

Presumptive Relationship between Oxidoreduction Potential and Both Antibacterial and Antioxidant Activities of Herbs and Spices: Oxidoreduction Potential as a Companion Tool for Measuring the Antioxidant Activity

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Abstract

Antibacterial and antioxidant activities form an essential component of the bioactive properties of plant products. The antibacterial and the antioxidant properties of three types of aqueous extracts of four herbs and spices were evaluated. While the total phenolic content, DPPH radical scavenging activity and oxidoreduction potential methods were used for the antioxidant properties determination, the inhibition diameters of extracts toward the growth of *Staphylococcus warneri*, *Bacillus cereus*, *Enterobacter cloacae* and *Proteus vulgaris* were evaluated. Although the phenolic content did not correlate with the antibacterial activity of plant extracts, it linearly correlated ($R^2 = 0.827$) with DPPH scavenging activity of the studied plants following the order: sumac > ginger > rosemary > cinnamon. The oxidoreduction potential values linearly correlated with both the phenolic content ($R^2 \approx 0.88$) and DPPH scavenging activity values ($R^2 \approx 0.96$). The oxidoreduction potential could be proposed as a useful companion tool combined with other techniques when determining the antioxidant activity of plant extracts and food products is considered.

Keywords: antibacterial activity; antioxidant activity; herbs and spices; oxidoreduction potential; phenolic content

Introduction

Herbs and spices have been traditionally added to many foods and used in folk medicine since ancient times by different nations. Their uses were for many objectives such as the improvement of the sensorial properties and the extension of the shelf life of food product by decreasing or eliminating the microbial load in product (Negi, 2012). These plants are characterized by their antimicrobial and antioxidant activities thanks to the richness of chemical composition in active compounds such as organic acids, phenolic compounds, essential oils, sulfur compounds and pigments (Proestos *et al.*, 2005). Many researches have been conducted to characterize these active compounds and their antioxidant and antimicrobial activities as well as their potential applications in pharmaceutical and food products.

The exploring of natural sources of bioactive molecules requires reliable methods for the antioxidant activity evaluation in product (Shahidi and Zhong, 2015). Different methods and tools used for measuring antioxidants activity have remarkably advanced during the last few decades.

Many of these methods have been proposed for the determination of antioxidant activities using different mechanisms and instruments (Shahidi and Zhong, 2015). The difference between these methods is characterized by the mechanism of action, the active reagent applied and the instrument used to log and interpret the data. The free radicals techniques are widely used in *in vitro* antioxidant analysis by researchers thanks to their simplicity and ease of manipulation. Among the free radical methods, the DPPH scavenging activity as well as the phenolic content are the commonly used ones (Alam *et al.*, 2013).

Oxidoreduction potential is defined as a physicochemical parameter that determines the oxidizing/reducing properties of the medium / product. Its value depends on the chemical composition of the food / medium *i.e.* thiol-containing amino acids, peptides, proteins, vitamins, dissolved oxygen, free radical content, phenolic compounds, content of reduced or oxidized couples such as $Fe^{+2/+3}$ and reducing sugars, the pH value, the temperature, and the dissolved oxygen content (Alwazeer *et al.*, 2003).

Although the biological activity of plants was widely studied in the literature but a less works were conducted on the presumed relationship between phenolic content and both the antibacterial and antioxidant activities of herbs and spices extracts (Shan *et al.*, 2007). A relationship assumed between both antioxidant and antibacterial activities and oxidoreduction potential was our first question to investigate. Another question was about the possibility of application of the oxidoreduction potential measurement as a supplementary method when the antioxidant activity is envisaged. To test these hypotheses, we evaluated different analytical measurements of the antibacterial and antioxidant activities for different types of plants and extracts.

The aims of the present study were to evaluate the antibacterial and the antioxidant activities of some herbs and spices commonly used in food preparation for the research of a relationship assumed between the oxidoreduction potential values and both the antimicrobial and the antioxidant activities. The possibility of using the oxidoreduction potential method as a companion tool for the antioxidant properties measurement of extract/product was also investigated.

Materials and Methods

Plant material

Sumac (*Rhus coriaria* L.) fruits, rosemary (*Rosmarinus officinalis* L.) leaves, cinnamon (*Cinnamomum zeylanicum*) barks and ginger (*Zingiber officinalis*) roots were purchased from a local supplier in dried form.

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Aldrich, USA), gallic acid (Lobachemie, India), sodium carbonate (Merck, Germany), glycerol (Merck, Germany), methanol (HPLC grade) and Folin-Ciocalteu (Merck, Germany) reagents were used for carrying out the assays.

