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ANTIOXIDANT PROPERTIES OF DIFFERENT EXTRACTS FROM FIVE MEDICINAL PLANTS

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ABSTRACT

Different extracts from five medicinal plants in terms of phenolic compounds and antioxidant properties were studied. By using four solvents, twenty extracts of the tested plants [clove (*Syzygium aromaticum*), rosemary (*Rosmarinus oficinalis*), thyme (*Thymus vulgaris*), sweet marjoram (*Origanum majorana*) and ginger (*Alpinia zerumbet*)] were obtained. Total phenolic compounds and total flavonoids were measured using Folin–Ciocalteu reagent and $AlCl_3$, respectively. In addition, total antioxidant capacity of the extracts was estimated by different methods including: DPPH (1,1-diphenyl-2 picrylhydrazyl radical), $ABTS^{\cdot+}$ (2, 2` azino bis-(3-ethylbenzthiazoline-6-sulfonic acid), and β-carotene/linoleic bleaching test. Clove extracts exhibited the strongest antioxidant capacity in all used assays, followed by rosemary and thyme extracts. In general, ethanol, water and hexane extracts showed comparable activity 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 of health promoting activities.

Keywords: *Syzygium aromaticum*, *Rosmarinus oficinalis*, *Thymus vulgaris*, *Origanum majorana*, *Alpinia zerumbet*, antioxidant activity, phenolic compounds.

INTRODUCTION

Antioxidant compounds such as flavonoids, tannins, coumarins, curcumanoids, xanthons, lignans and terpenoids are found in different plant parts (e.g., fruits, leaves and seeds). Therefore, there is growing interest in separating these bioactive compounds 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 oilseed meals (Jeong *et al.*, 2004). Various solvents have been used to extract polyphenols from plant materials (Vasso and Constantina, 2007). Extraction yield is dependent on both solvent and the method of extraction (Pinelo *et al.*, 2004). Water and aqueous mixtures of ethanol, methanol and acetone are commonly used in the extraction (Goli *et al.*, 2005). Wang and Helliwell (2001) reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. 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).

Aromatic and medicinal plants are rich sources of phenolic compounds with health-promoting activities. In this investigation five plants [clove (*Syzygium aromaticum*), rosemary (*Rosmarinus oficinalis*), thyme (*Thymus vulgaris*), sweet marjoram (*Origanum majorana*) and ginger (*Alpinia zerumbet*)] were selected to study their phenolic contents and antioxidant activities. While, thyme possesses various beneficial effects such as antiseptic, carminative, antimicrobial, and antioxidative properties (Baranauskiene *et al.*, 2003). As well as, the major aroma constituents of thyme were 2-isopropyl-5-methylphenol (thymol; 8.55 mg/g), 4-isopropyl-2-methylphenol (carvacrol; 0.681 mg/g), linalool (0.471 mg/g), α-terpineol (0.291 mg/g), and 1,8-cineole (0.245 mg/g). Twelve aroma constituents of thyme were examined for their antioxidant activities using the aldehyde/carboxylic acid assay. Eugenol, thymol,

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carvacrol, and 4-allylphenol showed stronger antioxidant activities than did the other components tested in the assay. They inhibited all the oxidation of hexanal by almost 100% for a period of 30 days at a concentration of 5μg/mL. Their antioxidant activities were comparable to those of the known antioxidants such as α-tocopherol and BHT (Lee *et al.*, 2005).

Ginger [*Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. (Family Zingiberaceae)] is a perennial ginger growing widely in the subtropics and tropics. It is used in folk medicine for its anti-inflammatory, bacteriostatic and fungistatic properties (Zoghbi *et al.*, 1999). The essential oil extracted from its leaves possessed both relaxant and antispasmodic actions in rate ileum (Bezerra *et al.*, 2000). The highest dihydro-5, 6-dehydrokawain content was found in the hexane extract of fresh rhizomes. Ethyl acetate extracts from leaves showed higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities than those from rhizomes. Ethyl acetate extract from wastewater of leaves possessed strong inhibition to β-carotene oxidation. Ferulic and *p*-hydroxybenzoic acids were the major phenolics present in these extracts (Elzaawely *et al.*, 2007).

