

Preliminary communication**PHENOLIC CONTENTS AND IN VITRO ANTIOXIDANT ACTIVITY
OF FOUR COMMONLY CONSUMED NUTS IN ALGERIA**

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This study was carried out to determine the phenolic contents and the antioxidant activity of four nuts with different solvent extract. Total phenolic compounds, flavonoids, and proanthocyanidin were quantified. Antioxidant activity was evaluated by various in vitro tests, including ferric reducing power, phosphomolybdenum method assay, and free radical scavenging activity. The results showed that the total phenolic contents varied between 0.30 g GAE/100 g (peanuts) and 1.65 g GAE/100 g (walnuts); the flavonoid contents varied between 0.17 g QE/100 g (peanuts) and 0.41 g QE/100 g (hazelnut). The phenolic contents of four nut extracts exhibit potent antioxidant activity. Indeed, walnuts were the richest in total phenolic content and demonstrated the highest potential for overall antioxidant capacity using ferric reducing power assay (FRP), phosphomolybdenum method assay, and free radical scavenging activity (FRSA). Phenolic amounts positively correlated with antioxidant activity tested.

Keywords: nuts, extraction solvent, phenolics, antioxidant activity

Nuts are consumed all over the world, not only as a fruit but also in a diversity of manufactured food products, such as snacks, chocolates, cereals, bakery and dairy products, salads, entrées, sauces, ice creams and other dessert formulations (AMARAL et al., 2006). Main nuts consumed are walnuts, hazelnuts, almonds, cashews, chestnuts, pistachios, and peanuts. In 2004, over 8.5 million tons of nuts were produced throughout the world (OLIVEIRA et al., 2008). Most nuts, left in their shell, have a remarkably long shelf life and can conveniently be stored for winter use (BLOMHOFF et al., 2006). Overall, previous studies have shown nuts to contain different types of phytochemical compounds such as polyphenols, monounsaturated and polyunsaturated fatty acids, and tocopherols (vitamin E) (KORNSTEINER et al., 2006). For instance, almonds are known to contain flavonoids such as catechins, flavonols, and flavonones in their aglycone and glycoside form, while peanuts and pistachios contain flavonoids and have a high concentration of resveratrol in comparison with the other nuts (GENTILE et al., 2007). Walnuts contain a wide variety of phenolics such as tocopherols and non-flavonoids such as ellagitannins (ANDERSON et al., 2001).

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Phenolic compounds have attracted considerable attention in the past few years due to their potential health benefits. There is considerable evidence that antioxidant compounds in fruit, vegetables, and beverages play a pivotal role in the maintenance of health and in the prevention of diseases (PEREIRA et al., 2008). Nuts contain an array of polyphenolic compounds such as flavonoids, proanthocyanidins, and stilbenes (BOLLING et al., 2011).

Nuts are nutritious snacks and a food with ingredients providing both nutrients and bioactive antioxidants. These fruits are an integral part of Mediterranean food patterns and their incorporation into the regular diet of human beings is believed to provide health benefits to the consumer. In Algeria, nut consumption is very important, especially at celebrations. In the present study, four nuts (walnut, hazelnut, almond, and peanut) were used to select the best aqueous solvent (acetone 80%, methanol 80%, or ethanol 80%) for extraction of phenolic compounds as well as to evaluate their antioxidant capacity by the ferric reducing power (FRP) method, phosphomolybdate ammonium method, and measuring free radical scavenging activity (FRSA).

1. Materials and methods

1.1. Samples preparation

Four commonly consumed nuts, walnuts (*Juglans regia*, USA), hazelnuts (*Corylus avellana*, Turkey), almonds (*Prunus amygdalus dulcis*, Spain), and peanuts (*Arachis hypogaea*, China) were purchased from the local market in Bejaia, Algeria. Raw nuts grown organically were manually cracked and shelled then ground with a coffee mill. The powder of each kind of nut was preserved in the refrigerator (4 °C) until analysis.

1.2. Preparation of extracts

Three solvents were used in order to extract the phenolic compounds: methanol/water (80/20, v/v), ethanol/water (80/20, v/v), and acetone/water (80/20, v/v). A quantity of 2 g of each sample was mixed with 20 ml of each solvent. After stirring for 3 min, the mixture was incubated in a water bath (Memmert Wb22, Germany) at 50 °C for 30 min. The extracts were centrifuged (centrifuge NF 200, Nuve, Belgium) at 4000 r.p.m. for 15 min and filtered.

