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Antioxidant Activity of Table Olives as Influenced by Processing Method

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Abstract

Table olives are a traditional product and one of the most important components of the Mediterranean diet. The first study aimed to the determination of the antioxidant substances in different preparations of Algerian table olive varieties (*Sigoise* and *Azerradj*) at three ripening stages and the investigation of their antioxidant potential is hereby reported. Fruits of *Sigoise* (Bejaia and Ain-Defla) and *Azerradj* cultivars were harvested at three ripening stages; they were processed following the Spanish style for green olives, including debittering (with alkali at 1.5%), washing and fermentation in brine (5%); turning olives were directly fermented in brine (2%) for three months and black olives were processed following the Greek style including fermentation in dry salt for 60 days. Phenolic extracts were obtained with methanol. Total phenolics, *ortho*-diphenols, flavonoids and anthocyans were determined and the antioxidant activity of extracts was evaluated by measuring antiradical activity and reducing power. Turning olives prepared naturally in brine are richer in total phenolics; *Sigoise* variety (Ain-Defla) showed the highest content in total phenols (4284.40 mg/100g), *O*-diphenols (261.09 mg/100g), flavonoids (71.03 mg/100g) and anthocyans (1681.23 mg/100g). The antioxidant activity of extracts followed the order: turning olives in brine > Greek style black olives> Californian black oxidized olives> Spanish style green olives. *Sigoise* variety (Ain-Defla) exerts the best antiradical activity (3.69 g QE/100g) and reducing power (1.85 g QE/100g). Consumption of turning olives of *Sigoise* variety from Ain-Defla, prepared naturally in brine is considered, to offer a high intake of antioxidants and so a health benefit for the prevention of many diseases linked to oxidative stress.

Keywords

Olea europaea, Antioxidant Activity, Phenolics, Processing

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1. Introduction

Diets rich in fruits and vegetables have been associated with lower risk of coronary heart disease and cancer. The notion that the deleterious effects of oxidative metabolism can be ameliorated through a diet rich in antioxidants has gained credibility (Berger, 2005). Table olives (*Olea europaea*) are one of the main parts of the Mediterranean diet together with olive oil. Production for the 2013- 2014 season amounted to 23 000 000 tons, the majority of which (40 %) is located in the

European Union. Algerian production is in increase; actually with 6% of the world production, Algeria takes up the 8th position (IOC, 2013).

Table olives are prepared from specifically cultivated fruit varieties harvested at the pre-determined ripening stage. The main commercial table olive preparations are the Spanish-style green olives, the Californian-style black oxidized olives and the Greek-style naturally black olives (Fernandez- Diez, 1979; Panagou et al., 2008). The Spanish processing method includes treatment with sodium hydroxide

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solution, for the total removal of the bitter compound oleuropein (generating hydroxytyrosol and elenolic acid glycoside), washing, brining and fermentation, sorting and packaging (Romero et al., 2004; Montano et al., 2010). The Californian treatment method includes brining, lye treatment, washing, iron salt treatment and air oxidation for colour improvement, washing, canning and sterilization (Toscano et al., 2003; Mafra et al., 2006). The Greek-style method of treatment is milder and includes natural anaerobic-fermentation in dry salt and packaging (Panagou, 2006). These procedures result in a decrease of the total amount of phenols. Different kinds of table olives are distinguished according to processing method, cultivar, irrigation regimes, ripening stage, fruits size and technological processing (Ryan et al., 1999; El Khaloui and Nouri, 2007).

Table olives are well known sources of phenolic compounds, the major of which are hydroxytyrosol, elenolic acid, tyrosol and caffeic acid; their concentration is depended upon the ripening degree and the treatment method of olive drupe till they became edible (Blekas et al., 2002; Charoenprasert and Michell, 2012).

So far, olives have been used in culinary purposes mainly because of their pleasant flavour and colour; their nutritive value has been rather overlooked. It has been claimed that table olives possess important biological proprieties, such as antioxidant activity (Balatsouras, 1997; Bianchi, 2003) with respect to the oxidation of low-density lipoproteins associated with lower risk of coronary heart disease (Soni et al., 2006), prevention of some types of cancer (Soler Rivas et al., 2000; Saija and Ucella, 2001) and diabetes. Gillani et al. (2006) reported spasmolytic (calcium antagonist) and spasmogenic (cholinergic) activities of olive fruit, and so, its action on gastrointestinal disorders. Hydroxytyrosol was shown to inhibit peroxynitrite-dependent DNA damage and to protect human erythrocytes against hydrogen peroxide-induced oxidative alterations (Blekas et al., 2002).

