Food Chemistry 245 (2018) 7–12

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antimicrobial and antioxidant properties of various Greek garlic genotypes

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ARTICLE INFO

Keywords:
Allium sativum L.
Antibacterial activity
Antioxidant activity
Antimicrobial activity
Escherichia coli
Fungicidal properties
Staphylococcus aureus
Total phenolics

ABSTRACT

Recent studies show a significant variation in antioxidant and antimicrobial properties between the various garlic genotypes mostly due to differences in chemical composition and bioactive compounds content. The aim of the present study was to evaluate antioxidant properties and antimicrobial activity of garlics collected from the main cultivation areas of Greece, as well as to correlate this activity with their total phenolics content. Genotype G5 showed the highest total phenolics content, which was significantly correlated with the lowest EC_{50} values for all the tested antioxidant activity assays. Antimicrobial activity was significant, especially against the bacteria *Proteus mirabilis* and Antibiotic resistant *Escherichia coli*. In conclusion, significant variation was observed between the studied garlic genotypes, indicating the importance of both growing conditions and genotype on bioactive properties of dry garlic bulbs. This variation could be further exploited in breeding programs in order to select elite genotypes with increased bioactive properties.

1. Introduction

Garlic (*Allium sativum* L.) is one of the most economically important species of the Allium genus and a very economically important vegetable with an annual production of 24,939,965 tonnes and a total harvested area of 1,547,381 hectares (FAO Statistics Division, 2014). Although, the species originates from Central Asia, it is widely distributed and well adapted throughout the Mediterranean basin, while in Greece there are many regions where garlic is the main agricultural product and a part of popular culture throughout the centuries.So far, there is great interest in its therapeutic properties and health benefits and many reports confirm the beneficial effects of garlic and garlic related products, e.g. aged garlic extracts (AGE), garlic oils, essential oils and so forth against various diseases (Casella, Leonardi, Melai, Fratini, & Pistelli, 2013; El-Hamidi & El-Shami, 2015; Kopec, Piatkowska, Leszczynska, & Sikora, 2013; Kyung, 2012; Martins, Petropoulos, & Ferreira, 2016; Zeng et al., 2017). Moreover, considering the vegetative propagation of garlic, there is a great interest for local landraces and ecotypes regarding their bioactive compounds content and the importance of genetic material conservation (Baghalian, Naghavi, Ziai, & Badi, 2006; Baghalian, Ziai, Naghavi, & Naghdi Badi, 2005; Gonzalez, Soto, Sance, Camargo, & Galmarini, 2009; Kamenetsky et al., 2005).One of the major beneficial effects of garlic is related with antioxidant properties which have been tested in animal models and clinical studies with various forms, e.g. raw garlic, garlic extracts, AGE, commercial supplements or herbal medicines (Hirsch et al., 2017; Santhosha, Jamuna, & Prabhavathi, 2013). The strong antioxidant properties of garlic have been associated with many therapeutic effects, including cancer prevention, antithrombotic effects, cardiovascular protection and anti-aging effects (Huang et al., 2015). Therefore, it is crucial to identify garlic genotypes with high inherent antioxidant potential, as well as growing and climate conditions that may increase these antioxidant properties. According to Kyung (2012) who evaluated 19 garlic parental lines and cultivars, there is a positive correlation between total phenolics and flavonoids content and antioxidant activity, as determined by DPPH assay, as well as a great variation in antioxidant properties of the studied genotypes. The strong correlation of total phenolics content and antioxidant activity, has been also confirmed by Chen et al. (2013), who tested five different assays and observed similar results, while Bozin, Mimica-Dukic, Samojlik, Goran, and Igic (2008) suggested that antioxidant activity of phenolic compounds of various garlic extracts from raw garlic bulbs, AGE and air-dried plants may show variable EC_{50} values, depending on the evaluated assay (DPPH, lipid peroxidation and hydrogen peroxide-scavenging activity). Similar results have been reported by Denre, Pal, Mazumdar, Chakravarty, and Bhattacharya (2013) who also observed a significant variation in antioxidant activity between various garlic cultivars and radical scavenging activity assays. Moreover, Beato, Orgaz, Mansilla,

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http://dx.doi.org/10.1016/j.foodchem.2017.10.078
Received 3 August 2017; Received in revised form 12 October 2017; Accepted 13 October 2017
Available online 16 October 2017

Table 1
Description of the tested genotypes regarding sampling region, type of cultivation and growing period (planting and harvesting time).