Rosemary extracts were categorized into three groups: phenolic diterpenes possessing abietic acid framework, flavonoids, and phenolic acids. Carnosic acid (CA) and carnosol, abietane-type diterpenes, and rosmarinic acid (RA), α-hydroxycinnamic acid ester were the main antioxidant compounds present in rosemary. CA was identified as a major component in the acetone extracts from mature shoots of rosemary (Caruso *et al.*, 2000). These compounds, together with other isoprenoids (sterols, isoprene, mono- and diterpenes, tocopherols or carotenoids) play a photoprotective role and are considered as bioactive constituents (Troncoso *et al.*, 2005; Vogelsang *et al.*, 2006).

Aroma extract from dried clove buds [*Syzygium aromaticum* (L.) Merr. et Perry] was obtained by steam-distillation under mild conditions. The aroma extract isolated from clove buds inhibited the oxidation of hexanal for 30 days at a level of 50 μg/ml. Clove bud extract inhibited malonaldehyde formation from cod liver oil by 93% at the 160 μg/ml level. Twenty-two compounds were identified in the extracts of clove buds by gas chromatography/mass spectrometry. The major aroma constituents of clove buds were eugenol (24.371 mg/g dry weight) and eugenyl acetate (2.354 mg/g dry weight). The antioxidant activity of clove bud extract and its major aroma components i.e., eugenol and eugenyl acetate, were comparable to that of α-tocopherol (Lee and Shibamoto, 2001). Clove oil is obtained by distillation of the flowers, stems and leaves of the clove tree. Clove oil was evaluated by employing various *in vitro* antioxidant assays. Clove oil inhibited 97.3% lipid peroxidation of linoleic acid emulsion at 15 μg/mL concentration. In addition, clove oil had an effective DPPH scavenging, $ABTS^{+}$ scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, ferric ions (Fe^{3+}) reducing power and ferrous ions (Fe^{2+}) chelating activities (Gülçin *et al.*, 2012).

Sweet marjoram *(Origanum majorana* L.*)* is a herbaceous and perennial plant native to southern Europe and the Mediterranean. For food uses, marjoram is employed to flavour sausages, meats, salads and soups (Novak *et al.*, 2000). Traditionally, it is used as a folk remedy against asthma, indigestion, headache and rheumatism. The fresh or dried highly aromatic leaves and flowering tops of marjoram are widely used to flavour many foods. Its essential oil and alcoholic extracts are applied in pharmaceuticals, perfumes and cosmetics (Bauer *et al.*, 1990). Little is known about the biologically active compounds of marjoram as a medicinal plant, except for its essential oil. The essential oil contains mainly terpinen-4-ol (>20%) which with (+)- cis-sabinene hydrate is responsible for the characteristic flavour and fragrance of marjoram oil. In addition to these compounds, α- and γ-terpinene and terpinolene were the other major components; thymol and carvacrol were determined in smaller amounts (Baratta *et al.*, 1998). Antioxidant activity of marjoram essential oil and its purified substance has been reported (Jun *et al.*, 2001). Its volatile oil possesses antimicrobial properties against food borne bacteria and mycotoxigenic fungi (Baratta *et al.*, 1998; Daferea *et al.*, 2000; Ezzeddine *et al.*, 2001).

Many medicinal plants were studied in the term of phenolic compounds and antioxidant activity.

However, most of these studies focused on using only one solvent to extract bioactive compounds. In the current research, we examined different plant extracts in the term of phenolic compounds and antioxidant activity. So in this investigation, we selected five medicinal plants which are commonly found in the national and international markets including clove, rosemary, thyme, sweet marjoram and ginger.

MATERIALS AND METHODS

Materials and Reagents

Five plant samples [clove (*Syzygium aromaticum*) (buds and flowers), rosemary (*Rosmarinus oficinalis*) (leaves), thyme (*Thymus vulgaris*) (leaves), sweet marjoram (*Origanum majorana*) (leaves) and ginger (*Alpinia purpurata*) (rhizomes)] have been obtained from a local markets (Zagazig, Egypt). Tert-Butyl hydroquinone (TBHQ), 1, 1-Diphenyl-2picrylhydrazyl (DPPH), 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), β-carotene, gallic acid and quercetin were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were analytical grade.

