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Antioxidant activities and total phenolics content in some Egyptian herbs

الأنشطة المضادة للأكسدة ومحتوى الفينولات الكلى في بعض الأعشاب المصرية

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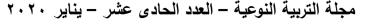
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المستخلص

الهدف من هذه الدراسة هو تحديد محتوى الفينولات الكلي في العديد من الأعشاب النامية في مصر، واستكشاف العلاقة بين المحتوى الفينولي والنشاط المضاد للأكسدة. أظهرت خلاصات الأعشاب ومزيجها اختلافات كبيرة في نشاط مضادات الأكسدة (90.98 - 80.11) عندما تم حسابها بواسطة الطرق الأربعة المختلفة المستخدمة في هذه الدراسة. أظهرت البردقوش نشاطًا قويًا بسبب محتواها من الفينولات العالي (40.000 40.000 40.000 والفينوليك الكلي (40.000 40.000 والفينوليك الكلي (40.000 40.000 والفينوليك الكلي والنشاط المضاد للأكسدة من الأعشاب المختارة أنه عندما تم تضمين الأعشاب المختارة في التحليل الإحصائي، كانت هناك ارتباطات موجبة (40.000 وهامة (40.000 وهامة (40.000 وهامة (40.000 وهامة المضاد للأكسدة الموالي. في الختام، يمكن أن تمثل بيانات الدراسة الحالية حجر الميل نحو التوسع في استخدام مسحوق أو مقتطفات من الأعشاب المختارة كمضادات الأكسدة الطبيعية في العديد من التطبيقات الغذائية والتغذوية المختلفة.

الكلمات الرئيسية:

النعناع، البردقوش، الزنجبيل، المركبات الكيميائية النباتية، مضادات الأكسدة الطبيعية.





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Antioxidant activities and total phenolics content in some Egyptian herbs

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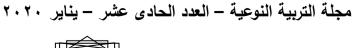
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Abstract:

The aim of this study was to determine the contents of total phenolics in several herbs growing in Egypt and to explore relationship(s) between phenolic content and antioxidant activity. Herbs extracts and their mixture showed considerable differences in antioxidant activity (AA= 80.11- 90.98%) when it was calculated by the four different methods used in this study. Marjoram showed strong activity because of their high phenolic content (938.87 mg/100 g d.b.). Spearmint and ginger showed relatively moderate antioxidant activity (AA= 83.21 and 80.11%) and total phenolics (376.82 and 298.43 mg/100 g d.b.). Data of relationship between total phenolic content and antioxidant activity of selected herbs indicated that when the selected herbs were included in the statistical analysis, there were positive correlations (r²) were recorded 0.8415, 0.8856, 0.7273 and 0.8978 and significant (p ≤ 0.05 , 0.01, 0.05 and 0.01) between total phenolics and antioxidant activity for spearmint, marjoram, ginger and their mixture, respectively. In conclusion, data of the present study could be represent the mile stone towards the extension of using the powder or extracts of the selected herbs as natural antioxidants in many different nutritional and food processing applications.

Keywords: spearmint, marjoram, ginger, Phytochemical compounds, natural antioxidants





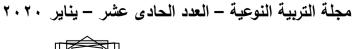
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Introduction

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as vitamins (carotenoids, and ascorbic acid) (Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997). Synthetic antioxidants such as butylated hydroxyanisole (BAH) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Branen, 1975; Ito *et al.*, 1983). Thus, the interest in natural antioxidants such phytochemicals has increased considerably (Loliger, 1991).

Phytochemical compounds which naturally occurring in food materials including herbs can be classified into several families include allyle sulfides, indoles, phenolic compounds, saponins and terpens. Amongst of different families of phytochemicals, phenolic compounds have been occupied the central position. The term of phenolic compound embraces a wide range of compound plant substances, which possess in common an aromatic ring bearing one or more hydroxyl substituents. They most frequently occur combined with sugar glycoside and usually located in the cell vacuole (Harborne, 1973). Phenolic acids are a group of phenolic compound, which may be identified as hydroxycarboxylic acids with phenolic hydroxyl groups (Misaghi, 1982). Phenolic acids and their derivatives are widely distributed in all plant derived food systems and in most diets. The levels vary dramatically, especially as influenced by factors such as the species, germination, ripening, storage and processing. For example, potato tubers were shown to have several kinds of phenolic acids and most of these phenolics are concentrated in the outer part (Reeve et al., 1969; Come., 1971; Kumar et al., 1991; Dao and Friedman ,1992; Onyeneho and Hettiarachchy, 1993; and Rodriguez et al., 1994 a-b. Many of phenolic acids exhibits their antioxidative (Majid et al., 1991; Deschamps et al., 1991; Laranjinha et al., 1994 and Khoteem, 2005), anticarcinogenic (Raj et al., 1983; Mukhtar et al., (1984; Camarasa et al., 1987; Gali et al., 1991; and Harttig et al., 1996), and antibacterial (Herald and Davidson, 1983; Nakane et al., 1990; Nowosielska et al., 1991; Ghaly, 2004 and Khoteem, 2005) effects.

