Antioxidant and Antimicrobial Activity of Thyme and Cinnamon Extracts

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ABSTRACT

Thyme (*Thymus vulgaris*) and cinnamon (*Cinnamomum cassia*) extracts were investigated for their phenolic compounds , antioxidant and antimicrobial activity. Moreover, the gross chemical composition for both herbs was determined. Polyphenols were found to be 21.22 and 16.13 mg gallic acid/g for thyme and cinnamon ,respectively. Data of HPLC revealed thirteen peaks of phenolic compounds in thyme extract, while cinnamon extract exhibited ten peaks only. Gallic, *p*-coumaric , vanillic, caffeic, ferulic acids were detected in both extracts, in addition to rosmarinic acid and thymol in thyme, also catechin was found in cinnamon. Both thyme and cinnamon extracts have high antioxidant activity, being 83.70 % and 73.60%, respectively as measured by DMPD, Radical Scavenging Activity. Boiling of extracts for 20 min resulted in a significant decline of phenolic compounds content and antioxidant activities of thyme and cinnamon extracts .Moreover, sunflower oil contained 400 ppm of thyme extract exhibited the least peroxide value (PV) when stored at 60ºC for 7 days. Sunflower oil contained 400 ppm of cinnamon extract or BHT had lower PV than that of the control. Thyme and cinnamon extracts explored considerable antimicrobial inhibition of each of *Bacillus subtilis, Escherichia coli* , *Saccharomyces cerevisiae* and *Aspergillus niger* . In this respect, cinnamon extract was more effective than thyme extract.

Key words: Thyme, cinnamon, gross composition, antioxidant activity. antimicrobial activity, polyphenols, HPLC, PV.

INTRODUCTION

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidants 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 and triplet oxygen, or decomposing peroxides (Muchuweti *et al*., 2007). The position and degree of hydroxylation of phenolic compounds is of primary importance in determining the antioxidant activity of phenolic compounds. The ortho and para position of hydroxyl groups contribute markedly to the antioxidant activity while the meta position has little or no effect as antioxidant (Rice-Evans *et al*., 1995).In general, there are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic ones, which are being restricted due to their carcinogenicity (Zheng & wang, 2001). Natural antioxidants of plant origin are generally classified as vitamins, phenolic compounds including flavonoids, phenolic acids and volatile compounds in herbs and spices. The differences in the antioxidant activity of the herbs could be due to the methods of cultivation used for the different spices and differences in environmental factors they were exposed to, such as climatic growth conditions and duration of storage (Namiki, 1990, Muchuweti *et al*., 2007).These natural antioxidants are becoming increasingly important, not only in food but also in preventive medicine (Halliwell *et al.,* 1992). Lipid oxidation decreases food safety and nutritional quality by formation of potentially toxic products and secondary reaction products during cooking or processing (Shahidi *et al.,* 1992). To prevent and retard lipid oxidation, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertialy- butylhydroquinone (TBH), and propyle gallate (PG) have been added to lipid-containing foods (Winata & Lorenz, 1996). However, potential health hazards of synthetic antioxidants in foods, including possible carcinogens, have been reported several times. Since then, the search for naturally occurring antioxidants in plants as alter-

natives to synthetic antioxidants is of great interest to researches (Frankel, 1996). Crude extract of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food . The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers toward functional food with specific health effects (Loliger, 1991, Ramadan & Al-Ghandi, 2012).

The use of spices is widely spread in Asian countries. Historically, spices were exploited for their antimicrobial properties to preserve meat products. (Muchuweti *et al.,* 2007) . Antimicrobials are used in food for two main reasons: (1) to control natural spoilage processes (food preservation), and (2) to prevent/control growth of micro-organisms, including pathogenic micro-organisms (food safety). Natural antimicrobials are derived from animal, plant and microbial sources (Burt, 2004, Brandi *et al*., 2006). Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savory, and fennel, have been successfully used alone or in combination with other preservation methods. They exert direct or indirect effects to extend foodstuff shelf life or as antimicrobial agent against a variety of Gram positive and Gram-negative bacteria. However, their efficacy depends on the pH, the storage temperature, the amount of oxygen, the essential oil concentration and active components (Burt *et al*., 2007) . Mixtures of cinnamon and clove oils were capable of suppressing the growth of major spoilage microorganisms of intermediate moisture foods (Matan *et al*., 2006).

Due to the concern towards synthetic antioxidants, the present study aimed to identify and determine phenolic compounds, antioxidant activity and antimicrobial effect of thyme and cinnamon as natural and safe antioxidants and antimicrobial agents in food processing.

MATERIALS AND METHODS

Materials

Thyme (*Thymus vulgaris)* and cinnamon (*Cinnamomum cassia*) were purchased from the local market, Alexandria, Egypt, in powder form and kept in polyethylene bags at room temperature (22±2°C) until used.