Preparation of the Extracts

Plant samples were dried in a vacuum oven at 40 °C and ground to a fine powder in a mill. Ground materials (10g) were extracted individually with different solvents (100mL) hexane, ethyl acetate, ethanol 80% and distilled water using magnetic stirrer at room temperature followed by filtration through Whatman No. 1 filter paper. The residues were re-extracted under the same conditions, then hexane and ethyl acetate combined filtrate were evaporated in a rotary evaporator (BÜCHI-water bath-B-480) below 40°C. Water and ethanol 80% extracts were freeze-dried (Thermo- Electron Corporation - Heto power dry LL300 Freeze Dryer). The dried extracts after evaporation of solvents were weighed to determine the yield and stored at -20°C until further use.

Determination of Total Phenolic Compounds

The concentration of total phenols in all extracts were measured by a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer), based on a colorimetric oxidation/reduction reaction, 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/1L) were added. The sample was incubated for 5 min at 50°C and 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:

$$y = 0.015x + 0.0533$$

$$R^2 = 0.9966$$

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

R^2=Correlation Coefficient.

Determination of Total Flavonoids

Total flavonoid content was determined by the method of Ordon *et al.* (2006) with some modification. A 1.5 mL aliquot of 20 g L^{-1} $AlCl_3$ ethanolic solution were added to 0.5mL of extract solution (10 mg in 10 mL solvent). After one hour of addition, the absorbance at 420 nm was measured at room temperature. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 1 mg mL^{-1}. Total flavonoid content expressed as quercetin equivalent (QE) was calculated using the following equation based on the calibration curve:

$$y = 0.02248x$$

$$R^2 = 0.992$$

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

R^2=Correlation Coefficient.

DPPH Radical-Scavenging Activity

The electron donation ability of the obtained extracts was measured by bleaching of the purple colored solution of DPPH according to the method of Hanato *et al.* (1988). One hundred μL of each extracts (10mg extract/10mL solvent) was added to 3 mL of 0.1 mM DPPH dissolved in toluene, ethyl acetate, ethanol and methanol according to the solvent used for

extraction. After incubation period of 30, 60 and 120 min at room temperature, the absorbance was determined against a control at 517 nm (Gulcin *et al.*, 2004). Percentage of antioxidant activity of free radical DPPH 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.

ABTS Radical-Scavenging Activity

For the ABTS 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 12–16 h at room temperature in the dark. One mL of the resulting $ABTS^{\bullet+}$ solution was diluted with 60 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 radical-scavenging activity (%) = $[(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100$

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

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). In brief, 0.2 mg of β-carotene in 1 mL of chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were placed in a round-bottom flask. After removal of the chloroform, 50 mL of distilled water were added and the resulting mixture was stirred vigorously. Aliquots (3mL) of the emulsion were transferred to tubes containing extract or synthetic antioxidant. Immediately after mixing 0.5mL 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 (Abs^0). The remaining samples were placed in a water bath at 50 °C for 2 h, then the absorbance at 470 nm was recorded (Abs^{120}). A control with no added extract was also analysed. Antioxidant activity was calculated as follows:

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

Where Abs^0_{sample} is the absorbance of sample at 0-time, Abs^{120}_{sample} is the absorbance after 120 min, $Abs^0_{control}$ is the absorbance of control at 0-time and $Abs^{120}_{control}$ is the absorbance of control after 120 min.

RESULTS AND DISCUSSION

One of the principal causes of food deterioration is lipid oxidation. Lipid oxidation results in the formation of reactive oxygen species and free radicals; which are purportedly associated with carcinogenesis, mutagenesis, inflammation, DNA changes, aging and cardiovascular diseases (Gordon 1991). The use of antioxidants in lipids and lipid-containing foods is one method to minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products (Siddhuraju and Becker, 2003; Shahid *et al.*, 2008).

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 wherein several natural sources are being examined (Ito *et al.*, 1982; Pin-Der and Gow-Chin, 1997; Shahidi 1997).

Yield of Extracts

The yield of extracts with different solvents varied from 1 to 18.5g extract/100g plant (Figure 1). Thyme, clove and sweet marjoram

had the highest yield when extracted with ethanol 80% followed by water. Rosemary and ginger gave high yield when extracted with water followed by ethanol. Owing to its high lipid content, rosemary and clove gave the highest yield as extracted with ethyl acetate followed by hexane. Variation in the extraction yields of different extracts is attributed to differences in polarity of compounds found in plants; such differences have been reported (Jayaprakasha *et al.*, 2001).