As far we know, this is the first report considering the antioxidant capacity of table olives at different ripening stages. The objective of the present work was to investigate the content of the antioxidant substances in different preparations of two Algerian varieties of table olives (*Sigoise* and *Azerradj*) and their antioxidant potential.

2. Material and Methods

2.1. Plant Material

Fruits of *Sigoise* (from Bejaia and Ain-Defla) and *Azerradj* (from Bejaia) cultivars were harvested at green, turning colour and black maturation stages. 1Kg of olives per variety and per ripening stage was used. Only olives free of blemishes cut and

insect's punctures were selected. 1kg of commercial black oxidised *Sigoise* variety (Californian Style) was also studied.

2.2. Processing

Green olives were processed according to the Codex Stan 66 (1987), following the Spanish style. Olives were debittered in an alkali solution (1.5%) for 12h, rinsed twice, then fermented in brine (5%) where a lactic fermentation reduces the pH to 4.5. Table olives were stored at 6°C. Turning olives are directly treated in the brine for three months. Black olives were fermented during 60 days in dry salt (40 Kg NaCl/100Kg of olives).

2.3. Water Content

Samples of approximately 5g of olive were dried at 105°C as described by Tovar et al. (2002).

2.4. Phenolic Compounds

2.4.1. Preparation of Extracts

The phenolic compounds were extracted according to the method described by McDonald et al. (2001) with slight modifications. 5g of dry weight olives were mixed with enough 25 ml of methanol and centrifuged at 3000 rpm/5 min (sigma 2-16 K, Germany). The residue was extracted again in the same conditions, and extracts were combined, washed with hexane. The extracts were then filtered.

2.4.2. Total Phenolics

The total phenolic content of the extracts was determined with Folin Ciocalteu reagent according to Malik and Bradford (2006). Total phenol values were expressed as gallic acid equivalents (mg/100g dry weight) from a calibration curve (y= 0.0036x; R^2 = 0.99).

2.4.3. Ortho-Diphenols

The content of *ortho*-diphenolics was determined according to Tovar et al. (2002). Sodium molybdate (1ml) was added to diluted aliquots of the methanolic extract, mixed and the absorbance was measured at 370 nm (Shimadzu UV-Vis mini1240 spectrophotometer). *O*-diphenol contents were expressed as caffeic acid equivalents (mg/100g dry weight) from a calibration curve (y=0.0672x; R²= 0.99).

2.4.4. Flavonoids

The flavonoid content of the extracts was estimated according to the method reported by Djeridane et al. (2006). 1.5 ml of the extracts was added to the same volume of aluminium chloride solution (2%). After 15 min, the absorbance was measured at 430 nm. Flavonoid contents were expressed as quercetin equivalents (mg/100g dry weight) from a calibration curve (y= 0.0438x; R²= 0.99).

2.4.5. Anthocyans

Anthocyans were extracted according to the method described by Piga et al. (2005); 5 g of olive paste and 0.1g of sodium metabisulphite were mixed with 7.5ml of methanol: water (80:20) for 2 min. After centrifugation at 8000 rpm for 15 min, the supernatant was collected and the extraction was repeated twice on the residue. The resulting supernatants were added to the first one and the flask was made up to volume of 25 ml with methanol: water. Anthocyans were estimated by a pH differential method as described by Iqbal et al. (2007). Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5, using:

A= $(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$, with molar extinction coefficient of cyanidin-3-glucoside of 29,600. Results were expressed as equivalents cyanidin-3-glucoside (mg/100g dry weight).

2.5. Antioxidant Capacity

2.5.1. Antiradical Activity

The procedure reported by Boskou et al. (2006) was adapted to estimate the effect of the olive extracts on the DPPH radical. An aliquot of the extract (0.5 ml) was added to DPPH solution (3.9 ml) and kept in dark for 30 min. The absorbance was measured at 515 nm and antiradical activity was expressed as quercetin equivalents (mg/100g dry weight) using a calibration curve (y = 0.0036x; $R^2 = 0.99$).

