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ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES OF DIFFERENT EXTRACTS OF GARDEN CRESS (*Lepidium sativum* L.)

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ABSTRACT

Two extracts from *Lepidium sativum L.* seed were studied in terms of phenolic compounds content, for their antioxidant and antibacterial properties. The extraction was done using ethanol 80% with assist of ultrasonic and by distilled water with assist of microwave. Total phenolics and total flavonoids were measured using Folin-Ciocalteu reagent and $AlCl_3$, respectively. Total antioxidant capacity of the extracts was estimated by different methods including DPPH (1,1-diphenyl-2-picrylhydrazyl radical), $ABTS^{\bullet+}$ (2,2` azino bis-(3-ethyl benzthiazoline-6- sulfonic acid), β-carotene/linoleic bleaching, and ferric reducing antioxidant power (FRAP). Antibacterial activity of extracts was determined using pathogen gram positive and gram negative bacteria by disc assay method. Extracts exhibited both antioxidant (54.66 % of β-carotene/linoleic bleaching assay) and antibacterial activities (20 mm with *Salmonella Enteritidis*) that were comparable to the synthetic antioxidants TBHQ. Therefore, these extracts could be used as preservative ingredients in the food and/or pharmaceutical industries. Data from this study could be used for developing natural antioxidants and bioactive agents to improve human health.

Key words: *Lepidium sativum*, phenolic compounds, antioxidant activity, antibacterial activity.

INTRODUCTION

Lepidium sativum L., commonly known as garden cress (GC) is a member of Brassicaceae family. It is indigenous to south west Asia and was referred to over many centuries ago in Western Europe (Sharma and Agarwal, 2011). The plant needs minimal agricultural resources, grows well in semi-arid regions and does not require much fertilizer (Diwakar *et al.*, 2008). So that, this plant can be cultivated in the new reclaimed soil in Egypt. It was consumed in Persia even before bread was known (Mahdi and Navaei, 2006). GC is a cool season annual and is an erect herbaceous plant which grows up to 50 cm rapidly and its seeds can be harvested within 70-90 days to give 800–1000 kg/hectare (Diwakar *et al*., 2008). The seeds, leaves and roots of GC have economic value but its crop is mainly cultivated for its seeds (Mohammed, 2013).

The use of plant extracts for the treatment of various diseases and for minimizing the effects of chemotherapeutic agent is growing (Jouad *et al.*, 2001). There is evidence that cress seed extract has antimicrobial, antihypertensive, antioxidant, antispasmodic, antidiarrheal, antiasthmatic, hypoglycemic, and hypolipidemic properties (Adam *et al.*, 2011; Rehman *et al.*, 2012; Gilani *et al.*, 2013). Plant mucilages are also used for thickening, binding, disintegrating, emulsifying, suspending, stabilizing, and as gelling agents (Malviya, 2011). These characteristics are related to their structural properties and metabolic functions in food, pharmaceutical, cosmetic, textile and biomedical products (Nishinari *et al.*, 2000).

Boiled seeds and the ground seeds are used in health drink formulations with added honey or in hot milk (Wadhwa *et al*., 2012). GC seeds contain approximately 21.54% oil (solvent extracted) and are a rich source of ω-3 fatty

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acid. The main fatty acids in garden cress seed oil (GCO) are α-linolenic acids (34%) and oleic (22%). The total tocopherol and carotenoid content of GCO are 327.42 and 1.0 µg/100 g oil, respectively (Diwakar *et al.,* 2010). The total phytosterol concentration in cress oil is 14.41 mg/g, which mainly consists of sitosterol, campesterol, and avenasterol (Moser *et al.*, 2009). GCO is considered to be fairly stable oil, since its component natural antioxidants (tocopherol, phytosterol, and carotenoids) protect the oil from rancidity (Diwakar *et al.*, 2010). GCO supplementation in the human diet is efficient in depressing cholesterol, triglycerides, α-linolenic acid and arachidonic acid levels in serum and liver tissues, and for converting linoleic acid into the long-chain fatty acids, eicosapentaenoic acid and docosahexaenoic acid in serum, liver, heart and brain tissue (Diwakar *et al.,* 2008).