Genotype	Region	Type of cultivation	Planting	Harvesting
G1	Evros Perfecture	Commercially cultivated	Late October	Mid to late June
G2	Evros Perfecture	Commercially cultivated	Late October	Mid to late June
G3	Laconia Perfecture	Local landrace	November	Early May
G4	Laconia Perfecture	Commercially cultivated	November	Early May
G5	Euboea Prefecture	Commercially cultivated	November	End of May–Start of June
G6	Magnissia Prefecture	Local landrace	December	End of June
G7	Magnissia Prefecture	Commercially cultivated	December	End of June
G8	Magnissia Prefecture	Commercial cultivar	December	End of June
G9	Arcadia Prefecture	Local landrace	Late November	Late July
G10	Arcadia Prefecture	Local landrace	Late November	Late July
G11	Arcadia Prefecture	Local landrace	Late November	Late July

and Montaño (2011) evaluated the same garlic cultivars at four different locations and confirmed the significant effect of growing conditions on total phenolic compounds content. In contrast, Soto, González, Sance, and Galmarini (2016) suggested that antioxidant activity of garlic is mostly associated with organosulfuric compounds, rather than total phenolic compounds content.Antimicrobial activity of garlic is known since the ancient times, where people used to use it as a basic ingredient in folk medicine against various infections (Lanzotti, Bonanomi, & Scala, 2013). Allicin is considered the most potent compound of garlic with significant antibacterial and fungicidal properties which have been confirmed through *in vitro* test with allicin in pure form (Ankri & Mirelman, 1999), while *in vivo* activity has not been well confirmed with preclinical and clinical studies so far (Marchese et al., 2016). Although volatile compounds are of outmost importance, especially organosulfuric compounds such as thiosulfinates, garlic bulbs contain a great variety of other bioactive compounds which contribute to their overall antimicrobial activity, including phenols, saponins, peptides and so forth (Kyung, 2012; Lanzotti, Barile, Antignani, Bonanomi, & Scala, 2012; Lanzotti et al., 2013), especially after processing of garlic. It has been reported that processing treatments may alter chemical composition and therefore affect significantly antimicrobial and antioxidant properties of garlic products, mostly due to the labile nature of organosulfuric compounds (de Queiroz et al., 2014; Horita et al., 2016). Therefore, garlic bulbs content in other bioactive compounds except for organosulfuric ones, is essential for their overall antimicrobial properties.Total phenolic compounds content of garlic bulbs shows a great variability depending on both growing condition and genetic factors. Hirata, Abdelrahman, Yamauchi, and Shigyo (2015) evaluated 103 garlic clones collected from various regions throughout the world regarding their the content of S-allyl-cysteine sulfoxide and total phenolics, and they reported a significant variation which could be attributed to the adaptation of the species under various growing conditions during the diffusion of the species throughout the world.

Beato et al. (2011), have also suggested that cultivar selection might be a useful means to improve quality by increasing the total phenolics and ferulic acid contents, regardless of the growing conditions, which mostly have a significant impact on specific compounds content, such as caffeic, vanillic, *p*-hydroxybenzoic and *p*-coumaric acids. In addition, Chen et al. (2013) studied 43 garlic cultivars for their phenolic compounds (total phenolics and flavonoids contents) and antioxidant activity through various assays [(DPPH [2,2-diphenyl-1-picrylhydrazyl] radical scavenging activity, HRSC (hydroxyl radical scavenging capacity), FRAP (ferric ion reducing antioxidant power), CUPRAC (cupric ion reducing antioxidant capacity), and MCA (metal chelating activity)]. The authors reported a significant variation among the tested cultivars and furthermore they distinguished three separate groups of cultivars, according to the observed differences in total phenolics and flavonoids contents, and antioxidant activity. In contrast, Beato et al. (2011) evaluated total phenolics content in white and purple garlics and although they found a higher content of total phenolics content in white comparing to purple colored garlics, these differences were not significant, indicating a genotype × environment interaction.

