

Analysis of bioactive compounds and antioxidant activities of cultivated garlic (*Allium sativum* L.) and red onion (*Allium cepa* L.) in Algeria

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Abstract

In all times, humankind has used several species of the genus *Allium* as food, spice, or herbal remedy. Some of these species have been cultivated, such as garlic (*Allium sativum*) or onion (*Allium cepa*). Today, their value for human health care is one of the most important aims of research. Up to now, many applications of *Allium* species are known for the use of phyto-pharmaceutical preparations. Therefore, the present study aimed to determine the phytochemical profile of cultivated garlic (*Allium sativum*), and red onion (*Allium cepa*) in Algeria, both quantitatively (total phenolic, total flavonoids, condensed and hydrolysable tannins contents) and qualitatively (phytochemical screening), to characterize the phenolic compounds using HPLC method and to evaluate the antioxidant properties using DPPH assay. Red onion gave the higher amounts of total phenolic compounds (86±1.00mg GAE/100g DM), flavonoids (43.33±0.57mg QE/100 g DM), condensed tannins (4.4±0.52 mg CE/100g DM) and hydrolyzable tannins (0.22±0.04mg TAE/100g DM) compared to garlic (45±1.00mg GAE/100g DM, 34.66±0.57mg QE/100g DM, 6.8±0.34mg CE/100g DM and 0.05±0.01mg TAE/100g DM) respectively. Five compounds were found in red onion extract and one compound in garlic extract after chromatographic analysis of the samples. Furthermore, red onion possessed the higher antioxidant activity (IC₅₀= 420.9±5.00 µg/ml) as compared to garlic (919.87±4.43 µg/ml). These findings provide ample evidence of the existence of bioactive compounds in garlic and red onion, both of which are rich in phenolics primarily flavonoids and tannins, have strong antioxidant activity, and can be further consumed directly or as food products.

Keywords: *Allium sativum* L., *Allium cepa* L., Phytochemistry, HPLC, DPPH, Algeria.

Introduction

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al.,

2008). Garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) are the most important species of the Amarylidaceae family and, for thousands of years, have been used for their characteristic flavour as spices or food, or for their medicinal properties (Takahashi and Shibamoto, 2008).

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During the last years, *Allium* spices were among the most studied vegetables and aroused great interest for food industries. These interests appear from the encouraging results of the antioxidant capacity of some of their compounds, which have been to be comparable to and sometimes higher than that of synthetic antioxidants used in food industry particularly BHA (butylated hydroxyanisole,) and BHT (butylated hydroxytoluene) (Barlow, 1990).

According to previous studies, both vegetables play a role in lowering the risk of chronic diseases like cardiovascular disease, cancer and aging-related disorders in which reactive oxygen species are involved (Moreno et al., 2014). Their beneficial effect on health was attributed to high contents of biologically active phytochemicals, such as phenolic compounds, especially flavonoids, and several organosulfur compounds (Goldman et al., 1996).

Garlic and red onion are especially common in Algeria with a total production of 103.627 and 1.526.339 tons respectively (Bouhenni et al., 2019). However, few studies were carried out concerning these important plants, in this concept, the objective of this paper was to determine and quantify the phenolic contents (flavonoids and tannins) of cultivated garlic (*Allium sativum*), and red onion (*Allium cepa*) in Algeria using HPLC method, as well as investigate their antioxidant activity. The originality of this study consists in the fact that it is the first report on polyphenolic composition of methanolic extract of cultivated garlic and red onion in Algeria.

Materials and Methods

Chemical Reagents

Methanol, Folin Ciocalteu reagent, quercetin, aluminium chloride, catechin, sulfuric acid, tannic acid, hydrochloric acid, chloroform, chlorhydric alcohol, isoamyl alcohol, acetic anhydride, glacial acetic acid and DPPH were purchased from Sigma-Aldrich, U.S.A. Gallic acid and vanillin were obtained from Merck, Germany, sodium carbonate was from Acros Organics, Belgium, and ferric chloride was purchased from Alfa Aesar, Germany.

In HPLC analysis, Fortis column was obtained from Fortis Technologies Ltd, UK. The used solvents (acetonitrile and formic acid) were purchased from Merck, Germany. Concerning the standards; caffeic acid, chlorogenic acid, ferulic acid, trans- p-coumaric acid, gallic acid, rosmarinic acid, salicin, apigenin, quercetin, quercitrin, isoquercitrin, hyperoside, luteolin -7-O-glucoside, luteolin, kaempferol were from Phytolab, Germany and ellagic acid, salicylic acid, chicoric acid, naringenin, chrysin, myricetin were purchased from Merck, Germany.