Alkaloids, flavonoids, cardiotonic glycosides, glucosinolates, sterols, tannins, and triterpenes are important phytochemical constituents, which impart pharmacological characteristics to garden cress seed (Ghante *et al.,* 2011). A four week treatment with 1 g GC seed powder (three times a day orally) improves the clinical symptoms and severity of asthmatic attacks and various other pulmonary functions in asthmatic patients (Archana and Mehta, 2006). Phenolic constituents such as sinapic acid and sinapin have been isolated from the methanolic extract of defatted seeds (Schultz and Gmelin, 1952). Cress seed extracts were widely studied as food hydrocolloid and medicinal plant products. The seed mucilage has been used as a replacement for tragacanth and Arabic gum. GC seed methanolic extract has also been used in combination with some antibiotics such as penicillin G and erythromycin to combat the antibiotic resistance of *Pseudomonas aeruginosa* (Aburjai *et al.,* 2001). In another study, the antimicrobial activity of the petroleum ether, methanol and water extracts of GC seed extracts was demonstrated against six opportunistic pathogens namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and one fungus *Candida albicans*. Petroleum ether has been shown to be the best solvent for extracting antimicrobial substances from GC seed compared with methanol and water (Adam *et al.,* 2011).

Antioxidant compounds such as flavonoids, tannins, coumarins, curcumanoids, xanthons, phenolics, lignans and terpenoids are found in various plant parts (*e.g.* fruits, leaves, seeds and oils). For this reason, there is growing interest in separating these plant antioxidants and using them as natural antioxidants. Processing of fruits, vegetables and oilseeds results in high amounts of waste materials such as peels, seeds, stones and oil seed meals (Jeong *et al.,* 2004). Extraction yield is dependent on both solvent and method of extraction (Pinelo *et al.,* 2004). Water and aqueous mixtures of ethanol, methanol and acetone are commonly used in plant extraction (Goli *et al.,* 2005). Wang and Helliwell (2001) reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. However, in another study, water was found to be a better solvent than methanol 80% or ethanol 70% for extracting tea catechins (Sun and Ho, 2005).

Garden cress (GC) was studied in the term of phenolic compounds, antioxidant and antibacterial activity. However, most of previous studies focused on using solvent extraction at room temperature to extract bioactive compounds. In the current research, this study examined the impact of different extraction assisted techniques including ultrasonic and microwave on the levels of phenolics as well as the antioxidant and antibacterial activity of GC.

MATERIALS AND METHODS

Materials and Reagents

Garden cress seeds (*Lepidium sativum* L.) were obtained from a local Egyptian markets. Tert-butyl hydroquinone (TBHQ), 1,1-Diphenyl-2picrylhydrazyl (DPPH$^{\cdot}$), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), β-carotene, gallic acid and quercetin were purchased from Sigma (St. Louis, MO, USA). Bacterial strains of *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ScottA, *Salmonella Enteritidis* PT4, *Escherichia coli* ATCC 8739 and *Serratia marcescens* were obtained from Egyptian Culture Collection (Cairo, Ain Shams University, MERCIN). All other chemicals used were analytical grade.

Preparation of the Ultrasonic-Assist Ethanol Extract

Seed sample was dried in a vacuum oven at 40°C and ground to a fine powder in a mill. Ground material (10 g) was extracted individually with (100 ml) ethanol 80% with assist of ultrasonic at power 140 watt (40Khz) at 40°C for 30 min followed by filtration through filter paper Whatman No. 1. Combined filtrate was evaporated in a rotary evaporator (BÜCHI-Rotavapor R-124 and water bath-B-480) below 40°C. The residue was freeze-dried (Thermo-Electron Corporation - Heto power dry LL300 Freeze Dryer). The dried extract was weighed to determine the yield and stored at -20°C until further use.

Preparation of the Microwave-Assist Water Extract

Ground seed (10 g) was extracted individually with (100 ml) distilled water with assist of DAEWOO microwave device at power 40% (medium low) for 60 min followed by filtration through Whatman No. 1 filter paper. The temperature in the beginning was 40°C, then at the end was 80°C. Combined filtrate was freeze-dried. The dried extract was weighed to determine the yield and stored at -20°C until further use.

Determination of Phenolic Compounds

The concentration of total phenols in two extracts were measured by a UV spectrophotometer (Jenway – UV – VIS Spectrophotometer), based on a colorimetric reduction of the reagent by phenolic compounds *via* the formation of a blue complex, as described by Škerget *et al.* (2005). The used oxidizing reagent was Folin-Ciocalteu reagent (AOAS, 1990). To 0.5 ml of diluted extract (10 mg in 10 ml solvent) 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 ml of Na_2CO_3 (75 g/l) were added. The sample was incubated for 5 min at 50°C then cooled. For a control sample, 0.5 ml of distilled water was used. The absorbance was measured at 760 nm. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$$y = 0.0149x + 0.0997$$
$$R^2 = 0.9969$$

Where y is the absorbance and x is the concentration (mg GAE g^{-1} extract).