The aim of the present study was to determine the antioxidant activity and antimicrobial properties from various Greek garlics. For this purpose, samples of local landraces and cultivars were collected from the main cultivation regions of Greece in order to determine the variability in their antimicrobial and antioxidant properties, as well as to compare them with commercial cultivars.

2. Materials and methods

2.1. Plant material

Samples of garlic bulbs were collected from commercial farms located at the most important regions for garlic cultivations, where especially for local landraces sample methodology was applied according to IPGRI descriptors for Allium species (IPGRI, 2001). Considering that sampling regions extend all over the Greece (Supplementary material Fig. S1), there is a great variation in planting and harvesting (sampling) dates between the studied genotypes. Garlic is a winter crop with a growth cycle that may extend up to 8–9 months. Planting starts usually in late October-early December, while harvesting starts in late May and extends up to late July, depending on the growing conditions. In any case, the season of the year was the same for all the growing regions and planting and harvesting was carried out according to best practice guides applied in every region. After harvesting, bulbs were subjected to curing in order to allow for leaves to dry out and bulb necks to become firmer. This is the usual process applied in garlic postharvest for increasing storage ability. Further details about each genotype are presented in Table 1.

The collected samples included: a) two samples from Evros Prefecture of local garlic cultivar “Nea Vissa” (samples G1 and G2), b) two samples from Laconia Prefecture, including one sample of the local garlic landrace of “Neapoli” and one sample of Chinese origin (samples G3 and G4, respectively), c) one sample from Euboea Prefecture of garlic from Chinese origin (sample G5), d) three samples from Magnissia Prefecture, including one sample of local garlic cultivar of “Platykampos” (sample G6), one sample of commercial garlic of Chinese origin (samples G7), and one sample of commercial garlic cultivar Gardos (AGER S.A.; sample G8), and e) three samples from Arcadia Prefecture of local garlic cultivar of “Tripoli” (samples G9-11, respectively). After collection, garlic bulbs were divided in cloves, followed by peeling of cloves, size reduction with a mortar and pestle by using liquid nitrogen and storage in deep-freezing conditions (−80 °C). Before the analyses, the already smashed cloves were lyophilized and reduced to a fine powder (20 mesh).

2.2. Antioxidant activity assays

For methanolic/water (80:20, v/v) extraction, one gram of lyophilized material was extracted twice for 1 h in a magnetic stirrer plate

Table 2
Total phenolic compounds content and antioxidant properties of the studied garlic samples (mean ± SD).

Samples*	Folin-Ciocalteu assay (mg GAE/g extract)	EC_{50} values (mg/mL)			
		DPPH radical-scavenging activity	Reducing power	β-Carotene bleaching inhibition	TBARS inhibition
G1	18.98 ± 0.03g	10.47 ± 0.20d	2.07 ± 0.02e	2.81 ± 0.06ab	0.157 ± 0.009d
G2	22.24 ± 0.22d	7.18 ± 0.65f	2.08 ± 0.02e	2.73 ± 0.10b	0.094 ± 0.004fg
G3	24.28 ± 0.54c	3.65 ± 0.12h	1.89 ± 0.01f	1.80 ± 0.02d	0.104 ± 0.001f
G4	16.96 ± 0.35h	16.90 ± 0.54b	2.05 ± 0.04e	1.68 ± 0.09d	0.18 ± 0.01c
G5	44.85 ± 0.19a	2.00 ± 0.08i	0.759 ± 0.009h	0.85 ± 0.01f	0.079 ± 0.011h
G6	21.54 ± 0.04e	13.06 ± 0.09c	2.16 ± 0.01d	1.54 ± 0.09e	0.14 ± 0.01e
G7	11.30 ± 0.23i	17.22 ± 0.32b	3.53 ± 0.01b	2.19 ± 0.08c	0.229 ± 0.001b
G8	8.59 ± 0.06j	20.09 ± 0.55a	5.42 ± 0.15a	2.91 ± 0.13a	0.271 ± 0.009a
G9	20.11 ± 0.08f	8.19 ± 0.17e	2.28 ± 0.03c	1.46 ± 0.13e	0.128 ± 0.005e
G10	33.89 ± 0.40b	3.34 ± 0.13h	1.17 ± 0.01g	0.98 ± 0.05f	0.083 ± 0.009gh
G11	23.90 ± 0.04c	6.40 ± 0.20g	2.23 ± 0.04cd	1.53 ± 0.05e	0.091 ± 0.001fgh