Determination of total phenolic content

According to the procedure defined by Singleton and Rossi, (1965), the method of Folin-Ciocalteu reagent has been used to estimate the total phenolic content. 0.5 ml of varying concentrations of each used extract and 2.5 ml of Folin-Ciocalteu (1/10 dilution in water) were mixed with 1ml of sodium carbonate (20%). This mixture was incubated in the

dark at room temperature for 30 min. The absorbance of the solution was measured at 765 nm using UV-Vis spectrophotometer HITACHI (Ratio Beam U-V 5100). A calibration curve was established using gallic acid as standard. The results were expressed as milligram of Gallic acid equivalent (GAE) per 100 g of Dry Matter.

Determination of total flavonoids content

The total flavonoids content of both extracts was determined using the aluminium chloride method as described by Zou et al., (2004). 1.5 ml of various concentrations of both extracts was mixed with 75µl of aluminium chloride solution and 0.5 ml of sodium acetate solution, the mixture was completed with distilled water until a volume of 2.5 ml. After an incubation period of 30 min at room temperature in the dark, the absorbance of the solution was measured at 415 nm using UV-Vis spectrophotometer. The results were expressed as milligram of Quercetin equivalent (QE) per 100 g of Dry Matter.

Determination of condensed tannins content

The analysis of condensed tannins was carried out according to Price et al., (1978). 1ml of each extract was mixed with 2.5 ml of 4% methanol vanillin solution and 2.5 ml of H₂SO₄. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents were expressed as milligram of Catechin equivalent (CE) per 100 g of Dry Matter.

Determination of hydrolysable tannins content

Hydrolysable tannins were estimated using method of Waterman, (1987). 500 µl of the extract was added to 3.5 ml of the ferric chloride solution. The contents were then quickly mixed and the absorbance read at 660 nm, 15 secs after the addition of the extract solution. Hydrolysable tannins content was expressed as milligram of Tannic acid equivalent (TAE) per 100 g of Dry Matter.

Phytochemical screening

Qualitative tests were realized to detect the presence of some secondary metabolites in plants extracts according to Trease and Evans, (1989); Sofowora (1993) (Table 1).

The analytical method used is high-performance liquid chromatography (HPLC), the identification of substances was performed according to their polarity in the solvents, the model of HPLC used for analytical control was: Shimadzu Nexera-I HPLC with autosampler and quaternary pump. Each extract was dissolved in methanol in a ratio of 1 part extract to 5 parts solvents. The extracts were analysed as such by injection into HPLC. The operating conditions are as follows: Column: silica gel-C18 type Fortis C18, 150 x 2.1 mm x 3 µm, Eluent: A = water, B = 0.1% formic acid, aqueous solution with pH = 2.5, and C = acetonitrile, Flow rate: 1 ml / min, Injected volume: 5 µl, Detector: DAD, spectrophotometric 220-400 nm, with chromatograms recorded at 254, 326 and 360 nm. The evaluation was based on a comparison of retention times and absorption maxima in the UV-Vis spectra. The resulting chromatographic profile is compared to standards (standard pure of

phytochemical molecules) injected into the same operating conditions as that of the sample. Retention time (Rt) of each component is determined by the integrator giving a peak on the chromatogram (Vlase et al., 2014).

Table 1. Phytochemical screening of garlic and red onion

Metabolites	Added reagent	Expected result
Flavonoïdes	KOH (50%)	Yellow color
Tannins	FeCl ₃ (1%)	Blue coloration
Alcaloids	HCl 2%+	Brown precipitate
Sterols and triterpenes	Wagner reagent	Red color (surface) + Greenish fluorescence
	Anhydride acetic + H ₂ SO ₄ (98%)	
Terpenoids	Chloroform + H ₂ SO ₄	Reddish brown coloration
Saponosides	Distilled water	Formation of foam
Anthocyanins	Chlorhydric alcohol+ isoamyl alcohol	Reddish brown Coloration
Cardiac glycosides	Glacial acetic acid + FeCl ₃ (5%)+ H ₂ SO ₄ (98%)	Brown ring
Reducing compounds	Fehlings (A+B)	Brownish-red precipitate

Determination of phenolic content by High Performance Liquid Chromatography (HPLC) analysis

Antioxidant activity

The antioxidant activity of extracts was measured with the DPPH method describing by Shimada et al., (1992). A solution of DPPH (0.1 mM) was freshly prepared by dissolving 4 mg DPPH in 100 ml methanol. Mother solution (1 mg/ml) was prepared and followed by serial dilution in order to obtain all increasing concentration needed (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml), from each extract 1 ml of each prepared diluted extract was added to 1 ml of DPPH (0.1 mM). The solutions were then incubated for 30 min at room temperature in the dark, and the absorbance was measured at 570 nm. The antioxidant activity was calculated according to the following formula:

% inhibition = [(A_{control} - A_{sample}) / A_{control}] × 100, where A_{control} is the absorbance of DPPH solution without extract and A_{sample} is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC₅₀) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

Statistical analysis

The data from phytochemical composition and antioxidant activity were analyzed with a statistical software program (SPSS version 20). Differences between plants were compared at P < 0.05 with One-Way ANOVA followed by Tukey's post hoc test in order to find the statistically significant differences.