2.5.2. FRAP Assay

The ferric-reducing antioxidant power (FRAP) of the extracts was measured as reported by Zhan et al. (2006). The extract (1

ml) was added to 2.5 ml of potassium ferricyanide, and the mixture was centrifuged at 3650 g for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride. The absorbance was then measured at 700 nm and the reducing power expressed as quercetin equivalents (mg/100g dry weight), was estimated from a calibration curve (y = 0.0131x; $R^2 = 0.98$).

2.6. Statistical Analysis

All the data represent the average of three measures. Data was analysed using ANOVA with STATISTICA logistic. Data was considered significant at p<0.05.

3. Results and Discussion

3.1. Moisture

Humidity levels of the samples are given in table 1. They differ following the cultivar, the origin and elaboration process; Spanish style green olives present the higher moisture content (70%), while turning olives in brine present low moisture: 64.6 and 55.5% for *Sigoise* from Bejaia and *Sigoise* from Ain-Defla varieties, respectively. Black olives in salt present the lowest moisture content. Moisture in table olives depends on the cultivar, the degree of maturation (2007) and mainly on the processing technology. Green olives process includes washing eliminating the excess of alkali that causes the increase in humidity. Black olives are prepared in dry salt involving the dryness of fruits. Sousa et al. (2011) have also indicated that water was the major component of the traditional table olives Northeast Portugal, varying from 58.9% to 80.0%.

 $\textbf{Table 1.} \ \ \textbf{Water (\%), total phenol}, \ O\text{-diphenols, flavonoids and anthocyans (mg/100g \ dry \ weight) content of table olives$

Processing method	Cultivar	Water content	Total phenol	O-diphenols	Flavonoids	Anthocyans
Spanish-style green olives	Sigoise (Bejaia)	72.32±1.19a	162.96±1.39h	16.27±0.07h	8.81±0.32f	271.49±24.63f
	Sigoise (Ain-Defla)	65.42±2.46c	390.33±5.54e	27.38±0.39f	11.99±0.32d	338.10±19.72d
	Azerradj (size 8)	70.61±1.7b	471.41±3.67d	43.49±0.39c	8.26±0.05g	56.83±0.00h
	Azerradj (size12/13)	70.60±1.7b	359.58±2.28f	28.93±0.11e	8.17±0.05g	104.18±26.79g
Turning olives naturally in brine	Sigoise (Bejaia)	64.56±3.36d	3307.08±9.44b	70.44±1.34b	54.31±0.46b	824.55±19.24b
	Sigoise (Ain-Defla)	55.47±3.62f	4284.4±14.58a	261.09±0.56a	71.03±0.91a	1681.23±8.84a
Greek-style black olives	Sigoise (Ain-Defla)	19.83±1.04h	1484.52±1.31c	33.88±0.35d	11.03±0.09e	315.91±4.91e
	Azerradj (size12/13)	20.39±1.34g	115.54±0.91i	4.48±0.24i	4.79±0.23h	ND
Californian-style oxidized olives	Sigoise (Ain-Defla)	63.70±2.68e	325.33±5.83g	22.10±0.10g	14.40±0.02c	475.36±10.84c

ND: not detected.

3.2. Phenolic Compounds

Table olives are important sources of monounsaturated fatty acids, as olive oil, recognized as a preventive factor in diseases in which free radicals are implicated, complemented by the amounts of vitamin E, with both antioxidant and vitamin action Sousa et al. (2011).

The mean values of phenolic, *O*-diphenol, flavonoid and anthocyan content in the four olive preparations are given in table 1. Phenolic contents differ significantly (p<0.05) with the processing method, origin and variety for the same preparation. Turning olives naturally in brine are richer in total phenols, *O*-diphenols, flavonoids and anthocyans; *Sigoise* variety from Ain-Defla shows the highest content in total

phenols (4284.40 mg/100g), *O*-diphenols (261.09 mg/100g), flavonoids (71.03 mg/100g) and anthocyans (1681.23 mg/100g). These findings confirm results obtained by Romero et al. (2004) and Brenes (2005) on Italian olive varieties (*Manzanilla, Hojiblanca* and *Gordal*), because there is no alkaline degradation of these compounds, and their diffusion outer olive drupe is lower.