GAE – gallic acid equivalents. The results are presented in EC_{50} values. This means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the reducing power assay.

* For details regarding the tested genotypes consult Table 1.

(25 °C at 150 rpm), with 30 mL of methanol/water (80:20, v/v), filtered through a Whatman No. 4 paper and vacuum-dried in a rotary evaporator (rotary evaporator Büchi R-210, Flawil, Switzerland) at 40 °C to remove the methanol. The extracts were further frozen and lyophilized. Afterwards, the extracts were re-dissolved in methanol/water (80:20, v/v) for *in vitro* antioxidant activity assays, at a final concentration of 20 mg/mL and further diluted to different concentrations.

The antioxidant activity was evaluated by DPPH radical-scavenging activity, reducing power, inhibition of beta-carotene bleaching in the presence of linoleic acid radicals and inhibition of lipid peroxidation using TBARS in brain homogenates (Petropoulos, Fernandes, Barros, Ferreira, & Ntatsi, 2015). The results were expressed in EC_{50} values (sample concentration providing 50% of antioxidant activity of DPPH radical-scavenging activity, inhibition of beta-carotene bleaching and TBARS assays, or 0.5 of absorbance for the reducing power assay) for antioxidant activity and Trolox was used as a positive control.

2.3. Total phenolic compounds

Total phenols were determined based on procedures previously described by Morales et al. (2014). Briefly, an aliquot of the garlic extract solution (0.5 mL) was mixed with Folin Ciocalteu reagent (2.5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/L 2 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for color development. Absorbance was then measured at 765 nm (AnalytikJena 200 spectrophotometer, Jena, Germany). Gallic acid was used to calculate the standard curve ($(10^{-5}$–4×10^{-4} mol L^{-1}; $Y = 2.8557X - 0.0021$; $R2 = 0.9999$), and the results were expressed as mg of gallic acid equivalents (GAEs) per gram of extract.

2.4. Antimicrobial activity

Antimicrobial activity was evaluated according to the procedure previously described by Glamočlija et al. (2015). More specifically, the Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), methicillin-resistant *Staphylococcus aureus* (MRSA 12), *Bacillus cereus* (clinical isolate) and *Micrococcus flavus* (ATCC 10240), and the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (clinical isolate), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), resistant *E. coli* (H2b) and *Enterobacter cloacae* (human isolate), and two fungi *Candida albicans* (IBRS MH4) and *C. krusei* (IBRS 1flac1) were used. The antimicrobial assay was carried out by a microdilution method (Tsukatani et al., 2012). In particular, the bacterial/fungi suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL (Glamočlija et al., 2015). The garlic extracts were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (5 mg/mL) and immediately added in Tryptic Soy broth (TSB) medium (100 μL) with bacterial/fungi inoculum (1.0×10^4 CFU per well). The lowest concentrations without visible growth (with the use of a binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MICs obtained from the susceptibility testing of various bacteria/fungi to tested extracts were determined also by a colorimetric microbial viability assay based on reduction of INT ((*p*-iodonitrotetrazolium violet) [2-(4-iodophenyl)-3-(4-nitrphenyl)-5-phenyltetrazolium chloride; Sigma]) color and compared with positive control for each microorganisms strains. The MBCs were determined by serial sub-cultivation of 2 μL into microtitre plates containing 100 μL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC/MFC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank (broth medium plus diluted compounds) and the positive control. Streptomycin (Sigma-Aldrich S6501) and Ampicillin (Sigma-Aldrich A9393) were used as positive controls (1 mg/mL in sterile physiological saline). Five percent DMSO was used as a negative control.