Microorganisms, growth media and standard antibiotic discs

A total of four foodborne bacteria were kindly provided by the Department of Microbiology, Faculty of Medicine, University of Homs, Syria. These strains of bacteria were used for antibacterial tests. The test bacteria included Gram-positive *Bacillus cereus* and *Staphylococcus warneri*, Gram-negative *Enterobacter cloacae* and *Proteus vulgaris*. Muller Hinton Agar (Biolab, Hungary) and Muller Hinton Broth (Oxoid Ltd, England) were used in the antibacterial screening test. Gentamicin and Penicillin discs (Oxoid, England) have been used in this study as a standard antibiotic. In preliminary studies, Gentamicin was found to be active against *Enterobacter cloacae* and *Proteus vulgaris*, while Penicillin was active against *Bacillus cereus* and *Staphylococcus warneri*.

Preparation of plant materials prior to extraction

The dried plants were kept in a refrigerator for no longer than three days prior to extraction. Before extraction, the plants were milled into a fine powder using a home mill.

Extraction and preparation of aqueous extracts

Five grams of each plant material were put in an Erlenmeyer, and then 100 mL of distilled water was added. Three extraction methods were employed: (1) maceration in water at room temperature for 24 hours, (2) maceration in water at 50 °C for 24 hours and (3) boiling in water for 2 min. The extracts were filtered using Whatman No. 4 filter paper (Whatman International, UK). The filtrated extracts were then concentrated to the tenth of its volume (1/10) using vacuum drier at 50 °C (Fratelli Galli, Italy). The obtained extracts were stored in a freezer at -18 °C until use. The pH of all samples was determined with a combined pH electrode (Inlab 427, Metler-Toledo).

Determination of antibacterial activity

Preparation of extract- and standard-loaded discs

Filter paper discs (Grade 54, diameter 6 mm, Whatman International, UK) were autoclaved at 121 °C for 20 min and oven-dried at 40 °C overnight. Each aqueous extract (20 µl) was loaded on a sterile filter paper disc. All impregnated discs were dried in sterile glass Petri dishes placed in an oven at 30 °C for 20 min. The discs were then allowed to condition to room temperature before using in the antibacterial test.

Screening of antibacterial activity of plant extracts

The antibacterial activity of extracts was determined by the disc agar diffusion method. Sterilized molten Mueller Hinton agar (20 ml) was dispensed to each sterile disposable Petri dish (diameter 9 cm) and allowed to solidify. Microbial suspension (100 µl) containing approximately 1.0×10^7 CFU was spread evenly onto the surface of the solidified medium. The plates were allowed to dry for 15 min before the test discs were placed at equidistance from each other. Each plate consisted of one standard antibiotic disc and three other discs impregnated with various extracts. After standing for 30 min, the Petri dishes were incubated in an inverted position at 37 °C for 18 to 24 hours. The diameter of the zone of inhibition (mm), defined by the clear area devoid of growth, was measured twice and compared to the diameter of the inhibition zone of the standard antibiotic in the same Petri dish.

Determination of the antioxidant activity

Determination of total phenolic content

The amount of phenolic compounds in the extracts was determined by the Folin-Ciocalteu colorimetric method (Macheix *et al.*, 1991). Determinations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. The total phenolic contents were expressed as mg gallic acid equivalents (mg GAE g⁻¹ extract).

Determination of DPPH free-radical scavenging activity

Antioxidant activity of the prepared extracts was determined with 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay (Singh *et al.*, 2002). The free-radical DPPH was used as an oxidizing molecule to be reduced by the antioxidant compounds present in the extracts. The extracts were dried at 50 °C using vacuum dryer (Fratelli Galli, Italy). One gram of dried extract was dissolved in 100 mL methanol. The same volumes of methanolic dissolved samples and DPPH

solution (60 μ M) were vortexed together. The final solution was allowed to react in the dark for 30 min. It was then centrifuged (5500 rpm (1165 \times g), Engelsdredde/leipzig DDR-7123 Centrifuge, Germany) for 15 minutes. The absorbance of the supernatant was measured at 517 nm with a UV spectrophotometer (Analytica, Germany). The tests were carried out in triplicates. The DPPH free-radical scavenging activity was calculated by the following formula:

$$\text{DPPH free radical scavenging activity (\%)} = [A_0 - A_1] / A_0 \times 100$$

Where A_0 is the absorbance of the control solution containing only DPPH after incubation and A_1 is the absorbance in the presence of plant extract in DPPH solution after incubation.

