemicals and Their Health Benefits. *Nutrition Journal*, **3**, 1-15. http://dx.doi.org/10.1186/1475-2891-3-5

[26] Panizzi, L. and Scarpati, M.L. (1954) Constitution of Cynarine, the Active Principle of the Artichoke. *Nature*, **174**, 1062-1063. http://dx.doi.org/10.1186/1475-2891-3-5

[27] Eberhardt, G. (1973) Action of Cynarin in Fatty Degeneration of the Hepatic Cells. *Zeitschrift fur Gastroenterologie*,

11, 183-186.

[28] Preziosi, P. (1969) Valutazione farmacologica dei principi attivi del carciofo. In: *Atti del I Congresso Internazionale di Studi sul Carciofo*, Edizioni Minerva Medica, Torino, 237-281.

[29] Barz, W., Koster, J., Weltring, K.-M. and Strack, D. (1985) Recent Advances in the Metabolism and Degradation of Phenolic Compounds in Plants and Animals. In: Van Sumere, C.F. and Lea, P.J., Eds., *Annual Proceedings of the Phytochemical Society of Europe*, Clarendon Press, Oxford, 307-347.

[30] Strack, D. and Gross, W. (1990) Properties and Activity Changes of Chlorogenic Acid: Glucaric Acid Caffeoyltransferase from Tomato (*Lycopersicon esculentum*). *Plant Physiology*, **92**, 41-47. http://dx.doi.org/10.1104/pp.92.1.41

[31] Zhang, J., Du, G., Tian, M.-S., Su, H.-L., Fu-Cong, L.V. and Yang, H.Y. (2011) Determination of Chlorogenic Acid in Leaves of *Cynara scolymus* L. by UPLC. *Northern Horticulture*, **20**, 46-48.

[32] Gonthier, M.P., Verny, M.A., Besson, C., Remesy, C. and Scalbert, A. (2003) Chlorogenic Acid Bioavailability Largely Depends on Its Metabolism by the Gut Microflora in Rats. *Journal of Nutrition*, **133**, 1853-1859.

[33] Kim, D.H., Jung, E.A., Sohng, I.S., Han, J.A., Kim, T.H. and Han, M.J. (1998) Intestinal Bacterial Metabolism of Flavonoids and Its Relation to Some Biological Activities. *Archives of Pharmacology Researches*, **21**, 17-23. http://dx.doi.org/10.1007/BF03216747

[34] Rechner, A.R., Kuhnle, G., Bremner, P., Hubbard, G.P., Moore, K.P. and Rice-Evans, C.A. (2002) The Metabolic Fate of Dietary Polyphenols in Humans. *Free Radicals Biology and Medicine*, **33**, 220-235. http://dx.doi.org/10.1016/S0891-5849(02)00877-8

[35] Kaushik, G., Satya, S., Khandelwal, R.K. and Naik, S.N. (2010) Commonly Consumed Indian Plant Food Materials in the Management of Diabetes Mellitus. *Diabetes Metabolic Syndrome*: *Clinical Research Reviews*, **4**, 21-40. http://dx.doi.org/10.1016/j.dsx.2008.02.006

[36] González-Gallego, J., García-Mediavilla, M.V., Sánchez-Campos, S. and Tuñón, M.J. (2010) Fruit Polyphenols, Immunity and Inflammation. *British Journal of Nutrition*, **104**, S15-S27. http://dx.doi.org/10.1016/j.dsx.2008.02.006

[37] Meng, F., Liu, R., Bai, H., Liu, B.W. and Liu, Y. (2014) Inhibitory Effect of Quercetin, Rutin and Puerarin on HDL Oxidation Induced by Cu^{2+}. *Journal of Sichuan University Medical Science Edition*, **35**, 836-838.

[38] Valenzuela, A., Sanhueza, J., Alonso, P., Corbari, A. and Nieto, S. (2004) Inhibitory Action of Conventional Food-Grade Natural Antioxidants and of Natural Antioxidants of New Development on the Thermal-Induced Oxidation of Cholesterol. *International Journal of Food Science and Nutrition*, **55**, 155-162. http://dx.doi.org/10.1080/09637480410001666496

[39] Olszanecki, R., Gebska, A., Kozlovski, V.I. and Gryglewski, R.J. (2002) Flavonoids and Nitric Oxide Synthetase. *Journal of Physiology and Pharmacology*, **53**, 5712-5584.

[40] Rossoni, G., Grande, S., Galli, C. and Visioli, F. (2005) Wild Artichoke Prevents the Age-Associated Loss of Vasomotor Function. *Journal of Agricultural and Food Chemistry*, **53**, 10291-10296. http://dx.doi.org/10.1021/jf052499s

[41] Faleiro, M.L. (2011) The Mode of Antibacterial Action of Essential Oils. In: Mendez-Vilas, A., Ed., *Science against Microbial Pathogens*: *Communicating Current Research and Technological Advances*, Formatex Research Center, Badajoz, 1143-1156.

[42] Ionescu, D., Predan, G., Rizea, G.D., Mihele, D., Dune, A., Ivopol, G. and Ioniță, C. (2013) Antimicrobial Activity of Some Hydroalcoholic Extracts of Artichoke (*Cynara scolymus*), Burdock (*Arctium lappa*) and Dandelion (*Taraxacum officinale*). *Bulletin of the Transilvania University of Braşov*, *Series II*: *Forestry*, *Wood Industry*, *Agricultural*, *Food Engineering*, **6**, 113-120.

[43] Kukic, J., Popovic, V., Petrovic, S., Mucaji, P., Ciric, A., Stojkovic, D. and Sokovic, M. (2008) Antioxidant and Antimicrobial Activity of *Cynara cardunculus* Extracts, *Food Chemistry*, **107**, 861-868. http://dx.doi.org/10.1016/j.foodchem.2007.09.005

[44] Castillo, S.L., Heredia, N., Cotreras, J.F. and Garcia, S. (2011) Extracts of Edible and Medicinal Plants in Inhibition of Growth, Adherence and Cytotoxin Production of *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Food Science*, **76**, M421-M426.

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or Online Submission Portal.