The assays were carried out with three repetitions and the results were expressed as mean values and standard deviation.

Results and Discussion

Phytochemical analysis

The results of extraction yield, total phenolic, total flavonoids, condensed and hydrolysable tannins content of garlic and red onion extracts were summarized in Table 2.

The extraction yield (mass of extract/mass of dry matter) was used as an indicator of the effects of the extraction conditions. According to the findings, the extract yield of garlic using maceration method and methanol 70% as solvent was higher (62.87±0.50 %) than red onion (57.38±0.56%).

In the present study, the results showed that red onion extract had a higher phenolic content (86±1.00 GAE/100g DM) than garlic extract (45±1.00mg GAE/100g DM). In addition, red onion extract had the highest total flavonoid content (43.33 ±0.57mg QE/100 g DM) compared to garlic extract (34.66 ±0.57mg QE/100g DM). In contrast to red onion (4.4 ±0.52 mg CE/100g DM), garlic has a higher value of condensed tannins (6.8 ±0.34mg CE /100g DM).

Table 2. Results of phytochemical analysis of garlic and red onion

Analysis	Garlic extract	Red onion extract
Extract yield (%)	62.51 ^b ±0.50	57.35 ^a ±0.56 ^{***}
TPC (mg GAE /100g DM)	45 ^a ±1.00	86 ^b ±1.00 ^{***}
TFC (mg QE /100g DM)	34.66 ^a ±0.57	43.33 ^b ±0.57 ^{***}
CTC (mg CE /100g DM)	6.8 ^b ±0.34	4.4 ^a ±0.52 ^{**}
HTC (mg TAE /100g DM)	0.05 ^a ±0.01	0.22 ^b ±0.04 ^{**}

TPC: Total Phenolic Content TFC: Total Flavonoids Content
CTC: Condensed Tannins Content HTC: Hydrolysable Tannins Content DM: Dry Matter

*** Significant at 0.001 or 0.1%

** Significant at 0.01 or 1%

a, b corresponds to the homogeneous groups obtained by the post-hoc Tukey test for each parameter.

Our percentage yield of garlic extract was higher than previous studies findings (Park and Chin, 2010), (Ali and Mohsen Sabri, 2014) and (Bhanot and Shri, 2010) which were 2.46%, 6% and 7% respectively. According to Kallel et al., (2014), aqueous garlic extract has a higher percentage of extract yield (26.5%) than ethanolic and methanolic garlic extracts, which were 4% and 7% respectively.

Although, Park and Chin, (2010) reported a percentage yield of 52.38% for red onion extract, (Bhanot and Shri, 2010) reported a much lower percentage yield of 6.8%. Statistically, there was a significant difference between garlic and red onion (p=0.000), however, this difference can be due to variety diversity, growing conditions, ripening degree and climate (Kaoru et al, 2006). Also, the particle size and shape of samples in extraction

process are important factors that affect the yield extraction, another factor that may have affected differences in yield between garlic and red onion is sample pre-treatment (Ali and Mohsen Sabri, 2014). The highest extraction yield with aqueous solutions can be attributed to the addition of water, which increases the polarity of the solvents (Kim et al., 2004).

The total phenolic compounds content of garlic was approximately comparable to that found in many studies (Chekki et al., 2014) and (Jastrzebski et al., 2007); with 43.6 mg GAE/100g and 49.3 mg GAE/100g respectively, while the present result was significantly higher than that reported by (Nuutila et al., 2003) and (Sarafa et al., 2016) with values of 11.5 mg GAE/100g and 0.42±0.02 mg GAE/100g respectively. However, the results found in the studies of Lenkova et al., (2016), Park et al., (2009), Chekki et al., (2014) and Kallel et al., (2014) were significantly higher with 105.1±18.09 mg GAE/100g, 562.6±1.93 mg GAE/100g, 500-4360 mg GAE/100g and 2283±1.69 mg GAE/100g respectively.