Determination of Total Flavonoids

Total flavonoid content was determined by the method of Ordon *et al.* (2006). A 1.5 ml aliquot of 20 g l^{-1} $AlCl_3$ ethanolic solution was added to 0.5 ml of extract solution (10 mg extract in 10 ml solvent). After 60 min of addition, the absorbance was measured at 420 nm at room temperature. A yellow color indicated the presence of flavonoids. Total flavonoid content expressed as quercetin equivalent (QE) was calculated using the following equation based on the calibration curve:

$$y = 0.0217x + 0.037$$
$$R^2 = 0.9964$$

Where x is the absorbance and y is the concentration (µg QE).

Determination of DPPH Radical-Scavenging Activity

The electron donation ability of the obtained extracts was measured by bleaching of the $DPPH^{\cdot}$ purple colored solution according to the method of Hanato *et al.* (1988). One hundred µl of each extracts (10 mg extract /10 ml solvent) was added to 3 ml of 0.1 mM $DPPH^{\cdot}$ dissolved in ethanol or methanol according to the solvent used for extraction. After incubation period of 30, 60 and 120 min at room temperature, the absorbance was measured against a control at 517 nm (Gulcin *et al.*, 2004). Percentage of antioxidant activity of free radical $DPPH^{\cdot}$ was calculated as follow:

Antioxidant activity (Inhibition %) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. TBHQ was used as a positive control. Samples were analyzed in triplicate.

Determination of ABTS Radical-scavenging Activity

For the $ABTS^{\bullet+}$ assay, the method of Re *et al.* (1999) was adopted. The stock solutions were

7 mmol l^{-1} ABTS solution and 2.4 mmol l^{-1} potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 hr., at room temperature in the dark. One ml of the resulting $ABTS^{\bullet+}$ solution was diluted with 50 ml of methanol. $ABTS^{\bullet+}$ solution was freshly prepared for each assay. Ten µl of each extract (10 mg extract /10 ml solvent) and (TBHQ solution) were allowed to react with 5 ml of $ABTS^{\bullet+}$ solution for 7 min, then the absorbance at 734 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows:

$ABTS^{\bullet+}$ radical-scavenging activity (%) = $[(A_{control} - A_{sample})/ A_{control}] \times 100$

Where $A_{control}$ is the absorbance of $ABTS^{\bullet+}$ radical+methanol and A_{sample} is the absorbance of $ABTS^{\bullet+}$ radical+extract/synthetic antioxidant.

Determination of β-Carotene/linoleic acid Bleaching

The ability of extracts and synthetic antioxidants to prevent the bleaching of β-carotene was assessed as described by Keyvan *et al.* (2007). Two mg of β-carotene in 10 ml of chloroform, 200 mg of linoleic acid and 200 mg of Tween 20 were placed in a round-bottom flask. After removal of the chloroform, 500 ml of distilled water was added and the resulting mixture was stirred vigorously. Aliquots (3 ml) of the emulsion were transferred to tubes containing extract or synthetic antioxidant. Immediately after mixing 0.5 ml of extract solution (10 mg extract /10 ml solvent), an aliquot from each tube was transferred to a cuvette and the absorbance at 470 nm was recorded (A^0). The remaining samples were placed in a water bath at 50°C for 2 hr., then the absorbance at 470 nm was recorded (A^{120}). A control with no added extract was also analyzed. Antioxidant activity was calculated as follows:

Antioxidant activity (%)=$[1-(A^0_{sample}-A^{120}_{sample})/ (A^0_{control} - A^{120}_{control})] \times 100$

Determination of Ferric Reducing Antioxidant Power (FRAP)

Reducing power of extracts was measured by method of Gülçin *et al.* (2010). The reduction of Fe^{+3} to Fe^{+2} was determined by measuring absorbance of the Prussian blue complex. For this purpose, 0.1 ml of each extracts (10 mg extract/10 ml solvent) mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml (1%) of potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 20 min. of incubation, the reaction mixture was acidified with 1 mL of trichloroacetic acid (10%). Finally, 250 µl of $FeCl_3$ (0.1%) was added to this solution. Distilled water was used as blank and for control. Absorbance of this mixture was measured at 700 nm using a UV spectrophotometer. Decreased absorbance indicates ferric reducing power capability of sample.