1.3. Phenolic compounds

Total phenolic contents (TPC) were estimated following the method reported by ALASALVAR and co-workers (2009). However, the total flavonoid contents (TFC) were determined according to the method reported by OZSOY and co-workers (2008). Likewise, the proanthocyanidin contents (PAs) was measured according to the method of MAKSIMOVIC and co-workers (2005) using butanol/HCl. The results were expressed as gram gallic acid equivalent per 100 grams (g GAE/100 g) for TPC, as gram of quercetin equivalent per 100 grams (g QE/100 g) for TFC, and as gram cyanidin-3-glucoside equivalent per 100 grams (g C3GE/100 g) for PAs.

1.4. Determination of antioxidant activities

The ferric reducing power (FRP) was measured according to the method of OYAIZU (1986). However, the phosphomolybdenum method assay (PM) was measured according to the

method of PRIETO and co-workers (1999). In addition, the free radical scavenging activity (FRSA) was determined by the method described by SOUSA and co-workers (2008). The results of FRP and PM were expressed as gram of gallic acid equivalent per 100 grams (GAE/100 g), however, the results of FRSA were expressed as percentage (%)

1.5. Statistical analysis

All analyses were carried out in triplicate, and the experimental data were expressed as means \pm standard deviation. The software STATISTICA® 5.5 was used to compare the different results by the analysis of variance with one factor (ANOVA). Differences between the means at * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$ were considered statistically significant.

2. Results and discussion

2.1. Phenolic compounds

Phenolic contents of different extracts showed differences depending on varieties and solvents (Table 1). The concentrations of total phenolic in acetone extracts were higher than those of the methanol and ethanol extracts. Therefore, acetone solvent had the capacity to extract the highest quantity of polyphenols. Total phenolic contents varied between 0.07 (ethanol extract of almond) and 1.650 g GAE/100 g (acetone extract of walnut). Statistically, no significant differences ($P < 0.05$) in phenolic contents were observed between almond and peanut in the acetone extract, and between hazelnut and peanut in ethanol extracts.

Table 1. Phenolic contents of nuts extracted by different solvents (g/100 g)

Nuts	TPC	TFC	PAs
Acetone 80%			
Walnuts	1.65 \pm 0.09 ^a	0.33 \pm 0.00 ^c	0.021 \pm 0.00 ^c
Hazelnuts	0.74 \pm 0.02 ^c	0.41 \pm 0.01 ^b	0.056 \pm 0.00 ^a
Almonds	0.42 \pm 0.01 ^d	0.24 \pm 0.01 ^d	0.031 \pm 0.00 ^b
Peanuts	0.30 \pm 0.00 ^d	0.17 \pm 0.01 ^e	0.011 \pm 0.00 ^d
Methanol 80%			
Walnuts	1.18 \pm 0.00 ^a	0.22 \pm 0.00 ^b	0.011 \pm 0.00 ^c
Hazelnuts	0.41 \pm 0.01 ^b	0.17 \pm 0.00 ^c	0.025 \pm 0.00 ^b
Almonds	0.08 \pm 0.00 ^d	0.06 \pm 0.00 ^e	0.005 \pm 0.00 ^d
Peanuts	0.21 \pm 0.00 ^c	0.09 \pm 0.00 ^d	0.006 \pm 0.00 ^d
Ethanol 80%			
Walnuts	0.66 \pm 0.02 ^a	0.17 \pm 0.01 ^c	0.005 \pm 0.00 ^c
Hazelnuts	0.24 \pm 0.01 ^c	0.25 \pm 0.00 ^b	0.022 \pm 0.00 ^b
Almonds	0.07 \pm 0.01 ^d	0.08 \pm 0.00 ^e	0.004 \pm 0.00 ^{cd}
Peanuts	0.22 \pm 0.00 ^c	0.11 \pm 0.00 ^d	0.003 \pm 0.00 ^d

TPC: total phenols content, TFC: total flavonoid content; PAs: proanthocyanidins
Different superscripts denote statistically different differences at ($P < 0.05$).