Lower mean values are found in black olives in dry salt; *Azerradj* variety exhibits the lowest content in total phenols (115.54 mg/100g), *O*-diphenols (4.48 mg/100g) and flavonoids (4.79mg/100g). Spanish style green olives show contents in total phenols ranged from 162.96 mg/100g (*Sigoise*, Bejaia) to 471.41 mg/100g (*Azerradj*, size 8), *O*-diphenols from 16.27 mg/100g (*Sigoise*, Bejaia) to 43.49 mg/100g (*Azerradj*, size 8) and flavonoids from 8.17 mg/100g (*Azerradj*) to 11.99 mg/100g (*Sigoise*, Ain-Defla). Oxidized *Sigoise* variety contains the smallest amounts of total phenols (325.33 mg /100g) and *O*-diphenols (22.10 mg/100g) and relatively low contents in flavonoids (14.40 mg/100g) and anthocyans (475.36 mg/100g).

In comparison to the results obtained by Blekas et al. (2002) on Italian variety (Chalkidini), and by Ben Othman et al. (2009) on Tunisian ones, our varieties, mainly Sigoise from Ain-Defla and Azerradj (size 8) contain high amounts of phenolic compounds. Spanish style green olives are prepared with olives picked at early maturation with low amounts in phenolic compounds. Lye treatment hydrolyses ester bonds of oleuropein and increases permeability of outer waxy layer of fruits, influencing the loss of polar phenolic compounds during washing and brining (Kiai and Hafidi, 2014). Glycosides, including luteolin 7-O-glucoside and elenolic acid glucoside, are either partially hydrolysed during lactic acid fermentation or pass completely into the brine (Soler Rivas et al., 2000; Blekas et al., 2002). Investigation of Parinos et al. (2007) was focused on the loss of phenolic compounds into the wastewaters produced from different stages of Spanishstyle green olives processing; these authors estimated the loss during debittering to 21.1 mg/100g, during washing to 44.6 mg/100g and to 18.2 mg/100g during brining.

A significant difference (p<0,05) is noted between the two samples of *Sigoise* variety; sample from Ain-Defla is richer in phenolic compounds than Bejaia one. This difference can be attributed to the geographical origin associated to environmental conditions which affect PAL (L-phenylalanine-ammonia-lyase) activity, involved in phenolic synthesis, being highered as deficit in irrigation increased (Patumi et al., 2002; Tognetti et al., 2006). A significant difference is also noted concerning content of total phenols and *O*-diphenols between the two sizes of *Azerradj* variety; the sample of size 8 is richer than the 12/13 size one. This observation is in agreement with study of Amiot et al. (1986) establishing an inverse

relationship between size of olive fruits and their phenolic content.

Results obtained for Greek-style naturally black olives of *Sigoise* and *Azerradj* show that these varieties are richer in phenolic compounds than *Thassos* variety studied by Blekas et al. (2002). Black olives contain middle amounts of phenolic compounds due to the processing treatment; dry salt causes a severe osmosis responsible of the loss of these polar substances. This can also be attributed to the effect of maturation in response to the decrease of PAL activity and to the increase of peroxidase and esterase activities (Gandul-Rojas et al., 2004).

The decrease is more pronounced for *O*-diphenolic compounds (87.02%, in *Sigoise* variety from Ain-Defla) in regard to total phenolics (65.3%). Amiot et al. (1986) claimed that caffeic acid is perhaps used either directly in the formation of anthocyans or indirectly in possible acylation of these molecules. The difference in total phenols, *O*-diphenols and flavonoids content between *Sigoise* and *Azerradj* cultivars can be attributed to a difference in their composition and/or to a difference in the geographical origin.

A significant quantitative difference is also observed in the anthocyans content. These pigments have not been detected in *Azerradj* variety; this is closely linked to structure and nature of anthocyans, presuming a glycosylated anthocyans on the 3rd carbon for *Sigoise* variety, giving a stable pseudo-basic form and hydroxylated one for *Azerradj* variety, giving non stable pseudo-basic form witch can not been regenerated with acidification because of the irreversible translation of the hydroxyl enole to a ketone.