Disc Assay Test

Antibacterial activity was assayed as described by Bauer *et al.* (1966) using nutrient agar media (lab-lemco powder) poured into Petri dishes. Two tested extracts were applied at one concentration (1 mg/ml) to Petri dishes containing nutrient agar infected with two pathogenic Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) and three Gram-negative bacteria (*E. coli*, *S. Enteritidis* and *S. marcescens*), incubated at 37°C for 12 hr., and the diameter of the resulting inhibition zones are recorded. The disc was saturated with 10 µl of extract solution. Negative control was exactly prepared as the treatments except that extract was replaced by the solvent that is used in extraction (ethanol 80% and distilled water). Positive control was exactly prepared as the treatments except that extract was replaced by TBHQ. It was observed that negative control samples did not produce any inhibition zones (data not shown).

RESULTS AND DISCUSSION

In foods containing lipids, antioxidants delay the onset of oxidation or slow the rate at which it proceeds. These substances can occur as natural constituents of foods, but they can also be intentionally added to products or formed during processing. Their role is not to enhance or improve the quality of foods, but they do maintain food quality and extend shelf life. Antioxidants for use in food processing must be

inexpensive, nontoxic, effective at low concentrations, stable, and capable of surviving processing (carry-through effect); color, flavor, and odor must be minimal (Giese, 1996).

Antioxidants not only extend shelf life of the products, but also reduce raw material waste, reduce nutritional losses, antimicrobial agents and widen the range of fats that can be used in specific products (Coppen, 1983). By extending maintaining quality and increasing the number of oils that can be used in food products, antioxidants allow processors to use more available and less costly oils for product formulation.

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) are widely used in the food industry because they are more effective and less expensive than natural antioxidants (Pitchaon *et al.,* 2007). Their safety, however, has been questioned. TBHQ is banned in Japan and certain European countries and BHA and BHT are reported to be carcinogenic. Hence research into safer and more effective natural antioxidants is under way and several natural sources are being examined (Shahidi, 1997).

Yield, Total Phenolic and Total Flavonoids of Extracts

The yield of extracts was ranged from 1.8 to 11.25 g extract /100 g seeds (Table 1). Variation in the yields of extracts is attributed to differences in polarity of compounds present in plants and assist methods, such differences have been reported (Jayaprakasha *et al.,* 2001).

The amount of total phenolics showed in Table 1 as mg GAE/g extract. Flavonoids possess a broad spectrum of chemical and biological activities, including radical-scavenging properties. For this reason, extracts were analyzed for total phenolic, flavonoid contents. Table 1 present the flavonoid contents of extracts as mg quercetin equivalent/g extract (mg QE/g). The results revealed that ethanol 80% with assist of ultrasonic extraction was better than distilled water with assist of microwave in extracting phenolic compounds and flavonoids. This might be due to the polarity, good solubility, the temperature and electromagnetic radiation of microwaves that used during extraction (Siddhuraju and Becker, 2003; Kequan and Liangli, 2004; Wieland *et al.,* 2006; Zhang *et al.,* 2011).

Antioxidant Activity of Plant Extracts

As mentioned by Frankel and Meyer (2000) and Huang *et al.* (2005) no single method is adequate for evaluating the antioxidant capacity of foods or extracts, since different methods can yield widely diverging results. Several methods based on different mechanisms must be used.

DPPH$^{\bullet}$ radical-scavenging activity

The effect of antioxidants on DPPH radical-scavenging is thought to be due to their hydrogen-donating ability, DPPH$^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule (Gulcin *et al.,* 2004). Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases (Dorman *et al.,* 2003). DPPH$^{\bullet}$ is considered to be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH$^{\bullet}$, reducing the number of DPPH$^{\bullet}$ free radicals to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH$^{\bullet}$ (Juan *et al.,* 2005). It is visually noticeable as a discoloration from purple to yellow. The scavenging activity of extracts against DPPH$^{\bullet}$ was concentration-dependent. The results of DPPH radical-scavenging activities of GC assist are represented in Fig. 1 with scavenging activity 37.15% for GC ultrasonic assist and 18.07% for microwave assist after 120 min of reaction. The results clearly indicated that both extracts exhibited antioxidant activity.

The extracts that contained the high amount of total phenolic compounds (Table 1) showed relatively high antioxidant activity against TBHQ (94.38%). It has been proven that the antioxidant activity of extracts is mainly ascribable to the concentration of phenolic compounds in the plant (Heim *et al.,* 2002).