Total Phenolic Compounds

The Folin–Ciocalteu method measures the reduction of the reagent by phenolic compounds *via* the formation of a blue complex that can be measured at 760 nm against gallic acid equivalent (GAE) as a standard (Imeh and Khokhar, 2002). The amount of total phenolic compounds varied in the different extracts, ranging from 17.5 to 293 mg GAE g^{-1} extract (Table 1). In general, the results revealed that ethanol and water were better than other solvents for extracting phenolic compounds owing to their higher polarity and good solubility (Siddhuraju and Becker, 2003; Kequan and Liangli, 2004; Wieland *et al.*, 2006). As not expected, hexane extract of clove was founded to posses the highest amount of total phenolics. Bagavan *et al.* (2011) detected 47 compounds in the hexane extract of *S. aromaticum*, of which chavibetol was predominantly present. The other major constituents present in the hexane extract were eugenol acetate (phenol, 2- methoxy-4-(2 propenyl)-, acetate (15.09%), caryophyllene- (I1) (2, 6, 10, 10-tetramethyl bicycle [7.2.0] undeca-1, 6-diene (13.75%), caryophyllene oxide (3.04%), 2,6,6,9 tetramethyl- 1, 4, 8-cycloundecatriene (1.67%), and copaene (1.33%). The data in Table 1 show that hexane was the best solvent for extracting phenolic compounds from clove, followed by ethanol and water, with respective values of 293, 230 and 230 mg GAE g^{-1} extract. The best solvent for thyme and sweet marjoram was ethanol followed by ethyl acetate and water, with respective values of 233, 204 (ethanol 80%), 172, 143 (ethyl acetate) and 125, 99.9 (water) mg GAE g^{-1} extract, respectively. Rosemary had the highest total phenolic compounds in water extract followed by ethanol and hexane extracts, with respective values of 213, 202 and 103 mg GAE g^{-1} extract. The best solvent for extracting phenolic compounds from ginger was hexane followed by ethyl acetate and ethanol, with respective values 76.6, 70.6 and 48.2 mg GAE g^{-1} extract.

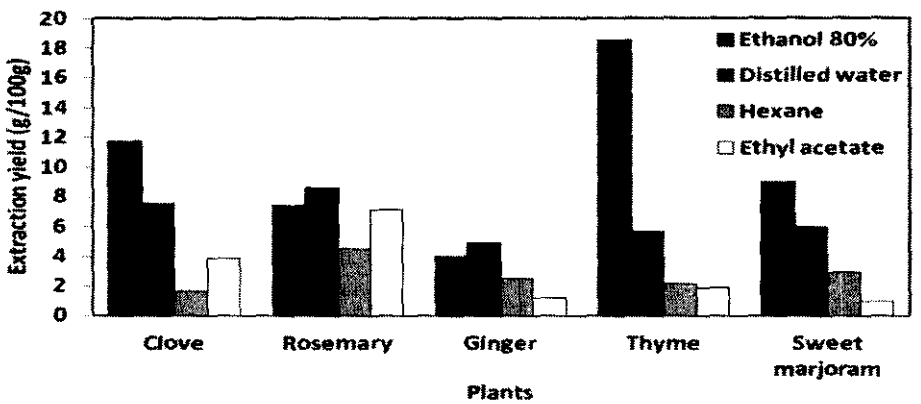

Figure 1. Yield of extracts (g/100g) for different plants

Table 1. Total phenolic compounds (mg gallic acid /g extract) in different plant extracts

Plant	Concentration (mg gallic acid / g extract)			
	Hexane	Ethyl acetate	Ethanol 80%	Distilled water
Clove	293	58.8	230	230
Rosemary	103	67.5	202	213
Ginger	76.6	70.6	48.2	17.5
Thyme	49.1	172	233	125
Sweet marjoram	10.6	143	204	99.9

Total Flavonoids

Flavonoids possess a broad spectrum of chemical and biological activities, including radical-scavenging properties. For this reason, all extracts were analyzed for total phenolic and flavonoid contents. Table 2 present the flavonoid contents of different extracts. The best solvent for extracting flavonoids was hexane. Sweet marjoram extract had the highest total flavonoid content (46.3 mg QE g^{-1} extract) followed by rosemary extract (43.2 mg QE g^{-1} extract) then thyme extracts (31.47 mg QE g^{-1} extract). On the other side, water extract was the best for clove (17.5 mg QE g^{-1} extract). High flavonoid content was also observed in extract with ethyl acetate for sweet marjoram (23.4 mg QE g^{-1} extract), while the lowest value was found in ethanol extract with ginger (0.99 mg QE g^{-1} extract). It is hard to find information in the literature to compare the obtained results with published data.