The antioxidant activity of several plant materials including herbs has recently been reported (Al-Saikhan *et al.*, 1995; Yen and Duh, 1995; Oomah and Mazza, 1996; Wang *et al.*, 1996; Cao *et al.*, 1996; Amarowicz *et al.*, 1996; Ghaly, 2004 and



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Khoteem, 2005); however, information on the relationship between antioxidant activity and phenolic content and composition of many food plant products is not available. Therefore, the aims of this study was to determine the contents of total phenolics in several herbs growing in Egypt and to explore relationship(s) between phenolic content and antioxidant activity.

Materials and Methods Materials

Plant materials: Three herbs ginger (Zingiber officinale), marjoram (Majorana hortensis) and spearmint (Mentha spicata L.) were obtained from Haras Company for Herbs Trading, Bab El-Khlek, Cairo, Egypt.. All samples were either ground or homogenized before they were freeze-dried to ensure equal moisture content.

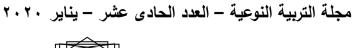
Experimental animals: Normal male albino rats (145±10g) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Standards: Gallic, β -hydroxytoluene and α -tocopherol were obtained from Sigma chemical Co, St Louis, MO,. Sodium tungstate and phosphomolybdic acid were obtained from Fischer, UK. All chemicals, reagents and solvents were of analytical grade and purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Folin-Ciocalteu reagent: One gram of sodium tungstate, 20 g of phosphomolybdic acid, and 750 ml of distilled water are transferred to a 1000 ml flask fitted with a reflux condenser. The solution is refluxed for 10 h; transferred to a graduated flask of 1000 ml capacity and made up to the mark with distilled water.

Preparation of herbs extracts

Powders of the selected herbs were used for their different types extracts as follow: A 20 g from dried plant powder plus 180 ml methanol (80%, v/v) were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of was removed under reduced pressure at 45°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany) and the extract could be ready for the basil diet blending purpose.





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Methods

Determination of antioxidant activity and total phenolics Extraction

Ground samples (100 g) were extracted with 80% aqueous methanol (750 ml) on an orbital shaker for 120 min at $70~^{0}$ C. The mixture was subsequently filtered (Whatman No. 5) on a Buchner funnel, and the filtrate was assayed for antioxidant activity.

Determination of antioxidant activity

Antioxidant activity of plant extyracts and standards (α-tocopherol, BHA, ans BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β carotene bleaching method following a modification of the procedure described by Marco (1968). For a typical assay, 1mL of β -carotene (Sigma) solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) and 0.2 mL of Tween 20 (BDH Chemical Co., Toronto, On). Each mixture was then dosed with 0.2 mL of 80% MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autooxidation at 50 °C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of β -carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT, BHA, and α-tocopherol in 80% methanol was used as the control.

Antioxidant activity was calculated in four different ways. In the first, absorbance was plotted against time, as a kint curve, and the absolute value of slope was expressed as antioxidant value (AOX). Antioxidant activity (AA) was all calculated as percent inhibition ralative to control using the following equation (Al-Saikhan et al., 1995).

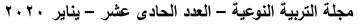
$$AA = (R_{control} - R_{sample}) / R_{control} \times 100$$

Where: $R_{control}$ and R_{sample} were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively.

The third method of expression based on the oxidation rate ratio (ORR) was calculated according to the method of Marinova et al., (1994) using the equation:

$$ORR = R_{sample} / R_{control}$$

Where: R control and R sample are the same in the previous equation.