Methods

Proximate chemical composition: Moisture, ash, crude protein (N × 6.25) and crude fat were determined as described in the A.O.A.C (1998) . Total carbohydrates were calculated by difference.

Extraction of total phenolic compounds: The powder of dry sample (10 g) was macerated in absolute methanol (100 ml) for 24 hours at room temperature according to Ziada (2002). The extracts were filtered, the filtrates were evaporated under vacuum using rotary evaporator at 45 ºC and weighed to determine the extract yield of thyme and cinnamon and these extracts were used for determination and identification of polyphenols.

Boiling of methanolic extracts: The extracts of thyme and cinnamon prepared by the previous method (without evaporation) were boiled in water bath for 0,5,10,15 and 20 min, and the residual antioxidant activity and total phenolic compounds were determined. (El Badawey *et al.,* 2010).

Determination of total phenolic content : The total phenolic content was determined in the extract following the Folin-Ciocalteu method (Singleton & Rossi, 1965). The reaction mixture contained 0.1 ml of extract, 7.9 ml distilled water, o.5 ml of Folin-Ciocalteu's reagent, and 1.5 ml of 20% anhydrous sodium carbonate solution. The contents were mixed and kept in dark for 2 hrs. The absorbance of the blue coloured samples was read at 765 nm. Total phenols content was calculated on dry weight basis as gallic acid equivalents (GAE) and values were expressed as mg of gallic acid/g of sample .

Identification of phenolic compounds by HPLC: Method of Shan *et al.* (2005) was used with some modifications in HPLC device as follows:- Phenolic compounds were extracted using 80% methanol containing 2 ml 0.1M sodium fluoride to prevent oxidation of phenolic compounds. Ten grams of sample were homogenized in 20 ml of extraction solution. The samples were centrifuged for 10 minutes at 12,000 xg and filtered through a 0.45 µm filter for analysis by HPLC. Separations of the phenolic compounds were carried out using HPLC system (Perkin Elmer Series 200) with a UV- Visible detector (Perkin Elmer Series 200) at 290 nm, the mobile phase was 5% formic acid in a gradient of methanol containing from 5 to 80%

final concentration. Compounds were identified by comparison with known standards (ferulic acid, caffeic acid, vanillic acid, *p*- coumaric acid, gallic acid, rosmarinic acid, thymol and catechin "Sigma-Aldrich Co.")

Antioxidant activity: Antioxidant activity was measured by the N, N-Dimethyl –*p*–phenylenediamine dihydrochloride (DMPD) . Two hundred and nine mg of DMPD were dissolved in 10 ml of deionized water. One ml of this solution was added to 100 ml of 0.1 M acetate buffer (pH = 5.25) then 0.2 ml of 0.05 M ferric chloride solution was added to obtain coloured radical cation ($DMPD^+$)as follows:

$$DMPD_{(uncoloured)} + \text{oxidant } (Fe^{3-}) + H^+ \rightarrow$$

$$DMPD^+ \text{ (purple coloured radical cation)}$$

$$DMPD^+_{(\text{purple coloured radical cation})} + AOH_{(\text{antioxidant material})} \rightarrow$$

$$DMPD_{(uncoloured)} + AO_{(\text{antioxidant compouds})}$$

One ml of this solution was directly placed in a 1 ml plastic cuvette and its absorbance was measured at 505 nm. Standard solution of the antioxidant compounds was prepared by dissolving 0.1 g ascorbic acid in 100 ml of deionized water.Antioxidant compounds were extracted from samples as follows: One g dried sample was added to 10 ml methanol , then centrifuged at 12,000 xg for 15 min. A volume of 50 µl of standard antioxidant or sample extraction was added in the spectrometric cuvette contained 1 ml of $DMPD^+$ solution, and after 10 min the absorbance was measured at 505 nm.A dose-response curve was derived for ascorbic acid, by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution according to the following equation:

$$\text{Inhibition of } A_{505}\,(\%) = (1 - A_F / A_0) \times 100$$

Where:

A_0= Absorbance of uninhibited radical cation.

A_F = Absorbance measured at 10 min after the addition of antioxidant samples. (Fogliano *et al.,* 1999).

Oxidative stability of sunflower oil : Oxidative stability was determined according to Ziada (2002) by oven test method for three different treatments of sunflower oil with natural and synthetic antioxidant as compared to the control as follows:-

A (control sample), B (sunflower oil + 400 ppm BHT), C (sunflower oil + 400 ppm thyme extract), and D (sunflower oil + 400 ppm cinnamon extract). Oil samples were stored in an incubator set at 60ºC for 7 days, during this period the peroxide values were determined daily during 7 days of storage.