Determination of oxidoreduction potential values

The oxidoreduction potential (Eh) value of extracts was measured with a combined redox electrode (Pt4805-DXX, Metler-Toledo, France) referred to the Ag/AgCl system and connected to a redox-controlled interface (Consort 835, Belgium). The Eh values were calculated according to Jacob (1970)(Jacob, 1970): $E_h = E_m + E_r$; where E_h is the electrode potential referred to the normal hydrogen electrode, E_m is the measured potential, and E_r is the potential of the reference electrode (Ag/AgCl). The Eh values at pH = 7 (E_{h7}) were obtained after correcting Eh values according to the pH value of the extract as follows:

$$E_{h7} = E_h - 59(7 - \text{pH}_m); \text{ where } \text{pH}_m \text{ is the measured pH value of the extract.}$$

Data and statistical analysis

All experiments were performed in different sets, with

each set in triplicate. Statistical analysis was performed for ANOVA (Analysis of variance). Values of P, which were \leq 0.05, were considered significant.

Results and Discussion

Antibacterial activity of aqueous extracts of plants

Antibacterial activity of aqueous extracts of sumac

Table 1 shows while the maceration 50 °C extracts has no effect against *Enterobacter cloacae* and *Proteus vulgaris*; this extract shows the highest inhibition zone recorded for *Staphylococcus warneri*. Furthermore, while the room temperature and 50 °C maceration extracts have similar effects against *Bacillus cereus*, the boiled aqueous extract shows the highest inhibition effect against these bacteria. It was reported that heating the aqueous extract of sumac fruits to 90 °C did not influence its antibacterial property (Gulmez *et al.*, 2006).

Otherwise, results show that the room temperature extract has the same effect on each *Enterobacter cloacae* and *Proteus vulgaris* (Table 1); whereas *Staphylococcus warneri* is the most sensible strain to this extract followed by *Bacillus cereus*. Other works found, similarly to our findings, the important effect of inhibition of aqueous sumac extracts on Gram-positive bacteria compared to Gram-negative ones (Nasar-Abbas and Halkman, 2004). On the contrary of Gram-positive bacteria, the resistance of Gram-negative bacteria to the sumac extracts could be described by the richness of lipid in the membrane of these bacteria which make it resistant to the diffusion of water-soluble active compounds such as organic acids and tannins inside the bacterial (Sung *et al.*, 2012).

Table 1. Antibacterial activity (diameter of inhibition zone) of aqueous extracts of species studied

Species	Extract type	Diameter of inhibition zone (mm)			
		<i>Bacillus cereus</i>	<i>Staphylococcus warneri</i>	<i>Proteus vulgaris</i>	<i>Enterobacter cloacae</i>
Sumac	Maceration at room temperature	^a 12.50 \pm 0.58 ^C	^a 16.88 \pm 1.03 ^B	^a 7.88 \pm 0.63 ^A	^a 8.63 \pm 0.48 ^A
	Maceration at 50 °C	^a 12.13 \pm 0.86 ^C	^b 20.50 \pm 0.58 ^B	^b 00 ^A	^b 00 ^A
	Boiled aqueous	^b 13.50 \pm 0.58 ^C	^a 16.38 \pm 0.48 ^B	^a 7.38 \pm 0.48 ^A	^a 8.13 \pm 0.63 ^A
	Penicillin	25	35	-	-
	Gentamicin	-	-	10	20
Rosemary	Maceration at room temperature	^a 9.75 \pm 0.65 ^D	^a 12.75 \pm 0.50 ^C	^a 8.00 \pm 0.70 ^B	^a 00 ^A
	Maceration at 50 °C	^a 9.63 \pm 0.48 ^D	^a 12.88 \pm 0.25 ^C	^a 8.00 \pm 0.00 ^B	^a 00 ^A
	Boiled aqueous	^a 10.25 \pm 0.29 ^D	^a 12.25 \pm 0.50 ^C	^a 8.88 \pm 0.63 ^B	^a 00 ^A
	Penicillin	25	35	-	-
	Gentamicin	-	-	10	20
Cinnamon	Maceration at room temperature	^a 10.25 \pm 0.50 ^C	^a 14.88 \pm 0.85 ^B	^a 00 ^A	^a 00 ^A
	Maceration at 50 °C	^a 10.5 \pm 0.58 ^D	^a 16.5 \pm 1.23 ^C	^b 7.25 \pm 0.50 ^B	^a 00 ^A
	Boiled aqueous	^a 10.63 \pm 0.75 ^C	^a 17.00 \pm 1.16 ^B	^a 00 ^A	^a 00 ^A
	Penicillin	25	35	-	-
	Gentamicin	-	-	10	20
Ginger	Maceration at room temperature	^a 00 ^A	^a 8.75 \pm 0.96 ^B	^a 7.75 \pm 0.29 ^B	^a 00 ^A
	Maceration at 50 °C	^a 00 ^A	^b 7.25 \pm 0.50 ^B	^b 7.00 \pm 0.00 ^B	^a 00 ^A
	Boiled aqueous	^a 00 ^A	^{ab} 7.88 \pm 0.25 ^B	^c 0 ^A	^a 00 ^A
	Penicillin	25	35	-	-
	Gentamicin	-	-	10	20