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In the fourth method, the antioxidant activity coefficient (AAC) was calculated as described by Mallet et al., (1994).

$$(AAC) = (Abs_{S120} - Abs_{C120}) / Abs_{C0} - Abs_{C120}) \times 1000$$

Where: Abs $_{S\ 120}$ was the absorbance of the antioxidant mixture at time 120 min, Abs $_{C\ 120}$ was the absorbance of the control at time 120 min, Abs $_{C\ 0}$ was the absorbance of the control at zero time.

β-carotene bleaching (BCB) assay

For β -carotene bleaching (BCB) assay, antioxidant activity (AA) against time (every 10 min thereafter for 120 min) for the all tested vegetables processing by-product extracts was measured/constructed according to Marco, (1968). The AA was all calculated as percent inhibition (bleaching rates of β -carotene in reactant mixture of plant part extracts) relative to control (bleaching rates of β -carotene in reactant mixture of without plant part extracts) such as described by Al-Saikhan *et al.*, (1995).

Determination of total phenolics

Total phenolics were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Two hundred milligrams of sample was extracted for 2 h with 2 mL of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 mL vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 0C for 5 min; 0.75 ml of sodium bicarbonate (60g/L) solution was added to the mixture after 90 min at 22 0 C, absorbance was measured at 725 nm. Results are expressed as ferulic and equivalents.

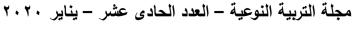
Statistical Analysis

All measurements were done in triplicate and recorded as mean \pm SD. Statistical analysis was performed with the Student *t*-test and MINITAB-12 computer program (Minitab Inc., State College, PA).

Results and Discussion

Antioxidant activities of selected herbs

The antioxidant activities and total phenolics of three herbs and their mixture extracts are shown in Figure (1). From such data it could be noticed that the herbs extracts and their mixture showed considerable differences in antioxidant activity (AA= 80.11- 90.98%) when it was calculated by the four different methods used in this study. Marjoram showed strong activity because of their high phenolic content





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(938.87 mg/100 g d.b.). Spearmint and ginger showed relatively moderate antioxidant activity (AA= 83.21 and 80.11%) and total phenolics (376.82 and 298.43 mg/100 g d.b.).

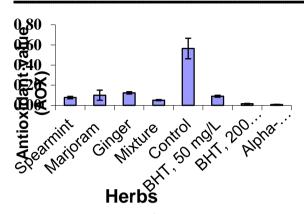
The decrease in absorbance of β -carotene in the presence of different methanolic herbs extracts (and well-known antioxidants used as standards) with the oxidation of β -carotene and linoleic acid is shown in Table (2) and Figure (9). Such data indicated that herbs mixture extract recorded the lowest decreasing followed by marjoram, ginger and spearmint extracts, respectively. The values of marjoram extracts absorbance's through 120 min are coming well i.e. closing the line of 50 mg/L of both BHT (50 mg/L) and α -tocopherol followed by ginger and spearmint extracts. These data proved the high stability of the all tested plant by-products when comparing with that more common standards BHT and α -tocopherol.

In a similar study, Velioglu *et al.*, (1998) determined the antioxidant activities and total phenolics of 28 herbs products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, several fruits, vegetables, and medicinal herbss and found that the total phenolics content varied from 169 to 10548 mg/100 g of dry product. Antioxidant activity of methanolic extract evaluated according to the β -carotene bleaching method expressed as AOX, AA , ORR, and AAC ranged from 0.05, 53.7, 0.009, and 51.7 to 0.26, 99.1, 0.46, and 969.3, respectively. Also, the present data are in accordance with the obtained by Ghaly, (2004) and Khoteem, (2005) who studied antioxidant activity of many spices and vegetable extracts commonly distributed in local markets.

Also, Elhassaneen *et al.*, (2013) indicated that the antioxidant activities of control and enriched MPP biscuits. The antioxidant activity (AA) in control biscuits was 31.34% which increased to 37.92 and 45.12% with the incorporation of MPP by 5 and 10%, respectively. The same date and antioxidant behavior for ROSP, and PPP are recorded by Shalaby (2015) and Ahmed, (2015).

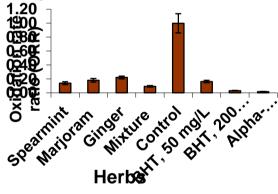


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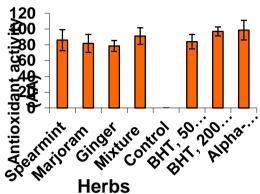


Antioxidant value (AOX*, A/h) of methanolic extracts of selected herbs.