It is known that phenolic compounds extraction of plant material depends on the solvent employed. More polar solvent mixtures were used with the aim of obtaining the highest

antioxidant capacity of nuts. The phenolic contents of nuts studied were similar to those obtained by KORNSTEINER and co-workers (2006). However, MIRALIKBARI and SHAHIDI (2008) recorded values widely higher than those found in our study. Also, YANG and co-workers (2009) revealed higher phenolic contents for walnut, hazelnut, almond, and peanut with values of 5.88, 3.15, 2.13, 1.23, and 6.46 g GAE/100 g, respectively. However, the values obtained for the extracts of hazelnut with acetone 80% were lower than those reported by CONTINI and co-workers (2008) (2.06 g GAE/100 g). Furthermore, BOLLING and co-workers (2010) obtained higher levels of total phenolic contents (7.10 g/100 g). BOLLING and co-workers (2011) reported that the phenolic contents of almonds, hazelnuts, and walnuts were 287, 687, and 1576 mg GAE/100 mg, respectively.

As illustrated in Table 1, flavonoid contents varied by nuts and solvents used. Among the samples tested with acetone extraction, hazelnut had the highest flavonoid contents (0.41 g QE/100 g) followed by walnuts, almonds, and peanuts. Methanol extract of almonds had the lowest phenolic amounts (0.006 g QE/100 g). Significant difference ($P < 0.001$) was observed between TFCs of nuts. The flavonoid levels of studied nuts were different from those reported by YANG and co-workers (2009) and CONTINI and co-workers (2008). BOLLING and co-workers (2011) reported that the TFC of almonds, hazelnuts, and walnuts were 15.25, 11.99, and 2.74 mg/100 g, respectively.

Proanthocyanidin content in the nut extracts oscillated between 0.003 (ethanol extract of peanuts) and 0.056 g C3GE/100 g (acetone extract of hazelnut). Statistical analysis revealed significant difference ($P < 0.01$) between the PA contents of the four nuts studied. The acetone extract of hazelnut showed the highest content followed by almond, walnut, and peanut with values of 0.031, 0.021, and 0.011 g C3GE/100 g, respectively. BOLLING and co-workers (2011) reported that total proanthocyanidins of almonds, hazelnuts, and walnuts were 184.02, 500.66, and 67.25 mg/100 g, respectively. In addition, GU and co-workers (2004) reported that the proanthocyanidins contents of hazelnuts, almonds, walnuts, peanuts roasted, and peanut butter were 500.7, 184.0, 67.3, 15.6, and 13.2 mg/100 g, respectively.

These differences could be attributed to several factors such as harvest year, nut variety, process methods, in-shell or without shell, cultivation conditions (soil, fertilizer, temperature, and cultivation technique), environmental conditions, method of extraction, and/or storage conditions.

2.2. Antioxidant activities

The results of FRP, PM, and FRSA are shown in Table 2. Walnuts showed higher reducing capacity than other nut extracts with a value of 1.38 g AAE/100 g. Likewise, methanol extracts of almonds showed the lowest reducing power with a value of 0.04 g AAE/100 g. The antioxidant activity of nuts studied is attributed to their bioactive compounds. These substances, which are electron donors, react with free radicals and convert them to more stable products terminating radical chain reactions. Our results were lower than those reported by ALASALVAR and co-workers (2009) and PEREIRA and co-workers (2008) for almonds and walnuts, respectively.

The values of phosphomolybdenum assay varied between 0.29 (methanol extract of almond) and 1.660 g GAE/100 g (acetone extract of a walnut). However, walnuts had the highest capacity of all the extracts to reduce molybdate. Hazelnuts and peanuts showed similar antioxidant activities. The nature of solvent had no effect on the results of the phosphomolybdenum method of differentiating samples. Data on phosphomolybdenum activity of nuts are not available in the literature.