Each value represents mean \pm SD (n = 4). The same small letters in the same column for each species show no significant effects between extracts for the indicated strain at p < 0.05 according to Tukey. The same capital letters in the same line show no significant effects between bacterial strains for the indicated extract at p < 0.05 according to Tukey.

Antibacterial activity of aqueous extracts of rosemary

While the three types of aqueous extracts of rosemary show no significant effect of inhibition on *Enterobacter cloacae*, the other studied strains are inhibited when exposed to rosemary extracts but with a similar effect of the extraction method ($P < 0.05$) (Table 1). On the other hand, results show a significant difference ($P < 0.05$) among the bacteria for the same type of extract indicated; with *Enterobacter cloacae* among other studied strains is the most resistant, while *Staphylococcus warneri* is the most sensitive one (Table 1). The Gram-positive strains are then more sensitive than Gram-negative ones for rosemary aqueous extracts. These findings agree with results obtained by Moreno *et al.* (2006) who found that while water extract of rosemary exhibited an antibacterial activity against *S. aureus* (Gram-positive) it did not show any antibacterial activity against *E. coli* (Gram-negative) (Moreno *et al.*, 2006).

Antibacterial activity of aqueous extracts of cinnamon

Results show no significant effect of extraction method on the antibacterial activity except the 50 °C maceration extract which demonstrates an inhibition against *Proteus vulgaris* (Table 1). On the other hand, findings show that *Staphylococcus warneri* is the most sensitive strain followed by *Bacillus cereus* (Table 1). The antimicrobial activity of cinnamon is generally attributed to its essential oils content. This could explain the low antibacterial activity of the aqueous extracts of cinnamon prepared in this study due to its low essential oils content. Similar results reported by Mukhtar and Ghori (2012) who revealed an antibacterial activity of aqueous extract of cinnamon (10-40%, macerated at 40-50 °C) against *B. subtilis*, whereas no effect was observed for *E. coli* (Mukhtar and Ghori, 2012).

Antibacterial activity of aqueous extracts of ginger

Results show no effect of the ginger aqueous extract on the antibacterial activity of *Enterobacter cloacae* and *Bacillus cereus* (Table 1). Otherwise, the room temperature and 50°C maceration extracts exhibit significant inhibitory effect against both *Proteus vulgaris* and *Staphylococcus warneri* compared to other extract types with a highly significant effect for the room temperature aqueous extract (Table 1). The low antibacterial activity of boiled water extract of ginger could be explained by the fact that some antibacterial components found in spice plants such as ginger are heat labile (Chen *et al.*, 1985). The cold-water extract of ginger was reported to have an inhibition effect on both *Staphylococcus aureus* and *Pseudomonas aeruginosa* at different concentrations studied (Chrubasik *et al.*, 2005).

Correlation between phenolic content and antibacterial activity of all types of plant extracts

In the present study, a correlation analysis was conducted to explore the relationships assumed between phenolic content and antibacterial activity of plant extracts studied using a regression analysis method. This analysis showed that the results of the phenolic content values don't correlate with the antibacterial activity found in our study (data not shown). From these findings we couldn't correlate the inhibition effect of extracts against the studied bacterial

strains only with the presence of phenolic content in extracts. Essential oils (EOs) were described as the principal responsible for the antimicrobial activities in plants, herbs and spices (Tajkarimi *et al.*, 2010). Another hypothesis to interpret the absence of defined correlation between the phenolic content and antibacterial activity could be explained by the reduced effectiveness attributed to the use of crude extracts instead of pure compounds, as these crude extracts generally contain flavonoids in glycosidic form where the sugar present in them decreases inhibition effectiveness against some bacteria (Negi, 2012).