* AOX (A/h) = The absolute value of slope (Abs was plotted against time).

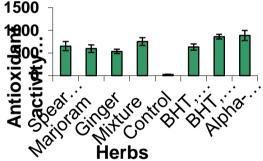


Oxidation rate ratio (ORR*, %) of methanolic extracts of selected herbs.



Antioxidant activity (AA*, %) of methanolic extracts of selected herbs.

*Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of betacarotene in reactant mixture without antioxidant and with plant extract, respectively.



Antioxidant activity coefficient (AAC*) of methanolic extracts of selected herbs.

* Antioxidant activity coefficient (AAC) = (Abs S 120 - Abs C 120) / Abs C 0 - Abs C 120) x 1000 where: .Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C 0 was the absorbance of the control at zero time.



^{*} Oxidation rate ratio (ORR) = R sample / R control

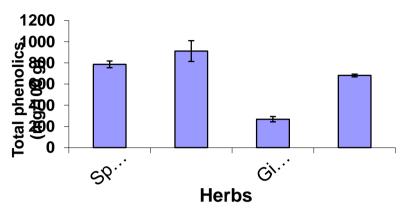


Figure 2. Total phenolics content in selected herbs

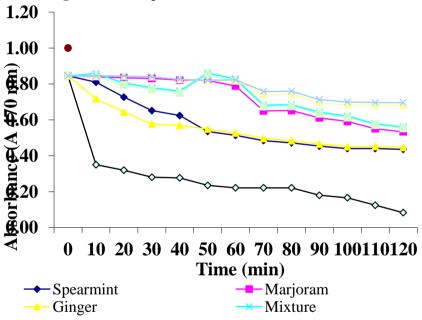


Figure 3. Antioxidant activity of methanolic extracts of selected herbs assayed by the β -carotene bleaching method (BHT α -tocopherol at 50 mg/L concentration was used as a reference)



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Relationship between phenolic contents and antioxidant activity

The total phenolic content of the selected herbs extracts investigated in this study varied from 298.43-938.87 mg/100 g of dry product (Figure 2). The relationship between total phenolic content and antioxidant activity of selected herbs is shown in Table (1) and Figure (3). The results indicated that when the selected herbs were included in the statistical analysis, there were positive correlations (r^2) were recorded 0.8415, 0.8856, 0.7273 and 0.8978 and significant ($p \le 0.05$, 0.01, 0.05 and 0.01) between total phenolics and antioxidant activity for spearmint, marjoram, ginger and their mixture, respectively. This indicates that phenolics can play a major role in the antioxidant activity of selected herbs. In similar study, Velioglu *et al.*, (1998) reported that the correlation coefficient between total phenolics and antioxidative activities of 28 herbs products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, several fruits, vegetables, and medicinal herbs was statistically significant. Also, Lee *et al.*, (1995) reported that phenolic compounds including flavonoids, correlated well with antioxidant activity (r^2 =0.86) in 5 cultivars of fresh pepper (*Capsicum annuum*).

Also, many studies indicated that there was a positive and significant ($p \le 0.01$) relationship between total phenolics and antioxidant activity in different plant parts (El-Mokadem, 2010; El-Safty, 2008 and Hegazy, 2009).

The data of this study with the others proved the importance of using selected herbs extracts as natural antioxidants in both therapy and food technology applications. Regarding to food technology applications, Serag El-Din, (2001) found that adding of phenolic acid (cinnamics) to vegetable oils leads to significant decrease in the rate of hydrolysis, rancidity and formation of the toxic and carcinogenic substances during the deep frying process. Also, Dewan, (2003) and Elhassaneen *et al.*,(2004) found that the washing of oils used in deep-fat frying of Tamia and potato for 12 h

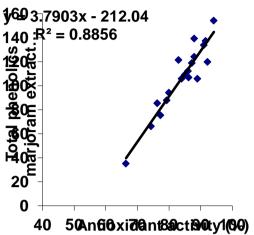
Table 1. Relationship between antioxidant activities and total phenolic contents of herbs

| Herbs | n | Relationship with total phenolics | r ² |
|---------------|----|-----------------------------------|---------------------|
| Spearmint | 16 | y = 1.4569x - 52.142 | 0.8415 ^a |
| Marjoram | 19 | y = 3.7903x - 212.04 | 0.8856 b |
| Ginger | 18 | y = 0.7845x - 14.863 | 0.7273 ^a |
| Herbs mixture | 18 | y = 2.3879x - 134.12 | 0.8978 ^b |