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. Thus, several methods based on different mechanisms should be used. Here we applied assays of ABTS radical-scavenging activity, DPPH radical-scavenging activity and β-carotene/linoleic acid bleaching test to each extract.

DPPH Radical-Scavenging Activity

The effect of antioxidants on DPPH radical-scavenging is thought to be due to their hydrogen-donating ability, $DPPH^{\cdot}$ 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). $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$, reducing the number of $DPPH^{\cdot}$ free radicals to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual $DPPH^{\cdot}$ (Juan *et al.*, 2005). It is visually noticeable as a discolouration from purple to yellow. The scavenging activity of extracts against $DPPH^{\cdot}$ was concentration-dependent. The results of DPPH radical-scavenging activities of various plants extracts are represented in (Figure 2). The results clearly indicated that all extracts exhibited antioxidant activity. The extracts that contained high amount of total phenolic compounds Table 1 showed relatively high antioxidant activity. In general, ethanol followed by water, hexane then ethyl acetate extract showed radical scavenging activity as strong as that of TBHQ (Figure 2: A, B, C and D). It has been proven that the antioxidant activity of plant extracts is mainly ascribable to the concentration of phenolic compounds in the plant (Heim *et al.*, 2002). The extracts antioxidant activity with different solvents varied from 91.4 to 5.50% after 120 min. The highest antioxidant activity was observed with hexane, water and ethanol 80% extracts of clove, with respective values 91.4%, 90.7% and 89.3%, followed by water, ethanol and hexane extracts of rosemary, with respective values 90.2%, 86.5% and 74.9%. Ethanol, water and ethyl acetate extracts of thyme has respective values 87.6%, 78.5% and 67.3%, followed by ethanol, ethyl acetate and water extracts of sweet marjoram, with respective values 86.9%, 57.1% and 38.1%. Ginger had antioxidant activity in its ethyl acetate, hexane and ethanol extracts, with respective values of 46.8%, 37.0% and 20.9%. The results of the $DPPH^{\cdot}$ free radical scavenging assay suggest that components involving the extracts are capable of scavenging free radicals *via* electron- or hydrogen-donating mechanisms and thus might 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 Radical-Scavenging Activity

Although the $DPPH^{\cdot}$ free radical is ubiquitously used to estimate the potential free radical-scavenging activity of natural products, the $ABTS^{\cdot+}$ free radical is commonly used when issues of solubility or interference arise and the use of $DPPH^{\cdot}$ based assays becomes inappropriate (Arnao, 2000; Dorman *et al.*, 2003).

Table 2. Total flavonoids content (mg QE/g extract) in different extracts

Plant	Concentration (mg QE/ g extract)			
	Hexane	Ethyl acetate	Ethanol 80%	Distilled water
Clove	4.58	4.72	12.0	17.5
Rosemary	43.2	4.00	7.06	4.99
Ginger	9.62	12.6	0.99	1.39
Sweet marjoram	46.3	23.4	7.24	4.94
Thyme	31.4	10.2	8.36	7.46