Nuutila et al., (2003) found that Giant onion had a total phenolic content of 84.5 mg GAE/100g, which was close to the current result. Petropoulos et al., (2015) result was lower in the range of 8.05-10.8 mg GAE/100g. Although several studies have been carried out to estimate the amount of total phenolic contents present in red onion; Sarafa et al., (2016), Lu et al., (2011), Cheng et al., (2013), Skerget et al., (2009) and Singh et al., (2009) found a higher result than our result, with amounts of 103±0.00 mg GAE/100g, 428 mg GAE/100g, 571±0.20 mg GAE/100g, 6362±2.03 mg GAE/100g and 38470±5.0 mg GAE/100g respectively. The high total phenolic content of red onion compared to garlic ($p=0.000$) may be due to differences in the method of sample extraction (e.g., solvent used), wherever, these contradictory results are most likely due to differences in the methodology and the experimental conditions used in the different studies (Nuutila et al., 2003).

In general, red onion had higher phenolic content than garlic; variations found between these two plants may be due to differences in their genetic composition and growing conditions, which have a strong influence on the levels of phenolic compounds (Soto et al., 2016).

Total flavonoids analysis revealed that garlic contains significantly more total flavonoids content than that reported by Soto et al., (2016) which was in the range of 7±0.007 - 11±0.02 mg QE/100 g. On the other hand, it was approximately similar to the findings of Chekki et al., (2014) and Shuxia chen et al., (2013), which were in the range of 0.42-59.5 mg QE/100 g and 7.5-67.5 mg QE/100 g, respectively. Kallel et al., (2014), Sarafa et al., (2016) and Moumen et al., (2016) found an increased amount; 60 mg QE/100 g, 113±0.01 mg QE/100 g, and 1521±0.93 mg QE/100 g respectively.

Significant variations in total flavonoids content were also found in red onion compared to previous studies of Soto et al., (2016) and Abuga (2014) with values of 8±0.008-18±0.033 mg QE/100 g, and 10±0.69 mg QE/100 g, respectively. Other researchers, Cheng et al., (2013), Sarafa et al., (2016), Skerget et al., (2009) and Singh et al., (2009) found higher contents; 165.8±0.41 mg QE/100 g, 366±0.01 mg QE/100 g, 1376±0.41 mg QE/100 g and 16520±3.2 mg QE/100 g, respectively.

TPC and TFC variability in garlic can be due to numerous cultivar characteristics, but clove size must be taken into account because it has an indirect effect on the final concentration of phenolic compounds (Lu et al., 2011). Different garlic cultivars had different phenolic contents, according to previous study (Chen et al., 2013). The present data revealed a highly significant difference in total flavonoids between the two plants ($p=0.000$), which can be explained by several factors, including experimental parameters and natural qualitative and quantitative variability in the raw material (Chen et al., 2013).

The presence of condensed tannins in garlic agreed with the report of Nwinuka et al., (2005) and Sarafa et al., (2016) with significant differences; 0.01±0.0mg CE/100g, 0.82±0.01mg CE/100g respectively. Moumen et al., (2016) observed that garlic methanolic extract showed the highest number of condensed tannins 3.01±0.39 mg CE/100g compared to aqueous and ethanolic extract; 1.35±0.5mg CE/100g and 0.69±0.2 mg CE/100g respectively.

Furthermore, a lower condensed tannins content was recorded in red onion in comparison with garlic ($p=0.003$), the present result was similar to Abuga (2014) result; 4.99±0.06 mg CE/100g, higher to Nwinuka et al., (2005) result; 0.01±0.01 mg CE/100g and lower to Sarafa et al., (2016) result; 9.82±0.02 mg CE/100g. This may be attributed to genetic and climatic factors rather than storage time, processing and extraction methods (Sarafa et al., 2016). Condensed tannins are water-soluble phenolic metabolites commonly found in almost all plants parts (Kunyangana et al., 2014).

For hydrolysable tannins contents, there was a significant difference between these two plants ($p=0.002$), these findings suggest that the level of hydrolysable tannins is greatly influenced by tissue type, solvents (different polarities), and extraction conditions (Saleha, 2019).

The results of the qualitative assay of samples were shown in Table 3. They revealed the presence of flavonoids, tannins, terpenoids in garlic, as well as anthocyanins and cardiac glycosides in red onion. While, alkaloids, sterols, triterpenes, saponosides and reducing compounds were absent in both extracts.

Results of the phytochemical screening of methanolic extracts of the samples did not concur with Gazuwa et al., (2013) data, who reported the absence of tannins, saponins and phenolics in red onion and garlic. The presence of flavonoids and tannins in garlic and red onion agreed with the report

of Nwinuka et al., (2005), but contradicted the results of Green et al., (1997). This implied that the studied spices are potential sources of phytochemicals, many of which have been confirmed to have medicinal activity as well as physiological activity (De and James, 2002). However, the presence of these vital chemical substances supported the observation of Pandey (1980) that plants have some vital chemical substances (alkaloids, carbon compounds, glycosides, tannins and others).