On the other hand, a study found positive linear correlations between antibacterial activity and total phenolic content of 46 extracts from dietary spices and medicinal herbs (Shan *et al.*, 2007). The R^2 values were between 0.93 and 0.73, and decreased in the following order: *S. aureus* > *B. cereus* > *E. coli* > *S. anatum* > *L. monocytogenes*. This difference of results between our study and the latter study prove the arguments about the effect of extraction method on the potency of antibacterial activity of phenolic compounds. The extraction solvent of the latter work was 80% methanol that produced different active compounds extracted compared to our method. The identity of phenolic compounds extracted changes according to the solvent and the extraction factors applied. It was reported in literature that the acidified aqueous methanol solvent was very effective for the extraction of conjugate phenolic compounds (the major phenolics in plants) compared to other solvents (water, methanol, ethanol, acetone) used singly or in aqueous form (Wang *et al.*, 2017).

*Antioxidant activity of aqueous extracts of plants**Total phenolic compounds content of extracts*

The content of the phenolic components of the studied spices differs according to both the extraction method and spice type (Table 2). The phenolic content of the studied plants was as follows: sumac > ginger > rosemary > cinnamon. These findings confirm with the results reported by Ünver *et al.* (2009), who demonstrated that the total phenolic contents of sumac was higher than rosemary when extraction was conducted in mixture of 90% methanol + 9% water + 1% acetic acid at 24 °C for 24 h (Ünver *et al.*, 2009). Results show also that the room temperature aqueous extracts has the lowest content of phenolic compounds followed with a similar effect of both 50 °C maceration and boiled aqueous extracts (Table 2). This increase of the phenolic compounds with the increase of temperature could be explained by the fact that the temperature of extraction led to the increase of the liberation of some phenolic molecules from spice. On the other hand, the low content of phenolic content in heated extracts of cinnamon could be explained by the formation of gelatinous extract which led to difficulties in the filtration and maybe in the separation of phenolic compounds.

Measurement of DPPH free-radical scavenging activity

Results presented in Table 3 demonstrate an increase in the DPPH free-radical scavenging activity of sumac, rosemary and ginger extracts with the increase of the temperature of extraction.

Table 2. The total phenolic content of different aqueous extracts of sumac, rosemary, cinnamon and ginger

Species	Extraction method	Phenolic content (mg GAE g ⁻¹ extract)
Sumac	Maceration at room temperature	^a 41.45 ± 1.44 ^A
	Maceration at 50°C	^b 49.97 ± 0.85 ^A
	Boiled aqueous	^b 51.63 ± 0.96 ^A
Rosemary	Maceration at room temperature	^a 31.67 ± 0.15 ^B
	Maceration at 50°C	^{ab} 34.34 ± 1.54 ^B
	Boiled aqueous	^b 37.08 ± 0.48 ^B
Cinnamon	Maceration at room temperature	^a 26.56 ± 0.62 ^C
	Maceration at 50°C	^b 22.68 ± 0.51 ^C
	Boiled aqueous	^b 24.17 ± 0.07 ^C
Ginger	Maceration at room temperature	^a 41.76 ± 0.08 ^A
	Maceration at 50°C	^b 43.04 ± 0.46 ^D
	Boiled aqueous	^b 43.68 ± 0.22 ^D

Each value represents mean ± SD (n=4). The same small letters for the same species show no significant effects between extracts at p < 0.05 according to Tukey. The same capital letters show no significant effects between all species for the indicated extract type at p < 0.05 according to Tukey.

Table 3. DPPH free-radical scavenging activity of different aqueous extracts of sumac, rosemary, cinnamon and ginger

Species	Extraction method	DPPH free-radical scavenging activity (%)
Sumac	Maceration at room temperature	^a 75.29 ± 0.68 ^A
	Maceration at 50°C	^b 81.36 ± 0.67 ^A
	Boiled aqueous	^c 84.84 ± 0.83 ^A
Rosemary	Maceration at room temperature	^a 69.24 ± 0.19 ^B
	Maceration at 50°C	^{ab} 71.75 ± 1.74 ^B
	Boiled aqueous	^b 74 ± 0.01 ^B
Cinnamon	Maceration at room temperature	^a 38.17 ± 0.70 ^C
	Maceration at 50°C	^b 32.95 ± 1.32 ^C
	Boiled aqueous	^{ab} 34.70 ± 1.36 ^C
Ginger	Maceration at room temperature	^a 71.62 ± 0.43 ^D
	Maceration at 50°C	^b 73.95 ± 0.09 ^B
	Boiled aqueous	^c 76.10 ± 0.15 ^B

^a Each value represents mean ± SD

Same small letters show no significant effects between extracts for the indicated species at p < 0.05 according to Tukey.

Same capital letters show no significant effects between different species for the indicated extract at p < 0.05 according to Tukey.