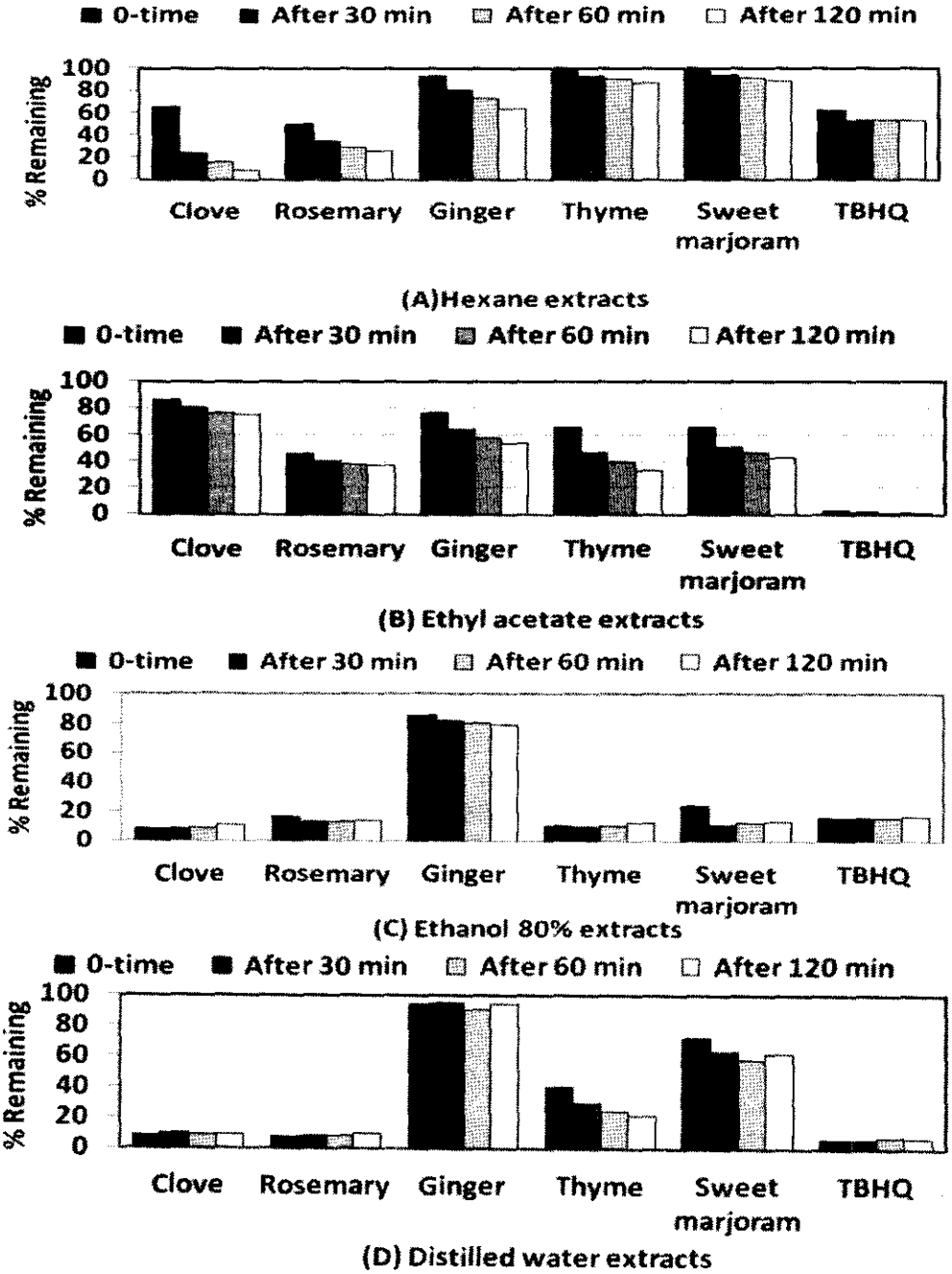

Figure 2. Scavenging activity of (A) hexane, (B) ethyl acetate, (C) ethanol 80%, (D) distilled water extracts against DPPH radical compared with TBHQ

Proton 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 proton radicals (Mathew and Abraham, 2006). Different extracts demonstrated a wide range of $ABTS^{\bullet+}$ scavenging activities from 1.12 to 99.5% (Figure 3). In our study, all extracts exhibited antioxidant activity. Ethanol extracts were the most efficient $ABTS^{\bullet+}$ scavengers in general, followed by water and hexane extract. The highest scavenging activity was observed with distilled water, hexane and ethanol extracts of clove with respective values 99.4%, 99.1% and 98.5%, followed by hexane, ethanol and water extracts of rosemary, with respective values 99.1%, 87.1% and 63.2%. Ethanol, ethyl acetate and water extracts of thyme had values of 99.5%, 78.3% and 59.2%, followed by ethanol, ethyl acetate and water extracts of sweet marjoram, with values of 81.1%, 72.3% and 33.3%. Ginger had scavenging activity in ethyl acetate, hexane and ethanol extracts, with respective values of 91.6%, 72.3% and 30.4%. Similar results were found by Djeridane *et al.* (2006) for some medicinal plants. In general, it can be said that extracts of high-polarity solvents are considerably more effective radical scavengers than those obtained using low-polarity solvents, indicating that antioxidants or active compounds of different polarities could be present in the extracts with high antioxidant capacity. Change in the polarity of a solvent alters its ability to dissolve a selected group of antioxidant compounds and influences activity estimation (Kequan and Liangli, 2004).