2.5. Statistical analysis

For all the antioxidant and antimicrobial properties analyses, three samples were analyzed for each treatment and all the assays were carried out in triplicate (n = 9). The results were expressed as mean values and standard deviations (SD). Statistical analysis of data was performed using SPSS v. 22.0 program (IBM Corp., Armonk, NY, USA) and one-way analysis of variance (ANOVA), while for means where a statistical difference was detected, means comparisons were carried out with Tukey's HSD Test at alpha = 0.05.

3. Results and discussion

The antioxidant properties and total phenolic compounds (TPC) content of the studied garlic genotypes are presented in Table 2. Significant differences were observed between the various tested genotypes, with genotype G5 having the lowest and genotype G8 the highest EC_{50} values for all the tested assays, indicating a strong and weak antioxidant activity, respectively. Apart from G5, other genotypes with significant antioxidant properties include genotype G10 and G3 (the latter for all the assays apart from β-carotene bleaching inhibition), whereas G7 showed the second lowest antioxidant activity for all the assays apart from β-carotene bleaching inhibition. Regarding TPC content, a reverse trend was observed for the same genotypes with G5

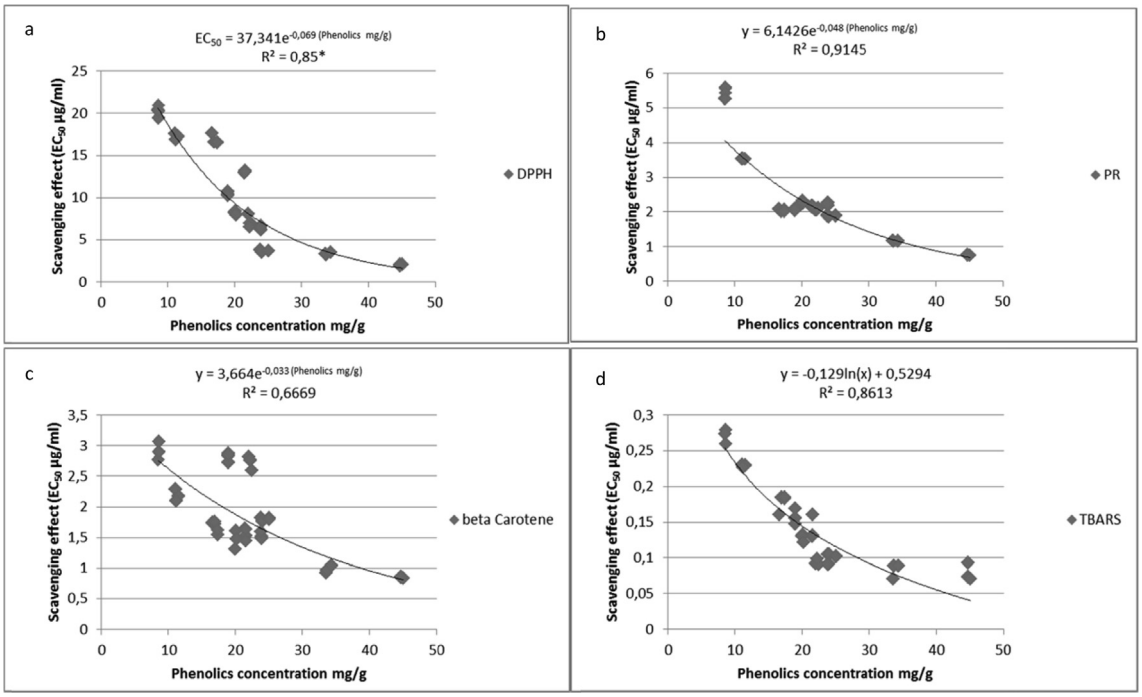

Fig. 1. Correlation established between total phenolics content and scavenging effect on (a) DPPH radicals, (b) reducing power, (c) b-carotene bleaching inhibition, and (d) lipid peroxidation inhibition.