Many parameters were generally reported to have an effect on the amount and composition of antioxidants in extracts such as solvent, ratio of solvent to product, temperature and extraction time (Makanjuola, 2017). However, the room temperature aqueous extract of cinnamon exhibits a higher value of DPPH inhibition than boiled aqueous with 38.17 and 34.70%, respectively (P < 0.05). This dissimilarity in results between cinnamon and other spices could be due to the formation of gelatinous extract in the case of cinnamon which led to difficulties in filtration and maybe in the separation of antioxidant compounds like phenolic compounds.

The potency of DPPH free-radical scavenging activity of the studied plants was as follows: sumac > ginger ≈ rosemary > cinnamon. Similar results showed that the antioxidant activity of sumac determined by DPPH method for the methanol/acetic acid/water extracts was higher than sage and rosemary (Ünver *et al.*, 2009).

Determination of the oxidoreduction potential values

Oxidoreduction potential (or Eh) is used generally to determine the redox value of the medium/product. This value represents the sum of the total oxidant/reductant

compounds found in the medium/product. The higher the oxidant compounds concentration is, the higher the Eh value of the medium is, and *vice versa*. The antioxidant activities of studied spices according to Eh method are as follows (Table 4): sumac > ginger > rosemary > cinnamon.

The Eh₇ values of sumac extracts are lower (more reducing properties) than other spices (Table 4). This could be explained by the high phenolic content of sumac extracts compared to other plants that could confirm this hypothesis. Otherwise, the decrease of the Eh₇ value is observed when the temperature of extraction increases except cinnamon. This maybe occurs due to the liberation of some reducing property molecules such as phenolic compounds especially flavonoids, vitamin C and molecules containing -SH group during the heating phase.

Correlation between DPPH assay and oxidoreduction potential values

The correlation coefficient (R²) between DPPH assay and oxidoreduction potential values of the aqueous extracts of the studied plant was determined (Fig. 1A). Results show a good positive linear correlation between DPPH assay and oxidoreduction potential values (R² ≈ 0.96).

Table 4. Oxidoreduction potential values of different aqueous extracts of sumac, rosemary, cinnamon and ginger

Species	Extraction method	Oxidoreduction potential (Eh ₇ , mV)
Sumac	Maceration at room temperature	+ 63
	Maceration at 50°C	+ 55
	Boiled aqueous	+ 52
Rosemary	Maceration at room temperature	+ 96
	Maceration at 50°C	+ 91
	Boiled aqueous	+ 88
Cinnamon	Maceration at room temperature	+ 152
	Maceration at 50°C	+ 180
	Boiled aqueous	+ 179
Ginger	Maceration at room temperature	+ 66
	Maceration at 50°C	+ 65
	Boiled aqueous	+ 65

When the value of DPPH scavenging activity of extracts increases, their Eh value decreases. For example, the lowest Eh₇ value is for boiling extract of sumac with +52 mV and a DPPH value of 84.84%; whereas, the highest Eh₇ value is for 50 °C maceration of cinnamon with +180 mV and a DPPH value of 32.95% (Table 4). The antioxidant activity of extracts combines with the high content of bioactive molecules possessing low oxidoreduction potential values such as phenolic compounds, vitamin C and E, molecules containing -SH group. These bioactive molecules with reducing properties are responsible for the decrease of Eh value of the medium.

Correlation between phenolic content assay and oxidoreduction potential values

To correlate the results obtained from the phenolic content assay and oxidoreduction potential methods, a regression analysis (correlation coefficient, R²) was performed (Fig. 1B). Results show a positive linear correlation between phenolic content values and oxidoreduction potential ones (R² ≈ 0.88). When the phenolic content of the extract increases its Eh₇ value decreases.

Good correlations were found between redox potential values and antioxidant properties using cyclic voltammetry method (Firuzi *et al.*, 2005). Authors found an inverse correlation between FRAP assay and electrochemical measurement values, and they concluded that while good correlations were not found between the total number of hydroxyl groups of flavonoids and FRAP values (R = 0.656) nor electrochemical values (R = 0.676), good correlations were found in the group of flavonols between these parameters (R = 0.96). Other researchers reported that the antioxidant activity of flavonoids is inversely proportional to their oxidoreduction values *i.e.* the lower oxidoreduction value of flavonoids is, the higher the antioxidant is (Yang *et al.*, 2001). The results of the two latter reports are in agreement with our findings.