Scavenging of the $ABTS^{\bullet+}$ radical by extracts was found to be higher than that of the DPPH radical. 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 the case in 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.

β-Carotene/Linoleic Acid Bleaching Assay

Synthetic free radical-scavenging models (ABTS and DPPH) 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 Figure 4, all extracts were capable of inhibiting the bleaching of β-carotene by scavenging linoleate-derived free radicals.

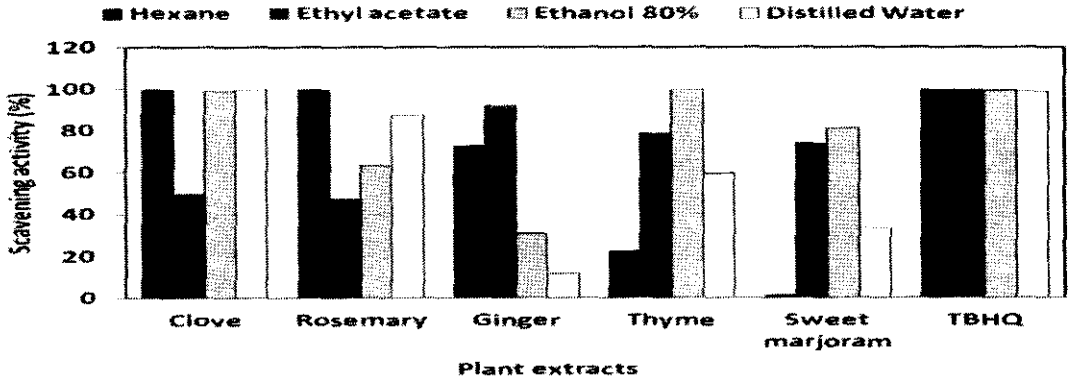

Figure 3. Scavenging activity of plant extracts against $ABTS^{\bullet+}$ radical compared with TBHQ

The order of decreasing efficacy at a dose of 200 μg mL^{-1} was TBHQ> clove> thyme> ginger> rosemary> sweet marjoram extracts. The results revealed that, overall, hexane and ethanol extracts had comparable scavenging ability to the synthetic antioxidants TBHQ. 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 all extracts studied here, a finding which has also been reported previously (Koleva *et al.*, 2003). According to the data of β-carotene/linoleic acid bleaching test, the extracts were 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.

Considering the results of all three assays, the extracts prepared from clove had the highest antioxidant activity, followed by those prepared from thyme, ginger and rosemary, while sweet marjoram extracts were less effective. The highest antioxidant activity was observed with hexane, water and ethanol extracts of clove, with respective values 95.0%, 79.3% and 77.3%, followed by water, ethanol and hexane extracts of thyme, with respective values 70.0%, 66.0% and 43.7%. Ethyl acetate, hexane and ethanol extracts of ginger had values of 81.3%, 64.5% and 37.3%, followed by hexane, ethyl acetate and ethanol extracts of rosemary, with values of 50.8%, 49.2% and 26.4%. Sweet marjoram has antioxidant activity in hexane extract, with value 19.28%. 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. Our results 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 neutralising free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994).

Conclusion

Clove, rosemary, thyme, sweet marjoram and ginger extracts were prepared using different solvents, and the *in vitro* antioxidant activity of each extract was investigated. Various 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. In general, it was observed extracts with higher antioxidant capacity were in parallel to their higher polyphenolic contents. It could be concluded that the obtained extracts using higher-polarity solvents were more effective radical scavengers than those obtained using lower-polarity solvents. Ethanol showed slightly better characteristics than distilled water as a solvent for phenolic compounds and flavonoids extraction.