The results of the DPPH$^{\bullet}$ free radical scavenging assay suggest that components

involving the extracts are capable of scavenging free radicals *via* electron- or hydrogen-donating

Table 1. Yield, total phenolic and flavonoids of GC seed extracts

	Ultrasonic assist	Microwave assist
Yield of extract with g/100 g seeds	011.25	01.80
Total phenolics as mg GAE/g extract	126.24	88.08
Total flavonoids as mg QE/g extract	007.21	00.65

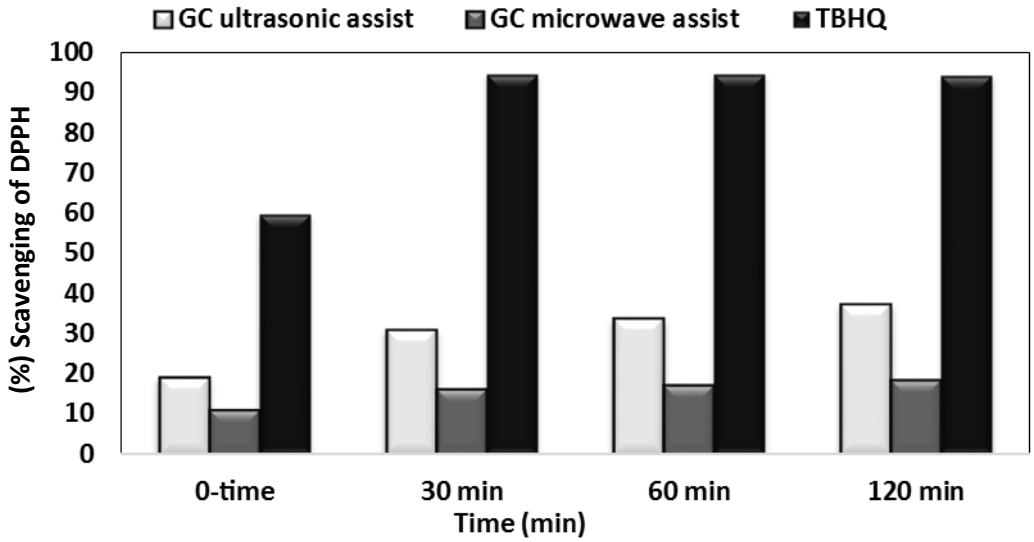

Fig. 1. Scavenging activity of GC ultrasonic-assisted and microwave-assisted extracts against DPPH radical compared with TBHQ

mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices. This further shows the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

$ABTS^{\bullet+}$ radical-scavenging activity

Although the $DPPH^{\bullet}$ free radical is ubiquitously used to estimate the potential free radical-scavenging activity of natural products, the $ABTS^{\bullet+}$ free radical is commonly used when issues of solubility or interference arise and the use of $DPPH^{\bullet}$ based assays becomes inappropriate (Arnao, 2000; Dorman *et al.*, 2003). Hydrogen radical scavenging is an important attribute of antioxidants. $ABTS^{\bullet+}$, a protonated radical, has a characteristic absorbance maximum at 734 nm that decreases with the scavenging of hydrogen radicals (Mathew and Abraham, 2006). Scavenging of the $ABTS^{\bullet+}$ radical by extracts was found to be fairly close to $ABTS^{\bullet+}$ than that of the DPPH radical (Fig. 2). Factors such as the stereoselectivity of radicals and the solubility of extracts indifferent test systems have been reported to affect the capacity of extracts to react with and quench different radicals. Wang *et al*. (1998) found that some compounds possessing $ABTS^{\bullet+}$-scavenging activity did not show DPPH-scavenging activity. This was not in agreement with the present study.

The $ABTS^{\bullet+}$ scavenging data suggest that components within the extracts are capable of scavenging free radicals *via* a mechanism of electron/hydrogen donation and should be able to protect susceptible matrices from free radical-mediated oxidative degradation. The percent of inhibition of GC extracts were 24.61% for GC

ultrasonic assist and 21.14 for microwave extract compared with TBHQ (72.27%).