Table 3. Results of phytochemical screening of garlic and red onion

Analysis	Garlic extract	Red onion extract
Flavonoids	+	++
Tannins	+	+++
Alkaloids	-	-
Sterols and triterpenes	-	-
Terpenoids	++	+++
Saponosides	-	-
Anthocyanins	-	++
Cardiac glycosides	-	+
Reducing compounds	-	-

(-): absent ;(+): low presence; (++): medium presence; (+++); high presence

Determination of phenolic content by HPLC analysis

The molecular separation of garlic and red onion methanolic extracts using HPLC was realized in three different wave lengths 254nm, 326nm and 360nm. The chromatograms with peaks and retention time of each molecule are shown in Figure 1- 4.

HPLC results revealed the presence of five components in red onion extract (Fig. 1-3) and one component in garlic extract (Fig. 4). The identification of molecules found in the samples is based on comparing their retention times (Rt) with that of pure standards under the same experimental conditions. Table 4 lists the compounds identified in methanolic extracts of garlic and red onion.

Chromatographic analysis of the samples identified five phytochemical molecules for red onion extract namely: Gallic acid, Quercetin, Rutin, Hyperoside and Karemperol and one molecule for garlic extract which is Gallic acid. The rest of the compounds that appeared on the chromatograms could not be identified.

Table 4. The polyphenolic compounds of garlic and red onion analysed by HPLC

Extract	Compounds	Retention time (min)
Red onion	Gallic acid	3.137
	Unknown	3.687
	Quercetin	10.728
	Rutin	14.734
	Hyperoside	15.490
	Unknown	16.451
	Karemperol	17.967
Garlic	Gallic acid	5.904

The polyphenols separated from the red onion extract at retention times of 3.137 min and 3.687 min are of the tannin class, probably Gallic acid derivatives, according to the spectra and absorption maxima. Flavonoids are isolated from the same extract at retention times of over 10 min, with the ones from 14.734; 16.451; and 17.967 min being probably Quercetin derivatives with maximum absorption at over 350 nm. Among the majority flavonoids in the red onion extract, the flavonoid from the minute 14.734 represents 48.7%. The flavonoid from minute 10.728 represents 26.5%, with the rest being in the proportion of less than 10%. There are not many polyphenols in the garlic extract. The only observable component of minute 5.904 is in very low concentration.

Under the same experimental conditions, a comparison of the retention times (Rt) of molecules found in the samples with those of pure standards identified five compounds in the methanolic extracts of red onion (Gallic acid, Quercetin, Rutin, Hyperoside, and Karemperol), as well as one compound in garlic (Gallic acid) and two other compounds that could not be identified.

Previous study concerning characterization of secondary metabolites in red onion observed the presence of Quercetin, Protocatechuic acid, Spiraoside, Tyrosine, Vanillic acid and Hydroxybenzoic acid (Lachman et al., 1997). Afterwards, Lachman et al., (2002) found that phytochemical characterization of different cultivars of onion (red, yellow and white) revealed the presence of six phenolic compounds with Spiraoside, Rutin and Quercetin as major constituents, as well as three other unidentified compounds. Different onion varieties (Nirvana, DPS 1032, Yellow 2025, King-Midas, and SBO 133) are one of the highly rich sources of main flavonols, Quercetin (Sellappan and Akoh, 2002). In contrast to other vegetables, onions have a 5–10 times higher overall Quercetin content (347 mg/kg). The most common flavonol, Quercetin, is present in both bound and free forms (Leighton et al., 1992).