This study revealed a parodoxal result, showing that content of anthocyans is higher in turning olives than in black olives; dry salt causes a drastic decrease in anthocyans due to the diffusion of these polar compounds out of the flesh. It has been reported that at pH superior to 8 or in the presence of sodium metabisulfite, anthocyans are destroyed by oxidation and ring opening yielding a sulfonic chromen, and that the acidification, occurred by acidified methanol, regenerate only partially the reactive form. In the other hand, this observation can be attributed to a possible polymerisation of anthocyans in black olives. The stability of colour is explained by Piga et al. (2005) with the replacement of monomeric anthocyans by other more stable pigments.

Californian-style black oxidized olives contain low amounts of phenolic compounds. This preparation, although popular, deprives the product from the valuable *O*-diphenols (Brenes-Balbuena et al., 1992). During darkening process, *O*-diphenols, such as hydroxyl-tyrosol and caffeic acid, are oxidized into quinons, a further polymerisation of these products is possible.

3.3. Antioxidant Activity

Methanolic extracts of olives were tested for their antioxidant activity by estimating the antiradical activity using the stable free radical DPPH, and the reducing power. Figures 1 and 2 show the scavenging effect on DPPH and FRAP values of the extracts. The extracts of turning olives naturally in brine exhibited the highest antioxidant potential. Sigoise variety from Ain-Defla exerts the best antiradical activity (3.69 g QE/100g) and reducing power (1.85 g QE/100g). Greek-style black olives in dry salt exhibited lower antiradical activity: 0.45 and 0.02g QE/100g for Sigoise and Azerradi, respectively and lower reducing power: 366 and 66 mg QE/100g. Californian-style black oxidized olives show fewer antiradical activity (0.49g QE/100g) and reducing power (0.17 g QE/100g) and Spanish-style green olives exhibited the lowest antioxidant activities ranged from 0.09 to 0.66 g QE/100g for antiradical activity, and from 0.09 to 0.26 g QE/100g for FRAP test). As demonstrated by Ziogas et al. (2010) and Fu et al. (2011), reducing power of fresh green olives are strongly cultivar and altitude dependant. Nadour et al. (2012) associate the difference in reducing capacity to the phenolic profile of the cultivars.

The scavenging effect on DPPH radical increases with concentration in the range 0-12 μ g/ml (figure 3). The extract of *Sigoise* (Ain-Defla) variety is efficient in scavenging the DPPH radical, with an effective concentration (EC₅₀) of 8.7 μ g/ml, corresponding to 1.65 μ g of phenolics or 0.04g of pulp in comparison to results published by Boskou et al. (2006) reporting an EC₅₀ of 587 μ g phenolics corresponding to 0.3 g of pulp for *Thrubes crete* variety naturally in brine. Sousa et al. obtained EC₅₀ ranged from 0.34 to 1.72 mg/ml for green table olives (*Alcaparra*). Antiradical activity of extracts exhibited a significant correlation (r=0.76), which is in agreement with the finding of Piscopo et al. (2014).

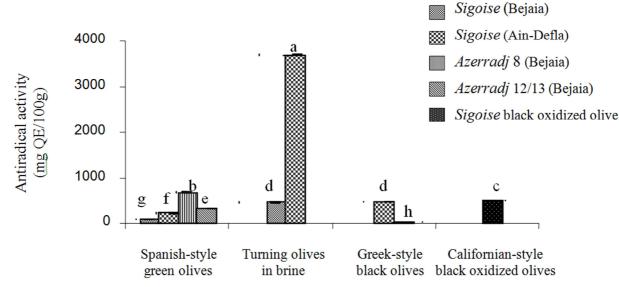


Figure 1. Antiradical activity of different preparations of table olives.

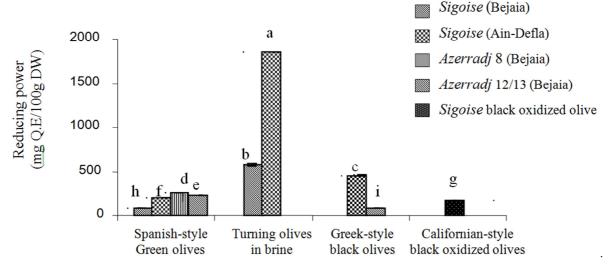


Figure 2. Reducing power of different preparations of table olives.