Table 2. Antioxidant activity of nuts extracted by different solvents

Nuts	FRP (g /100 g)	FRSA (%)	PM (g /100 g)
Acetone 80%			
Walnuts	1.38±0.02 ^a	85.68±4.59 ^a	1.06±0.01 ^a
Hazelnuts	0.54±0.03 ^b	58.7±11.68 ^b	0.78±0.03 ^b
Almonds	0.21±0.01 ^c	15.44±3.18 ^d	0.79±0.01 ^b
Peanuts	0.17±0.00 ^c	10.01±1.55 ^c	0.49±0.10 ^c
Methanol 80%			
Walnuts	1.10±0.01 ^a	58.22±0.93 ^a	1.66±0.02 ^a
Hazelnuts	0.25±0.01 ^c	11.78±0.11 ^b	0.63±0.08 ^{bc}
Almonds	0.04±0.00 ^e	7.62±1.81 ^c	0.29±0.00 ^d
Peanuts	0.09±0.00 ^d	4.65±0.80 ^d	0.55±0.03 ^c
Ethanol 80%			
Walnuts	0.73±0.01 ^a	24.30±0.74 ^b	1.17±0.11 ^a
Hazelnuts	0.21±0.00 ^c	32.34±3.02 ^a	0.74±0.05 ^b
Almonds	0.14±0.00 ^d	4.87±3.95 ^c	0.57±0.02 ^c
Peanuts	0.07±0.00 ^e	2.93±2.04 ^d	0.70±0.02 ^b

FRP: ferric reducing power; FRSA: free radical scavenging activity; PM: phosphomolybdenum method
Different superscripts denote statistically different differences at (P<0.05).

The free radical scavenging capacity was tested using the DPPH method assay, which is used primarily for the evaluation of the antioxidant properties of natural products. DPPH is a stable free radical and accepts an electron or hydrogen to become a stable molecule. Statistical analysis revealed a significant difference (P<0.05) between the antiradical activity of the four nuts studied. These results were in accordance with published data of MIRALIAKBARI and SHAHIDI (2008). Acetone extracts were more effective of DPPH radical scavenging than other extracts, which had the same overall scavenging activity. Likewise, walnuts showed the highest DPPH radical scavenging capacity (85.68%), followed by hazelnuts (58.7%), almonds (15.44%), and peanuts (10.01%).

The difference in the antioxidant capacity of different extracts may be attributed to differences in their phenolics compounds. In addition, the solvent type and the polarity of the extracts may affect the single electron transfer and the hydrogen atom transfer, which are key aspects in the measurements of antioxidant capacity (JAYAPRAKASHA et al., 2008). BOLLING and co-workers (2010) reported that the antioxidant activity of almonds might be more dependent on cultivar than on seasonal differences.

Correlation analysis was used to explore the relationships between different antioxidants measured and the antioxidant activities evaluated (Table 3). The analysis showed a positive relationship between phenolic compounds and the antioxidant activity tested of the three extraction solvents used. Indeed, a positive correlation (P<0.001) was observed between total phenolic content and ferric reducing power (r=0.85 for acetone, r=0.99 for methanol, and r=0.70 for ethanol). Linear correlations (P<0.001) were also obtained between phosphomolybdenum and phenolic content of acetone (r=0.87), methanol (r=0.99), and

ethanol ($r=0.94$) extracts. Hence, the antioxidant capacity of the samples was influenced by the content of their bioactive compounds. This good relationship can be explained by the significant contribution of phenolic amounts to the antioxidant activity tested. These results were in agreement with those reported by YANG and co-workers (2009). Moreover, BOLLING and co-workers (2010) reported that polyphenols identified from almonds showed a strong correlation with DPPH radical scavenging activity.

Table 3. Correlations between phenolic compounds and antioxidant activities

Correlation between	Acetone 80%	Methanol 80%	Ethanol 80%
TFC - PAs	$r=0.87^{***}$	$r=0.82^{***}$	$r=0.95^{***}$
TPC - PM	$r=0.87^{***}$	$r=0.99^{***}$	$r=0.94^{***}$
TPC - FRP	$r=0.85^{***}$	$r=0.99^{***}$	$r=0.70^{**}$

TPC: total phenols content, TFC: total flavonoid content; PAs: proanthocyanidins; PM: phosphomolybdenum method; FRP: ferric reducing power; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

3. Conclusions

The present study demonstrated that walnuts and hazelnuts were rich sources of antioxidants and showed better antioxidant activities than almonds and peanuts. Aqueous acetone extracted more phenolics from nuts than methanol or ethanol. Correlation analysis showed a positive relationship between phenolic content and antioxidant activity. This study showed the potential of using nuts to develop functional foods with high antioxidant activity. Such studies may help elucidate the mechanisms explaining the effect of consuming nuts or their by-products on oxidative stress status and the resulting or associated disease conditions in humans.

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