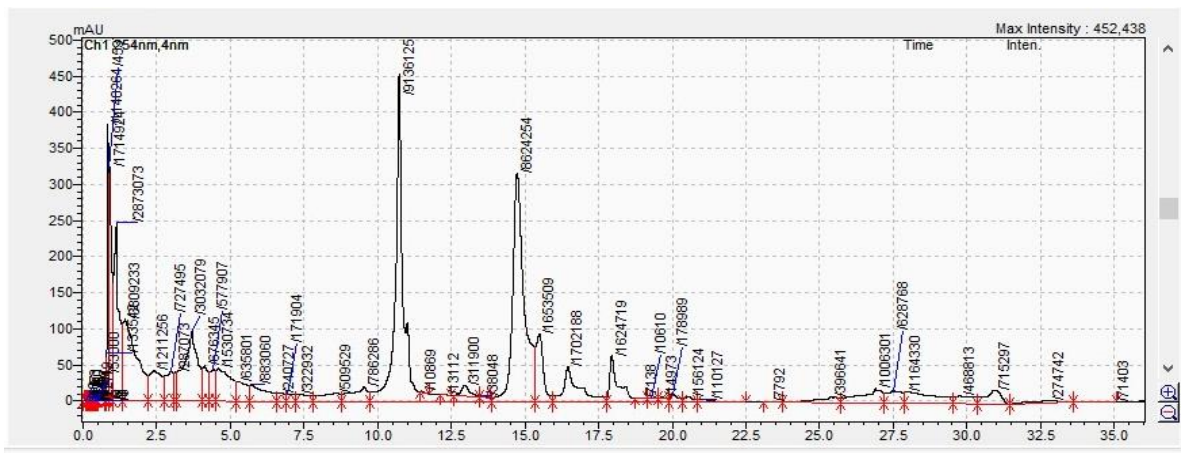


Figure 1. HPLC chromatogram of red onion dry extract at 254 nm

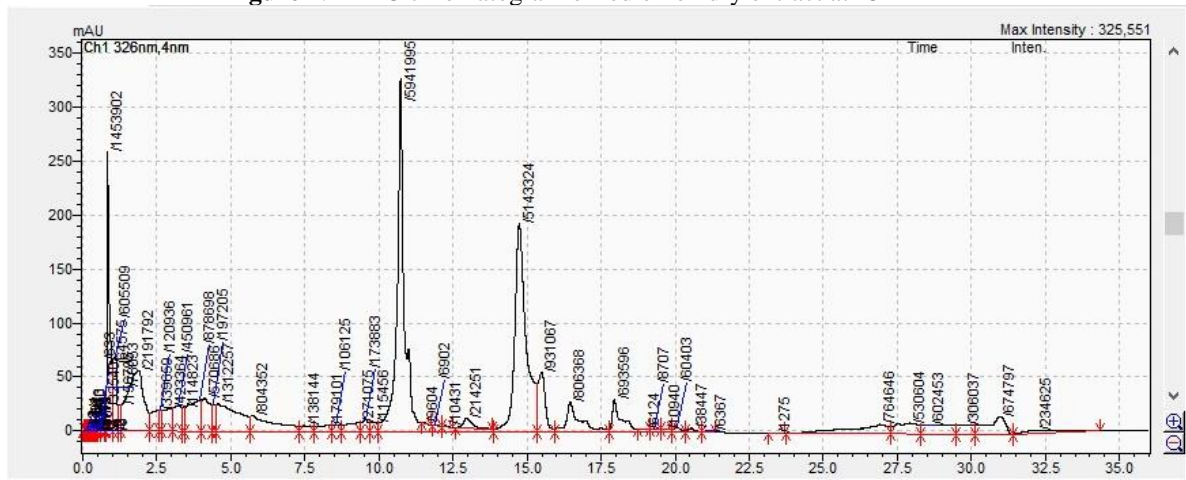


Figure 2. HPLC chromatogram of red onion dry extract at 326 nm

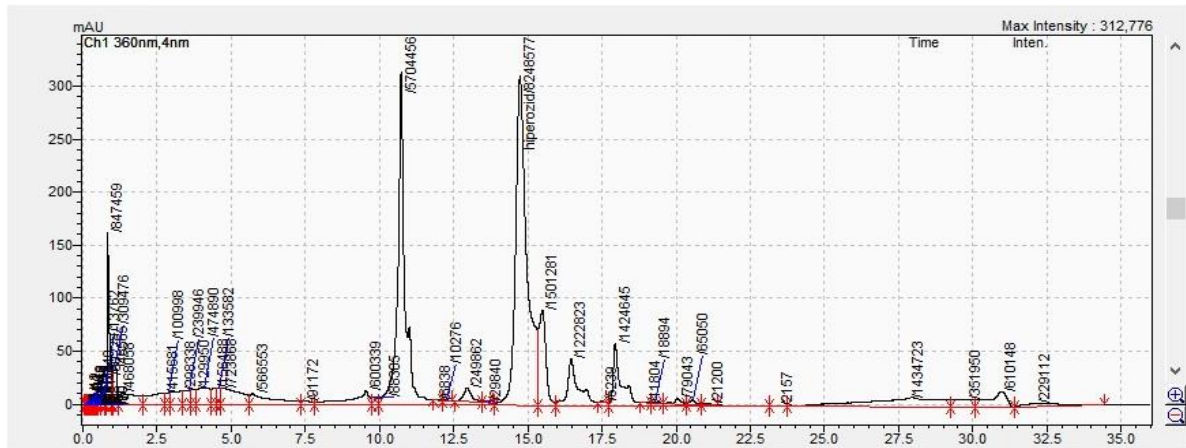


Figure 3. HPLC chromatogram of red onion dry extract at 360 nm

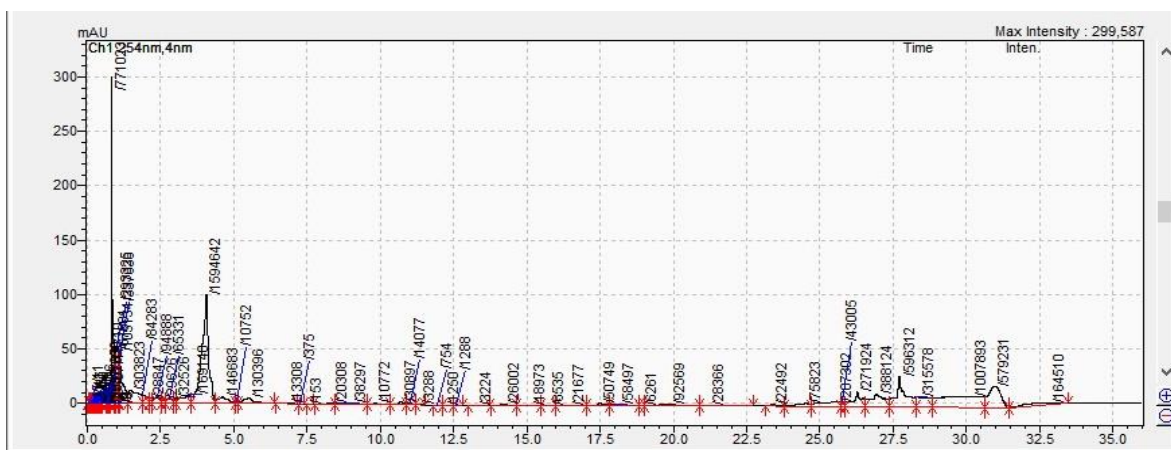


Figure 4. HPLC chromatogram of garlic dry extract at 254 nm

Quercetin-monglycosides spiraeoside (4'-O-β-D-glucoside), 3-O-β-D-glucoside, 3'-O-β-D-glucoside, and 7-O-β-D-glucoside are very highly manifested (Ioku et al., 2001). There are also kaempferol-glycosides present at minor amounts 3,4'-O-β-D-diglucoside, 7,4'-O-β-D-diglucoside, 3-O-sophoroside-7-O-β-D-glucuronide, 4'-O-β-Dglucoside. Another type of flavonols – isorhamnetin – is present only in yellow and red cultivars of onion in both free and bound form in glycosides as: 3,4'-O-β-D-diglucoside, 4'-O-β-D-glucoside and 3-O-β-D-glucoside (Park and Lee, 1996).

Eleven major phenol compounds were identified in peel and skin of some onion cultivars (Donna, Barito and Hy Park): Quercetin-3,7,4-triglucoside, p-hydroxybenzoic acid, Quercetin-7,4-diglucoside, Vanillic acid, Quercetin-3,4-diglucoside, Quercetin-3-O-glucoside, Kaempferol-3-O-glucoside, Isorhamnetin-3-O-glucoside, Quercetin-4-O-glucoside, Quercetin and Kaempferol (Burri et al., 2017).