having the highest and G8 the lowest content, while values ranged between 8.59 and 44.85 mg GAE g^{-1} dry extract or 277–1462 mg GAE 100 g^{-1} fw. The great variation in TPC content between the tested genotypes could be attributed to genotype effect when comparing different landraces and/or commercial cultivars cultivated in the same or different regions, as well as to differences in microclimate conditions and cultivation practices between the regions where the samples were collected. These results are in agreement with those reported by Bhandari, Yoon, and Kwak (2014), Chen et al. (2013), and Denre et al. (2013) who also reported a great variation between various garlic cultivars, as well as a positive correlation of antioxidant activity and TPC content. The correlation analysis of antioxidant activity and TPC content of our study showed significantly negative exponential correlations for all the tested assays, with reducing power assay having the highest determination coefficient (R^2 = 0.9145) and beta-carotene assay the lowest (R^2 = 0.6669) (Fig. 1). The variable response to the various tested assays is usually evidenced in plant extracts and according to Chen et al. (2013) it is due to different antioxidative mechanisms that may be involved in oxidative stress. Therefore, for better and more accurate results for antioxidant properties determination, it is important various assays to be tested. The TPC content of the studied genotypes was higher than that reported by Beato et al. (2011) (3.4–10.8 mg GAE g^{-1} dry weight) and similar to that of Chen et al. (2013) (17.16–42.53 mg GAE g^{-1} dry weight), ranging from 8.59 to 44.85 mg GAE g^{-1} dry weight. Nagella, Thiruvengadam, Ahmad, Yoon, and Chung (2014) who evaluated various garlics collected from different regions of Korea have reported TPC values in the upper range of our study (33.50–49.89 mg GAE g^{-1} extract), while they also observed significant differences in antioxidant activity between garlic samples for all the tested assays. Moreover, although that in the study of Beato et al. (2011) the authors reported that Chinese garlic had a higher TPC content comparing to other genotypes under the same conditions, in our study that was confirmed only for genotype G5 (of Chinese origin) which showed the highest overall TPC content. However, it was not possible to obtain samples from local landraces from the same region for direct comparisons, since garlic growers have abandoned them in favor of genotypes of foreign origin. In all the other cases where Chinese garlics were cultivated under the same conditions with local landraces (genotypes G3 and G4, and G6 and G7), local landraces had higher TPC content than garlics of Chinese origin, whereas the only commercial cultivar included in the present study (G8) had the lowest TPC content. This indicates that despite the higher yield and visual quality (usually larger and more uniform bulbs) that foreign genotypes may have, local cultivars and landraces showed highest antioxidant activity under the same growing conditions, which should be further in breeding programs for the selection of elite garlic genotypes.

Apart from the differences in antioxidant activity and TPC content between the various genotypes grown in different regions, significant variations were also observed between local cultivars (G1 and G2) and local landraces (G9–G11), which further supports the effect of genotype on these parameters apart from microclimate conditions and cultivations practices that usually differ between the various growing regions. Bhandari et al. (2014) have also reported significant differences in chemical composition and antioxidant activity of garlic lines and cultivars, which could be attributed to adaptation mechanisms that may be developed throughout their cultivation history, as well as to artificial selection through vegetative propagation (Hirata et al., 2015) and pre-harvest factors (Beato et al., 2011; Martins et al., 2016). Furthermore, Khar, Banerjee, Jadhav, and Lawande (2011) who evaluated chemical composition of various Indian garlic ecotypes, reported significant variation between different genotypes, as well as significant bulb to bulb variation, especially in local landraces where breeding status and uniformity is generally low. According to Qadir, Shahzadi, Bashir, Munir, and Shahzad (2017), TPC content and antioxidant activity of plant extracts is highly dependent on extraction process and solvent selection, with methanolic and ethanolic extracts showing the best results for most of the plant species and garlic in particular. Therefore, sampling and extraction process of garlic bulbs is very important for bioactive compounds evaluation, especially when local landraces and ecotypes are tested, and could justify the great variation of bioactive compounds amounts reported in various studies.