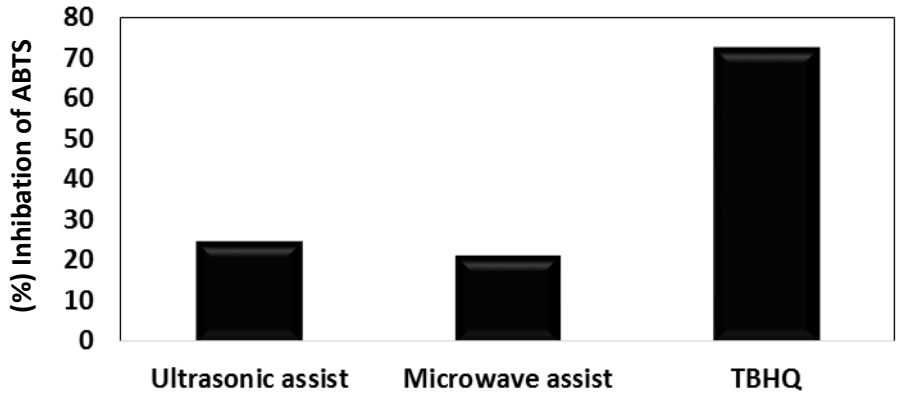

Fig. 2. Scavenging activity of extracts against $ABTS^{\bullet+}$ radical compared with TBHQ after 7 min.

β-Carotene/linoleic acid bleaching assay

Synthetic free radical-scavenging ($ABTS^{\bullet+}$ and $DPPH^{\cdot}$) models are valuable tools to indicate the potential antioxidant activity of plant extracts; however, these systems do not use a food or biologically relevant oxidisable substrate, so no direct information on an extract's protective action can be obtained (Dorman *et al.,* 2003). Therefore, it was considered important to assess the extracts in a β-carotene/linoleic acid lipid–water emulsion assay despite its reported limitations (Koleva *et al.*, 2002; Ley and Bertram, 2003). In this assay, oxidation of linoleic acid produces hydroperoxide-derived free radicals that attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant (Liyana-Pathirana and Shahidi, 2006). As can be seen in Fig. 3, extracts were capable of inhibiting the bleaching of β-carotene by scavenging linoleate-derived free radicals. The order of decreasing efficacy at a dose of 200 μgml^{-1} was TBHQ> GC microwave assist>GC ultrasonic assist. The results had revealed comparable scavenging ability 54.66% and 50.43% for GC microwave assist and ultrasonic assist to the synthetic antioxidants and TBHQ 65.69%. It has been suggested that the polarity of an extract is important in water–oil emulsions, in that non-polar extracts are more effective antioxidants than polar extracts owing to a concentrating effect within the lipid phase (Koleva *et al.,* 2002).

Thus, it would be expected that the less polar extracts would be more potent. This phenomenon was not observed in the case of extracts studied here, a finding which has also been reported previously (Koleva *et al.,* 2003). According to the data on β-carotene/linoleic acid bleaching, the extracts are capable of scavenging free radicals in a complex heterogeneous medium. This suggests that the extracts may have potential use as antioxidant preservatives in emulsion-type systems.

Ferric reducing antioxidant power (FRAP)

Antioxidant compounds cause the reduction of ferric (Fe^{+3}) form to the ferrous (Fe^{+2}) form because of their reductive capabilities. Prussian blue-colored complex is formed by adding $FeCl_3$ to the ferrous (Fe^{+2}) form. Therefore, reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm (Chang *et al.*, 2002). In this assay, yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power.

$$Fe(CN)_6^{-3} + \text{Reductive agent} \rightleftharpoons Fe(CN)_6^{-4}$$

$$Fe(CN)_6^{-4} + FeCl_3 \longrightarrow Fe_4[Fe(CN)_6]_3$$

As shown in Fig. 4, GC extracts showed ferric reducing power with the increased concentration as standard antioxidants (TBHQ). According to results of the present study, both

ferric reducing power and total phenolic content of GC ultrasonic assist were higher than those of microwave assist. Total phenolic content and ferric reducing power are related with each other.