Many studies on the phenolic profile of different onion cultivars reported that the only phenolic compound found in detectable quantities was Quercetin (Hertog et al., 1992; Mian and Mohamed (2001); Sultana and Anwar, 2008; and Zill-e-Huma et al., (2011). Quercetin and Gallic acid were the two phenol compounds found in the hydrolyzed extract of garlic and onion (Soto et al., 2016). Our results obtained for garlic cultivars were close to those of Sultana and Anwar (2008), who found no detectable amounts of Quercetin and Kaempferol.

The significant difference in the phenolic profile obtained by HPLC between garlic and red onion confirmed the previous results of total phenolic and flavonoids content. These differences may be due to many factors including genotype, maturity stage, growing and climate conditions, harvest period and even post-harvest conditions (Chun et al., 2006). Furthermore, the results of the characterization by HPLC depend on column's separating strength, flow velocity, and mobile phase composition (Johnson et al., 2011).

Antioxidant activity

The results of the antioxidant activity of plants extracts carried out by DPPH radical scavenging activities were summarized in Table 5. They showed that methanolic extract of red onion had the strongest radical-scavenging effect compared to garlic methanolic extract.

The results of our study showed that free radical scavenging activity of garlic was lower than red onion. In terms of IC₅₀, red onion had the lowest value (420.9±0.01 µg /ml), followed by garlic (919.87±0.01 µg/ml). However, these findings clearly show that red onion has more capacity to scavenge the free radicals compared to garlic (p=0.000).