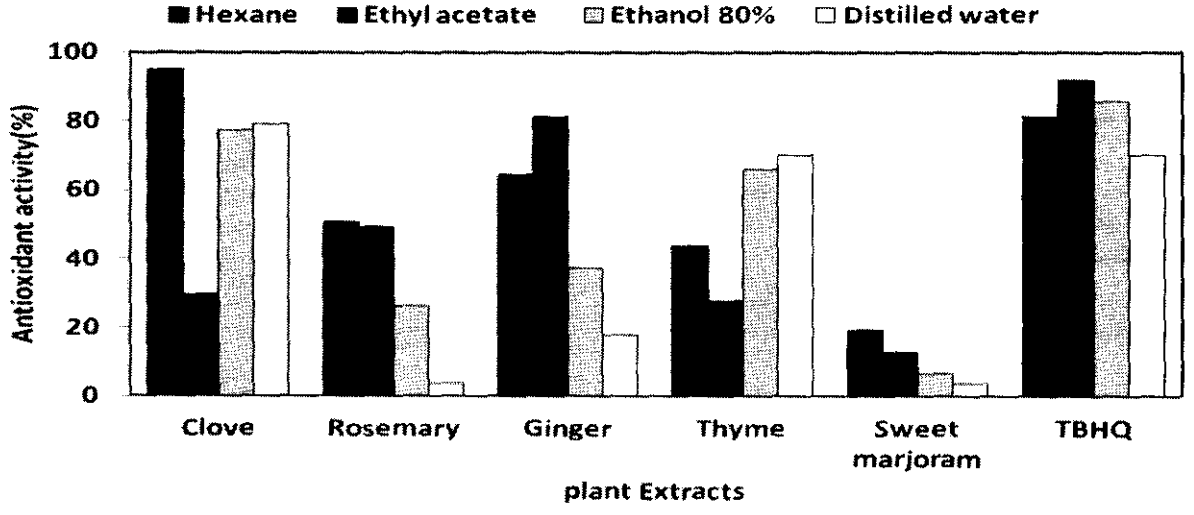

Figure 4. Antioxidant activity of plant extracts in β-carotene/linoleic acid system compared with TBHQ

Thus, for use in the food industry, ethanol would be a more appropriate solvent. Furthermore, it is notable that clove extracts exhibited strong antioxidant capacity in all assays used, followed by rosemary and thyme extracts. Overall, ethanol, distilled water and hexane extracts showed relatively comparable activity to TBHQ. Therefore, these extracts could be used as preservative ingredients in the food and/or pharmaceutical industries. Further research is required to identify phenolic compounds found in different extracts before such use can be proposed with confidence.

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الخصائص المختلفة لمستخلصات خمس نباتات طبية كمضادات للأكسدة

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الهدف من هذا البحث هو دراسة الخواص المضادة للأكسدة وكذلك المحتوي الكلي للمركبات الفينولية الموجودة في ٢٠ مستخلص نباتي للمذيبات المتمثلة في هكسان، ايثايل اسيتات، ايثانول ٨٠٪ والماء المقطر لخمسة أنواع من النباتات الطبية وهم: القرنفل، الروزماري، الزعتر، البردقوش والزنجبيل. وتم قياس كلا من المحتوي الكلي للمركبات الفينولية باستخدام طريقة فولن وتقدير الفلافونيدات الكلية باستخدام كلوريد الألومنيوم؛ كذلك تم تقدير الخواص المضادة للأكسدة لهذه المستخلصات باستخدام الشقوق الحرة مثل DPPH و ABTS وأيضاً تم استخدام طريقة قصر اللون لمستحلب البيتا كاروتين واللينوليك. وأوضحت النتائج أن القرنفل له أقوى خواص مضادة للأكسدة فى كل التقديرات تبعه الروزماري ثم الزعتر. وبالنسبة للمستخلصات؛ عموما فكان المستخلص الإيثانولى والمائي أعلى فى الخواص المضادة للأكسدة مقارنة بمضادات الأكسدة الصناعية (TBHQ). وبالتالى يمكن استخدام هذه المستخلصات كإضافات طبيعية لحفظ الأغذية وكمواد تستخدم فى التصنيع الدوائى كمضادات للأكسدة تحفظ للإنسان صحته.