Correlation between phenolic content and DPPH activity values of plant extracts

When subjecting the results of DPPH scavenging activity and phenolic content for all plant extracts studied to the regression analysis, a high correlation coefficient was observed between the phenolic content and the DPPH scavenging activity (Fig. 1C). Results show a positive linear

relationship between phenolic content and DPPH activity values for all types of plant extracts studied (R² = 0.827) indicating that phenolic compounds are the major contributors to the antioxidant properties of these plants. A study reported that the contribution of the different phenolic groups reached 87% of the antioxidant activity in pomegranate juice (Gil *et al.*, 2000). The latter finding was supported by another work in which the antioxidant capacity of tannins was reported to be 15-30 times more effective at quenching peroxy radicals than simple phenolics or Trolox (Gil *et al.*, 2000).

The linear reliability between the content of total phenolic compounds and the antioxidant activity of plant extracts has been similarly proved, like our finding, by some researchers. Wong *et al.* (2006) reported a significant and linear correlation coefficient between the antioxidant activity and the total phenolic content in both aqueous (R² = 0.7917, FRAP) and methanol (R² = 0.7584, FRAP) extracts of 30 Chinese medicinal plants, with phenolic compounds were thus a major contributor of antioxidant activity (Wong *et al.*, 2006). Similarly, a positive significant and linear correlation between antioxidant activity and total phenolic content of aqueous and methanolic extracts of 112 traditional Chinese medicinal plants (all R² values ≥ 0.95, DPPH)(Cai *et al.*, 2004), aqueous ethanolic extracts of some Algerian medicinal plants (R² = 0.7931, DPPH)(Djeridane *et al.*, 2006), Labiatae spice family (R² = 0.91, FRAP)(Wojdyło *et al.*, 2007), aqueous extracts of 30 plant of industrial interest (R² = 0.939, DPPH; R² = 0.966, ABTS and R² = 0.906, FRAP)(Dudonné *et al.*, 2009), methanolic extracts of Mediterranean herbs and aromatic plants (R² = 0.70-0.83, DPPH), aqueous extracts of 70 medicinal plants (R² = 0.9825, FRAP)(Katalinic *et al.*, 2006) was reported. These reports confirm the presence of significant linear correlation between the free radical scavenging activity determined by the DPPH, ABTS and FRAP methods, and total polyphenolic compounds (phenolic and flavonoids).

On the other hand, among three radical scavenging methods (Free radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion scavenging activity) used to determine the scavenging activity of plants, the highest correlation coefficients were found between the phenolic content and the DPPH scavenging activity, followed by the hydroxyl radical scavenging activity (Parejo *et al.*, 2002).