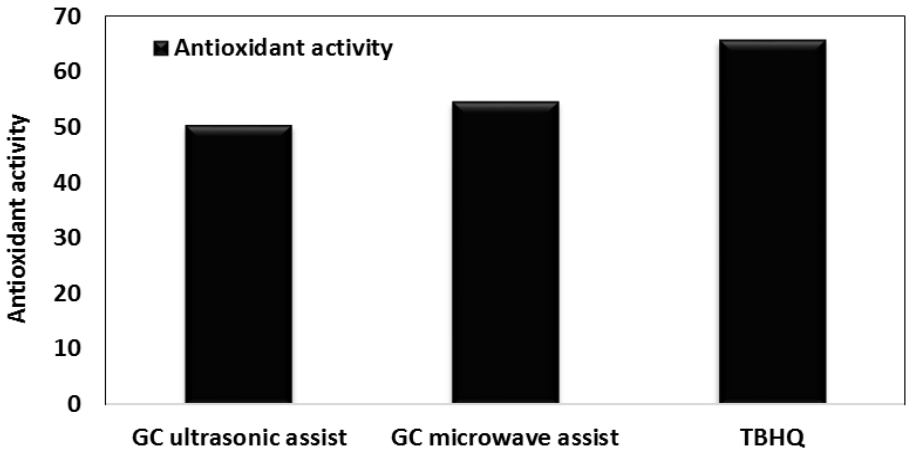

Fig. 3. Antioxidant activity of extracts in β-carotene/linoleic acid system compared with TBHQ

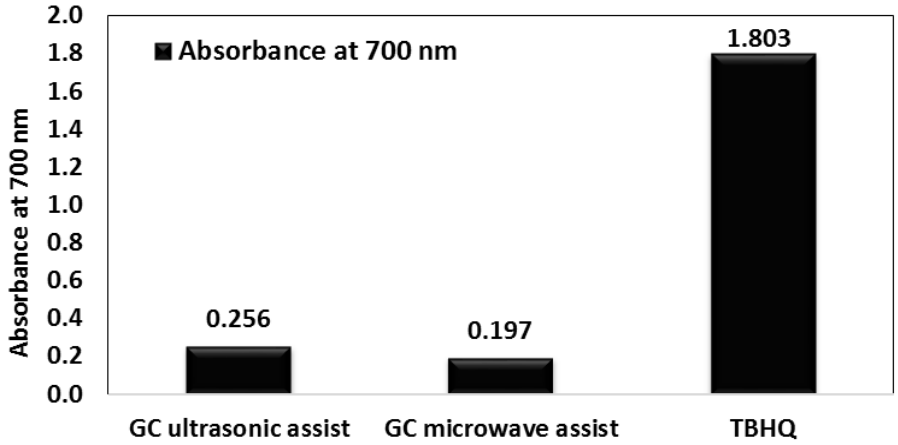

Fig. 4. Absorbance of ferric reducing power of GC extracts against TBHQ

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant activity (Dorman *et al.*, 2003). The extracts that contained high amount of total phenolic compounds (Table 1) showed relatively high antioxidant activity. In general, extracts showed ferric reducing power compared with TBHQ (Fig. 4). It has been proven that ferric reducing power of seed extracts is mainly ascribable to the concentration of phenolic compounds in the seed (Heim *et al.*, 2002). The ferric reducing power of both extracts were 0.256 and 0.197 compared with TBHQ (1.803).

Considering the results of all four assays, phenolic compounds can explain high antioxidant capacity (Fernandez-Pachon *et al.*, 2004; Mullen *et al.*, 2007), although some authors have reported that there is no correlation between the content of these main antioxidant compounds and radical-scavenging capacity, the results obtained in this study do not support this claim. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet, triplet oxygen or decomposing peroxides and reductive heavy metals with two or more valence states (Osawa, 1994).

Evaluation of the Antimicrobial Potential of Plant Extracts

Using agar disc assay (Fig. 5), the tested plant extracts induced inhibition zones against five tested bacteria (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ScottA, *Salmonella*

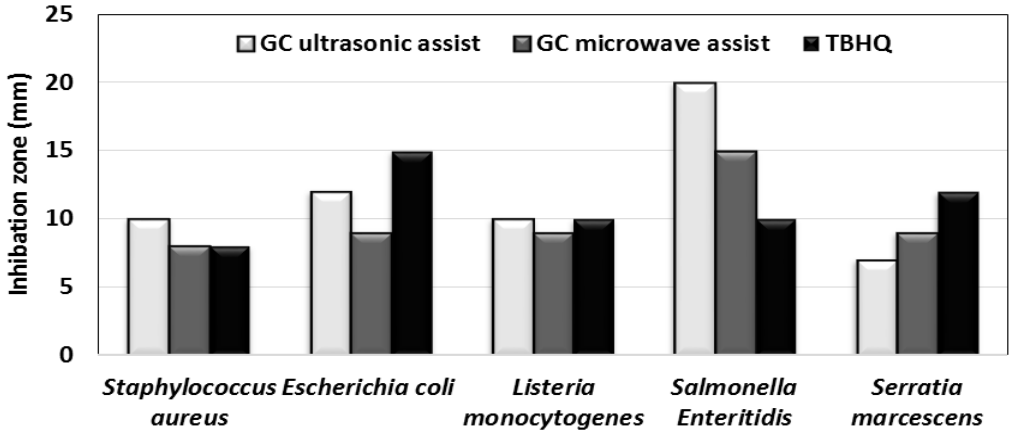

Fig. 5. Inhibition zones diameter (mm) of tested GC extracts with Gram positive and negative bacteria compared with TBHQ