In vitro antimicrobial properties of the studied garlic genotypes against various bacteria and fungi strains are presented in Table 3. Regarding the antibacterial activity, the bacteriostatic activity of all the

Table 3
In vitro antimicrobial activity of extracts (mg/ml) from the studied garlic samples.

Bacteria	G1* MIC/ MBC	G2 MIC/ MBC	G3 MIC/ MBC	G4 MIC/ MBC	G5 MIC/ MBC	G6 MIC/ MBC	G7 MIC/ MBC	G8 MIC/ MBC	G9 MIC/ MBC	G10 MIC/ MBC	G11 MIC/ MBC	Streptomycin MIC/ MBC	Ampicillin MIC/ MBC
Staphylococcus aureus	0.1	0.3	0.1	0.4	0.15	0.2	0.4	0.1	0.3	0.2	0.45	0.08	0.012
	0.3	0.6	0.3	0.6	0.3	0.45	0.6	0.3	0.45	0.3	0.6	0.16	0.025
Methicillin-resistant *Staphylococcus aureus*	0.15	0.2	0.15	0.2	0.15	0.15	0.2	0.15	0.15	0.15	0.2	0.1	–
	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	–	–
Bacillus cereus	0.05	0.075	0.04	0.075	0.05	0.075	0.075	0.05	0.075	0.075	0.075	0.0015	0.006
	0.15	0.15	0.075	0.15	0.075	0.15	0.15	0.15	0.15	0.15	0.15	0.003	0.025
Micrococcus flavus	0.075	0.1	0.075	0.1	0.1	0.1	0.1	0.075	0.1	0.1	0.1	0.025	0.25
	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.05	0.4
Pseudomonas aeruginosa	0.075	0.075	0.1	0.1	0.2	0.1	0.075	0.075	0.1	0.075	0.1	0.025	0.05
	0.15	0.15	0.15	0.15	0.3	0.15	0.15	0.15	0.15	0.15	0.15	0.05	0.1
Escherichia coli	0.075	0.1	0.075	0.1	0.1	0.1	0.075	0.075	0.075	0.075	0.1	0.05	0.1
	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.1	0.2
Antibiotic resistant *Escherichia coli*	0.04	0.05	0.05	0.05	0.075	0.075	0.05	0.04	0.04	0.05	0.1	0.1	0.2
	0.075	0.075	0.075	0.075	0.15	0.15	0.15	0.15	0.075	0.15	0.15	0.2	–
Proteus mirabilis	0.075	0.05	0.075	0.075	0.1	0.1	0.075	0.05	0.05	0.075	0.075	0.1	0.03
	0.15	0.15	0.15	0.15	0.15	0.2	0.15	0.15	0.075	0.15	0.15	0.2	0.06
Enterobacter cloacae	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.1	0.1	0.003	0.006
	0.15	0.15	0.15	0.15	0.3	0.15	0.15	0.15	0.15	0.15	0.15	0.006	0.012
Salmonella typhimurium	0.2	0.2	0.15	0.2	0.3	0.15	0.3	0.15	0.15	0.3	0.3	0.012	0.025
	0.45	0.45	0.3	0.45	0.45	0.3	0.45	0.3	0.3	0.45	0.6	0.025	0.05
Fungi	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	Nistadin MIC/ MFC	Fluconazole MIC/ MFC
Candida albicans	0.05	0.075	0.05	0.075	0.075	0.075	0.075	0.075	0.2	0.04	0.075	0.002	0.02
	0.15	0.15	0.075	0.15	0.15	0.15	0.15	0.15	0.3	0.075	0.15	0.003	0.04
Candida krusei	0.025	0.05	0.04	0.15	0.15	0.04	0.05	0.075	0.075	0.02	0.075	0.0007	0.03
	0.075	0.075	0.075	0.3	0.3	0.15	0.15	0.15	0.15	0.04	0.15	0.0015	0.06

MIC: Minimum Inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimal fungicidal concentration.
–: No activity detected.
* For details regarding the tested genotypes consult Table 1.