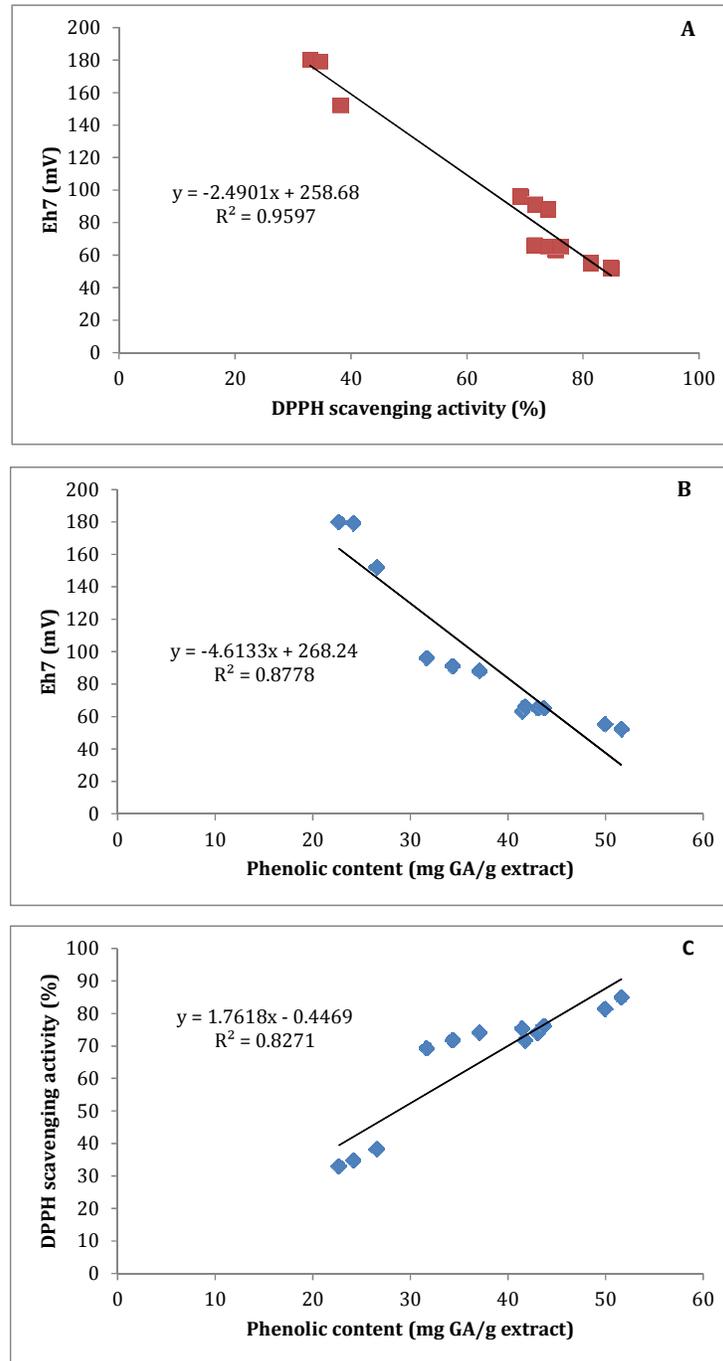


Fig. 1. Correlations between DPPH assay and oxidoreduction potential values (A), phenolic content and oxidoreduction potential values (B), and phenolic content and DPPH activity values (C) for all types of species extracts

This observation was confirmed by another study that demonstrated a weak correlation ($R^2 = 0.58$) between the phenolic content of different fruit juices and the total antioxidant activity estimated by the FRAP assay (Imeh and Khokhar, 2002).

The latter results elucidate the possibility of prediction of DPPH scavenging activity of a medium when its phenolic content is evaluated thanks to the ability of the two methods to donate hydrogen atoms (Katsube *et al.*, 2004).

It is important to indicate that the Folin-Ciocalteu reagent used in the phenolic content assays exhibits an ability to react with other compounds such as vitamins (ascorbic acid, folic acid, folinic acid, NADH, pyridoxine, retinoic acid, thiamine and Trolox (ascorbic and retinoic acids has the greatest reactivity), amino acids (tyrosine, tryptophan and cysteine), thiols, inorganic ions Fe^{+2} , Mn^{+2} , I^- and SO_3^{-2} (Everette *et al.*, 2010). Authors concluded that the Folin-Ciocalteu assay is a method of the determination of total antioxidant activity rather than phenolic content.

Phenolics, known as the widest abundant antioxidants in most plants, are generally classified into non-soluble phenolic compounds such as tannins, cell-wall bound phenolic compounds such as hydroxycinnamic acids, and soluble phenolic compounds such as phenolic acids, flavonoids and quinones (Rispaïl *et al.*, 2005).

The aqueous extraction of active compounds from plants could be described as a good choice, such the case of our study, if we take into account the free fraction of phenolic compounds dissolved in water and possesses high antioxidant activities compared with the soluble conjugate and insoluble fractions that generally require an acidified organic solvent (Sun *et al.*, 2012). Our findings could be also supported by another work where a positive correlation between antioxidant activity and total free phenolics ($R^2 = 0.96$) for seven cultivars of blueberry was found (Wang *et al.*, 2017). The latter study reported that the total conjugated phenolics compounds significantly correlated with both DPPH and FRAP showing R^2 value of 0.84 and 0.88, respectively.

Conclusions

In the present study, it was observed that the Gram-positive bacteria were more sensitive than Gram-negative bacteria toward the aqueous extracts of plant studied. It appears that the bioactive compounds extracted responsible for the antibacterial activity found in the aqueous extracts of the studied plant differ according to both the plant and extraction type. Nonlinear correlation has been observed between the phenolic content and the antibacterial activity of plant extracts prepared in this study. It was observed also that the antioxidant activity of the studied plants depends on the temperature of the extraction procedure. This increase in antioxidant activity was consistent with the increase of the phenolic content.

Many techniques have been developed to measure the antioxidant properties in food and biological systems using different methods such as the free radical scavenging activity, phenolic content and reducing/oxidizing capacity. Our finding proved a positive linear relationship between oxidoreduction potential values and those of both DPPH and phenolic content assays. Since previous studies advised at least two methods when screening the antioxidant activity of product (Schlesier *et al.*, 2002), we can then recommend to combine the oxidoreduction potential measurement method (reducing/oxidizing power) with other different antioxidant techniques such as FRAP, ABTS and DPPH assays generally applied when the measurement of antioxidant properties of samples is considered. Combination of oxidoreduction potential measurement with these methods can be a useful and easy tool for valid assessment of antioxidant activity.

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