Enteritidis PT4 and *Serratia marcescens*). It is noticed that extracts gave inhibition zones. Generally, there were no significant differences between the Gram-positive and negative bacteria in their susceptibility to the tested compounds referring to broad specificity antimicrobial action of the different extracts. However, GC ultrasonic assist had the best inhibition zone diameter with *S.* Enteritidis (20 mm) then microwave assist (15 mm) with the same bacteria then TBHQ, with 10 mm inhibition zone diameter, GC ultrasonic assist with 12 mm inhibition zone diameter with *E. coli*. The best inhibition zone diameter occurred with TBHQ with *E. coli* which had 15 mm inhibition zone diameter. TBHQ and GC ultrasonic assist were effective on *Listeria monocytogenes* bacteria with the same inhibition zone diameter (10 mm). However, GC ultrasonic assist was effective on *Staphylococcus aureus* bacteria inhibition zone diameter was 10 mm. Although, GC microwave assist and TBHQ had the same effect on *Staphylococcus aureus* with 8 mm inhibition zone. GC microwave assist was effective better than GC ultrasonic assist on *Serratia marcescens* bacteria with 9 and 7 mm inhibition zones, respectively, but TBHQ had the best inhibition zone diameter (12 mm). These results were in the same trend with those obtained by Adam *et al.,* (2011) who examined petroleum ether, methanol and water extracts of GC seed extracts against five pathogens bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and one fungus *Candida albicans*. Petroleum ether has been shown to be the best solvent for extracting antimicrobial substances from GC seed compared with methanol and water.

Conclusion

Seeds GC extracts showed varying degrees of antioxidant activity in different test systems in a dose-dependent manner. Furthermore, the pattern of activity of the extracts within the assays also differed. As observed, extracts with higher antioxidant capacity were in parallel to their higher phenolic contents. It can be concluded that the obtained extracts using highest phenolic compounds were more effective extracts obtained scavengers than those obtained using lowest phenolic content. Ethanol 80% showed slightly better characteristics than distilled water as a solvent for phenolic compounds and flavonoids and ultrasonic extraction was better than microwave extraction. So, for use in the food industry, ethanol would be a more appropriate solvent. Furthermore, it is notable that GC ultrasonic extract exhibited the

strongest antioxidant capacity in all assays used expect β-carotene linoleic emulsion assay. Overall, GC extracts showed comparable activity to TBHQ. Therefore, these extracts could be used as preservative ingredients in the food or pharmaceutical industries. However, further research is required before such use can be proposed with confidence.

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الخواص المضادة للأكسدة والمضادة للبكتريا لمستخلصات مختلفة من نبات حب الرشاد

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يهدف البحث إلى دراسة كمية المركبات الفينولية والخواص المضادة للاكسدة والبكتريا لبذور نبات حب الرشاد، حيث أجرى الاستخلاص بالإيثانول ٨٠% بمساعدة الموجات فوق الصوتية وبالماء المقطر بمساعدة موجات الميكروويف، وقدرت المركبات الفينولية الكلية باستخدام طريقة فولن وتقدير الفلافونيدات الكلية باستخدام كلوريد الألومنيوم، بالإضافة لتقدير الخواص المضادة للاكسدة مثل DPPH وABTS وطريقة قصر اللون لمستحلب البيتا كاروتين واختبار قدرة مضادات الاكسدة لاختزال الحديديك FRAP، أيضا تم تقدير الخواص المضادة للبكتريا باستخدام بكتريا مرضية موجبة وسالبة لجرام بواسطة اختبار فحص القرص Disc assay، وأظهرت النتائج أن لتلك المستخلصات خواص مضادة للاكسدة والبكتريا في جميع التجارب مقارنة بمضاد الأكسدة الصناعي TBHQ، وبالتالي يمكن استخدام تلك المستخلصات كمواد طبيعية لحفظ الأغذية وكمواد تستخدم في الصناعات الدوائية، أيضا فان النتائج المتحصل عليها من تلك الدراسة يمكن استخدامها لتعزيز استخدام مضادات الاكسدة الطبيعية والمركبات الفعالة لتحسين صحة الإنسان.

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