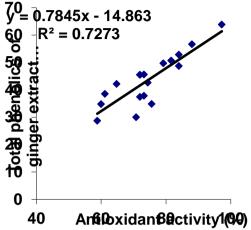
Where: y= Total Phenolics of herb extract (mg GAE/g extract), x= Antioxidant activity (%), a P \geq 0.05, b P \geq 0.01



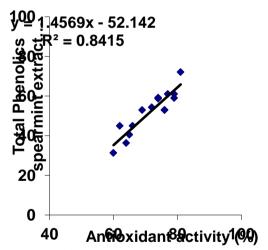
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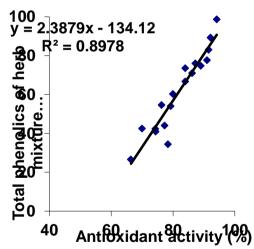
Relationship between total phenolic content and antioxidant activity of marjoram



Relationship between total phenolic content and antioxidant activity of ginger

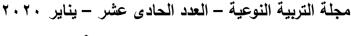


Relationship between total phenolic content and antioxidant activity of spearmint



Relationship between total phenolic content and antioxidant activity of herb mixture

Figure (3). Relationship between phenolic contents and antioxidant activity of selected herb mixture





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with water containing phenolic acids leads to significant improvement in all chemical and physical properties of that oil samples.

Also, some toxic compounds, i.e. mutagenic and carcinogenic substances formed during the deep-fat frying operation in oil samples have been removed partially after washing treatment. The effect of plant by-products treatment on the formation of PAH in charcoal broiled bread was studied by Mohammed, (2012). The addition of potato peel powder and red onion skin powder to the meatballs paste leads to significant decrease on the formation of polycyclic aromatic hydrocarbon compounds in its charcoal broiled product. Antioxidant compounds found in plant by-products such as used in the present study inhibited or delayed the oxidation of other molecules i.e. lipids, proteins, nucleic acid, and carbohydrates by inhibiting the initiation or propagation of oxidizing chain reactions (Shalaby, 2015).

Majid et al., (1991) found feeding of phenolic acid (ellagic) significantly increased the levels of reduced glutathione and glutathione reductase in liver and lungs of male and female mice as well as increase in inhibition of NADPH-dependent lipid peroxidation. The antioxidant activity of four phenolic acids, representative of three chemical groups present in human diet, upon low density lipoprotein peroxidation was studied *in vitro* in a low density lipoprotein (LDL) oxidation model by Laranjinha et al., (1994). The controlled oxidation of LDL was initiated by free radicals generated from a hydrophilic azo initiator and followed by monitoring the oxygen consumption and the fluorescence quenching of cis-parinaric acid previously incorporated into LDL. The hydroxycinnamic acid derivatives, chlorogenic and caffeic acids, have high stoichiometric numbers and reactivity with peroxyl radicals as compared with trolox, the water-soluble analogue of vitamin E, whereas ellagic acid (a tannic compound) compares with trolox effects. Protocatechuic acid (a hydroxybenzoic acid derivative) exhibits a complex reaction with peroxyl radicals, as indicated by UV spectroscopy, resulting in undefined inhibition periods of LDL oxidation and low reactivity with peroxyl radicals. Presumably, secondary radicals of these compounds are unable to initiate LDL oxidation.

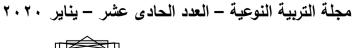
Data of the present study with that carried out by the others could be represent the mile stone towards the extension of using the powder or extracts of the selected herbs as natural antioxidants in many different nutritional and food processing applications. Synthetic antioxidants such as butylated hydroxyanisole (BAH) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of the last century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Reviewed in Al-Saikhan, 1995). Thus, the interest in natural antioxidants has increased considerably.



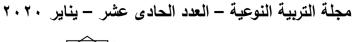
إعداد/أ.د. يوسف الحسانين، أ.م.د. عبير الخميسي، أ.م.د. ريعان عبد المنعم، أ. إيناس محمد مرزوق

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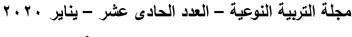


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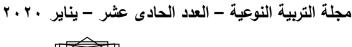


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