Che et al., (2011) found that garlic extract has a similar IC₅₀ to the current result with an amount of 0.95±0.01 mg/ml, however other researchers revealed a lower radical scavenging activity (Nuutila et al., 2003; IC₅₀=1000 mg/ml), (Moumen et al., 2016: IC₅₀=8.36mg/ml), (Lenkova et al., 2016: 17.17%±0.634) and (Fredotović et al. ,2017: IC₅₀ 82.64 mg/ml), while Kallel et al., (2014) study showed a higher radical scavenging activity of garlic: (IC₅₀ 0.64 mg/ml). Regarding red onion, previous studies showed that its radical scavenging activity was higher (Nuutila et al., 2003: IC₅₀=67 mg/ml) and (Fredotović et al., 2017: IC₅₀=77.13mg/ml).

Table 5. Results of evaluation of antioxidant activity of garlic and red onion

Extract	Extract concentration (µg/ml)	Inhibition (%)	IC ₅₀ (µg/ml)
Garlic	1000	54.85	919.87 ^b ±4.43 ***
	500	25.31	
	250	14.76	
	125	02.10	
Red onion	1000	80.79	420.9 ^a ±5.00
	500	68.31	
	250	40.19	
	125	24.35	

*** Significant at 0.001 or 0.1%

** Significant at 0.01 or 1%

a, b corresponds to the homogeneous groups obtained by the post-hoc Tukey test for each parameter

Similarly, according to Benkeblia (2005), garlic has higher free radical scavenging activity than red onion. Similar research conducted in other plants and fruits have shown that high radical scavenging activities are commonly associated with high TPC. For instance, Lim et al., (2006) reported that high phenolic content in extracts led to high radical scavenging activity. Several other studies have shown that phenolic compounds contribute to high radical scavenging activity. Mohd et al., (2006) suggested that free radical scavenging activity is not due to the phenolics only.

In contrast to our results, Miller et al., (2000) found that garlic has a six-fold higher antioxidant activity than onion. The difference is probably at least partially due to the different methods used. Miller et al., (2000) extracted the fresh vegetables using 50% methanol whereas, in our study, 70 % methanol was used for extraction. The high antioxidant activity of *Alliums* and especially high DPPH radical scavenger of garlic were reported by numerous investigators (Velioglu et al., 1998; Yin and Cheng, 1998). However, DPPH radical scavenger activity depended on both phenolics and sulfur compounds of *Alliums*. On the other hand, Nuutila et al., (2003) reported that the lowest antioxidant activity was detected in garlic. According to Benkeblia (2005) garlic extract reacted faster than other extracts and was the most effective DPPH radical scavenger, followed by purple, red and yellow onion extracts, while green onion extract showed the lowest DPPH radical scavenger. Previous study has suggested that garlic contains phenol, flavonoid, and various sulfur compounds such as disulfide (hydrophobic), and S-allyl-(L)-cysteine (SAC, hydrophilic), this latter has high radical scavenging activities (Colin-Gonzalez et al., 2012). The number of phenolic compounds and flavonoids has positive correlation with DPPH radical scavenging activities, which is due to hydrogen and electron donation from hydroxyl groups of these compounds' compounds (Rice-Evans et al., 1996).

Conclusion

The polyphenolic profile and the antioxidant activity for cultivated garlic (*Allium sativum*) and red onion (*Allium cepa*) in Algeria were evaluated in order to complete scientific data related to previous studies about proximate composition of these two plants. The phytochemical screening showed significant differences between these two species, both qualitatively and quantitatively. Red onion was

rich in polyphenols, flavonoids and tannins and possessed the higher antioxidant activity as compared to garlic which could be related to its high content of Quercitol derivatives. This variation is explained by difference in the genetic background of the plant material tested, rather than by differences in environmental conditions. The present study provides valuable information on phytochemical composition and functional activity of cultivated garlic and red onion species, which could be further used for their direct consumption or in the formulation of food products for human health. Future research should concentrate on the relationship between chemical structure and activity (SAR), as well as clinical trials to assess the potential effects both of the crude extracts and of the total extracts isolated compounds in human health.

Compliance with Ethical Standards

Conflicts of Interest: The authors declare no conflict of interest.

Author Contributions: Hasna Bouhenni, Koula Doukani / conception of the work; contribution in phytochemical analysis; writing the manuscript; Daniela Hanganu, Neli-Kinga Olah / contribution in HPLC analysis; analysis and interpretation of data; Nazım Sekeroglu, Sevgi Gezici contribution in phytochemical analysis; analysis and interpretation of data. All authors prepared and revised data, read and approved the manuscript.

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