studied genotypes ranged from 0.04 to 0.4 mg/mL, whereas positive controls of streptomycin and ampicillin presented MIC values ranging from 0.0015 to 0.1 mg/mL. Increased growth inhibitory properties of garlic methanolic extracts (80% methanol) against Gram positive and negative bacteria comparing to streptomycin have been also reported by Eadlapalli, Vemula, Bramhachari, and Vadde (2016). Similarly, bactericidal activity values ranged between 0.075 and 0.6 mg/mL, whereas positive controls MBC values ranged between 0.003 and 0.4 mg/mL. Moreover, genotypes G1 and G8 showed the lowest MIC values against most of the tested bacteria (8/10), while genotype G3 had the lowest MBC values against almost all the tested bacteria (9/10). Bactericidal effect against methicillin-resistant *Staphylococcus aureus* (a gram positive bacteria) was higher than the reference agents (streptomycin and ampicillin) for all the genotypes, indicating a strong potency of garlic against this bacteria, while similar results were observed for bacteriostatic effects against antibiotic resistant *Escherichia coli* and *Proteus mirabilis* (both gram negative bacteria) for almost all the tested genotypes. Garlic extracts have been previously reported to be effective against both gram positive and negative bacteria (Karwowska, Świderski, & Waszkiewicz-robak, 2007; Mozaffari Nejad, Shabani, Bayat, & Hosseini, 2014), while (Ankri & Mirelman, 1999) attribute these properties to chemical reaction of allicin with thiol-containing enzymes, such as thioredoxin reductases, RNA polymerases, alcohol hydrogenases and cysteine proteinases which are essential for microbes activity. More specifically, Karwowska et al. (2007), Florinda et al. (2016) and Taherikalani, Hassanzadazar, Bahmani, Baharvand-Ahmadi, and Rafieian-Kopaei (2016) have reported that garlic extracts showed significant *in vitro* inhibiting properties against *Staphylococcus aureus* and *Escherichia coli*, while Mozaffari Nejad et al. (2014) detected strong inhibiting effect of garlic aqueous extracts against *Staphylococcus aureus* in hamburgers.

Fungistatic and fungicidal properties are presented in Table 3, with MIC values ranging between 0.075 and 0.2 mg/mL for *Candida albicans* and *C. krusei*, while positive controls of nistadin and fluconazole showed significantly lower values than any of the tested garlic genotypes (0.0007–0.03 mg/mL). The fungistatic properties of garlic against *Candida* species have been confirmed through *in vitro* studies with pure allicin on clinical isolates of fungi (Ankri and Mirelman, 1999). Similarly, MFC values of the tested garlic genotypes ranged between 0.075 and 0.04 mg/mL, whereas positive controls were more effective, showing values between 0.0015 and 0.06 mg/mL. Genotypes G2, G3-G8 and G11 showed better fungistatic effects against *C. albicans* than the rest of the tested genotypes, while genotypes G1 and G10 were more potent against *C. krusei*. Significant differences were also observed between the tested genotypes regarding their fungicidal effects, with genotype G3 being more effective against *C. albicans* and genotype G10 against both the tested fungi. The fungicidal effects of garlic against *C. albicans* have been also reported by Karwowska et al. (2007).

4. Conclusions

The results of the present study indicate that despite the higher yield and visual quality that foreign genotypes may have, local cultivars and landraces showed highest antioxidant activity under the same growing conditions, which should be further valorized in breeding programs for the selection of elite garlic genotypes. Moreover, significant differences between the tested garlic genotypes were observed regarding the antimicrobial properties, while garlic extracts were more effective than positive controls against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*. Therefore, the collection and recording of the existing garlic local landraces needs to be intensified in order to evaluate and preserve promising genetic material, especially when considering the great variation that microclimate conditions of Greece offer, as well as the fact that local farmers do not have incentive

to retain local landraces and safeguard them for future generations. Further experiments are also required in order to determinate the effect of genotype × environment interaction on quality features, for better selection of elite genotypes.

Acknowledgment

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Program PT2020 for financial support to CIMO (UID/AGR/00690/2013), A. Fernandes grant (SFRH/BPD/114753/2016) and L. Barros contract. The authors are also grateful to the Serbian Ministry of Education, Science and Technological Development, grant number 173032 for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.10.078.

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