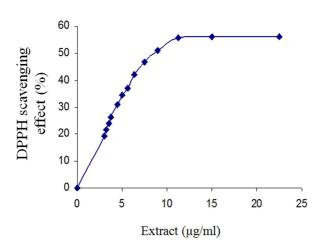


Figure 3. Scavenging effect of Sigoise table olive extract naturally in brine.

Statistical analysis revealed differences between the processing methods associated to ripening stage which control the nature of predominant phenolic compounds; at the turning and black stages, olives are richer in O-diphenolic compounds (hydroxytyrosol and caffeic acid), while at green stage, oleuropein and tyrosol are concentrated (Amiot et al., 1986; Bianchi, 2003). Otherwise, it was shown that O-diphenols exert potent antioxidant activity due to improved stabilisation of the phenoxyl radical (Visiolli and Galli, 1998; Lesage-Meessen et al., 2001; McDonald et al., 2001). Tuck and Hayball (2002) claimed that antioxidant activity of hydroxyl-tyrosol was higher than that of oleuropein and tyrosol. Degree of maturity and technological process determine also the presence of glycosidic forms; according to Garcia-Alonso et al. (2004), aglycons are more effective in scavenging free radicals than their corresponding glycosides where the sugar moiety reduces the antioxidant efficiency of adjacent hydroxyl groups due to steric hindrance.

Significant differences are also noted between varieties for the same technological process, that should certainly be related to differences in phenolic profiles of cultivars as reported by Sousa et al. and Vinha et al. (2005) including methylation, the involvement of other H-donating groups (-NH, -SH) (Son and Lewis, 2002), the presence of *O*-methoxy-phenols, and to the influence of sunlight and natural catecholases yielding phenolic derivatives with less antioxidant activity (Boskou et al., 2006).

We have recorded that samples richer in phenolic constituents exhibited higher anti-oxidant activity; a direct correlation is established between antioxidant efficiency of table olives and phenolic content of extracts (table 2) as demonstrated by linear regression analysis. Data of table 2 indicate high linear correlation levels in both tests analysed; the best correlation is reported between *O*-diphenolics content and FRAP test (r= 0.99), and antiradical activity (r= 0.98), suggesting a wide

dependence of antioxidant ability of table olives on O-diphenolics content. A good correlation levels are also observed for total phenolics, flavonoids and anthocyans content. Similar results were obtained by Sahan et al. (2013). Dourtoglou et al. (2006) established linear correlation between reducing power and phenolic content only with olives stored under CO_2 atmosphere. Linear correlation (r= 0.97) is also established between antiradical activity and FRAP assay (figure 4) confirming the compatibility of the two tests for antioxidant investigation of table olives.

Table 2. Correlations between antioxidant activities and phenolic contents.

	Total phenolics	O-diphenols	Flavonoids	Anthocyans
Antiradical activity	r= 0.76	r= 0.98	r= 0.80	r= 0.89
FRAP	r= 0.89	r= 0.99	r= 0.88	r= 0.93

4. Conclusion

The current work yielded information about antioxidant contents and antioxidant activity of two table olive varieties grown in Algeria. According to our results, the absolute concentration of biophenols in table olives is the result of a complex interaction between cultivar selection, degree of drupe maturation, processing technology, environmental conditions and size of fruits. The quantitative and the nature of phenolics in each type of table olives differentiate the total antioxidant capacity. With regard to phenolic level and antioxidant capacity of our samples, it can be concluded that these Algerian varieties are good sources of biophenols in comparison to results published.

In the future, other experiments should be carried out on other Algerian cultivars, with different sizes, at intermediate stages of ripening and prepared with different processing methods, to create a preparation with enhanced health benefits.

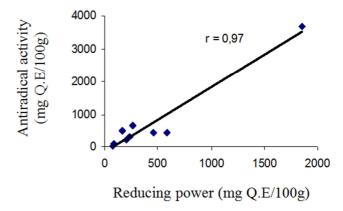


Figure 4. Correlation between antiradical activity and reducing power.

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