Peroxide value : Peroxide value was determined according to the A.O.A.C. (1998) and expressed as m.eq.O_2 / Kg.

Antimicrobial activity: Antimicrobial activity of thyme and cinnamon extracts was determined according to the method of Sahin *et al.* (2004) using disc diffusion assay as follows:-

Colonies of *Bacillus subtilis* B505 and *Escherichia coli* D H5x, cultured in Luria Bertani, *Aspergillus niger* (wild type) isolated from bread, and *Saccharomyces cerevisiae* ATCC 4126, cultured in Sabouroud medium strains were suspended in 5 ml 0.1% peptone water and 100µ l of suspension were swabbed on the entire surface of Plate Count Agar (PCA) for bacteria and Potato Dextrose Agar (PDA) for yeast and mold . Sterile 6-mm filter paper discs (Whatman, Kent, UK) immerged with thyme and cinnamon methanol extract were aseptically placed on the center of the inoculated plates. The diameters of inhibition zones were measured in mm after incubation at 37 ºC for 24 hr (for bacteria) and 30 ºC for 48 and 72 hr (for yeast and mold , respectively). All tests were performed in duplicate. A disc with methanol was used as a control.

Statistical procedures : F-test, and Analysis of Variance of treatments difference were performed according to Steel & Torrie (1980). Statistical analysis was done by ANOVA, FACTOR F-test, and L.S.D. procedures available within the SAS software package (Version 9.13 2008). Graphs were produced using Harvard graphics software (HG, version , 5. 2003).

RESULTS AND DISCUSSION

Gross chemical composition of thyme and cinnamon powder: The results shown in Table (1) indicate that there were considerable amounts of protein (9.44 and 4.07%) and total lipids (5.57 and 2.48%) in thyme and cinnamon (on dry weight basis), respectively, where thyme contained twice amount of protein and total lipids as compared with cinnamon. As for the ash content, thyme had three folds as compared to cinnamon , but cinnamon had higher content of carbohydrates (88.84 %) as compared with thyme which contained 71.21 %.

Table 1: Gross chemical composition of thyme and cinnamon (on dry weight basis)

Component (%)	Thyme	Cinnamon
Crude protein (N × 6.25)	9.44 ± 1.5	4.07 ±0.31
Crude fat	5.57 ± 0.38	2.48 ±0.23
Ash	13.78 ± 0.37	4.61± 0.52
Carbohydrates*	71.21 ± 0.62	88.84 ±0.91

Values are means of triplicates ± standard deviations.
Moisture content for thyme and cinnamon was 7.69 ± 0.38 and 9.5 ± 0.83, respectively.
* Calculated by difference.

Phenolic compounds content and antioxidant activity: The data presented in Table (2) reveal a high content of phenolic compounds (21.22 and 16.13 mg gallic acid /g) as well as high antioxidant activity(83.70% and 73.60%) for thyme and cinnamon extracts , respectively. These results indicate capability to use the two herbs as natural antioxidants in food processing. In accordance, data of Zheng & Wang (2001)showed that thyme (*T.vulgaris*) is known to have high antioxidant capacity, and some methylated flavones isolated from the thyme herbs were found to possess antioxidant activity. Meanwhile, Lee & Shibamoto (2002) found that the antioxidant activities of some herbs extracts decreased descendingly as follows: thyme > basil > rosmary > chamomile > lavender and cinnamon. Baranauskiene *et al.* (2003) reported that thyme possesses various beneficial effects as antiseptic, carminative, antimicrobial and antioxidants properties. Furthermore, Shan*et al.* (2005) showed that the most common spices in *Labiatae* family (rosemary, oregano, sage, basil, mint, and thyme) overall had very strong antioxidant capacity and cinnamon extracts had very high levels of phenolics and strong antioxidant capacity. Muchuweti *et al*. (2007) found that cinnamon had 13.66 mg/g of total phenolic compounds and 92.0% radical scavenging activities .

Table 2: Phenolic compounds content and antioxidant activity of thyme and cinnamon extracts

Polyphenols (mg gallic acid/g)		Antioxidant activity (%)	
Thyme	Cinnamon	Thyme	Cinnamon
21.22±0.9	16.13 ±0.7	83.70±1.3	73.60±1.6

* On dry weight basis
Values are means of triplicates ± standard deviations.

Identification of phenolic compounds by HPLC: Figures (1) and (2) show the phenolic compounds of methanolic extracts of thyme and cinnamon. Thyme extract exhibited thirteen numbers of separated phenolic compounds. Only, seven compounds could be identified as follows: gallic acid, *p*-coumaric acid, vanillic acid, caffeic acid, ferulic acid, rosmarinic acid and thymol . Notwithstanding, ten of phenolic compounds could be separated from cinnamon extract by HPLC. Only six compounds

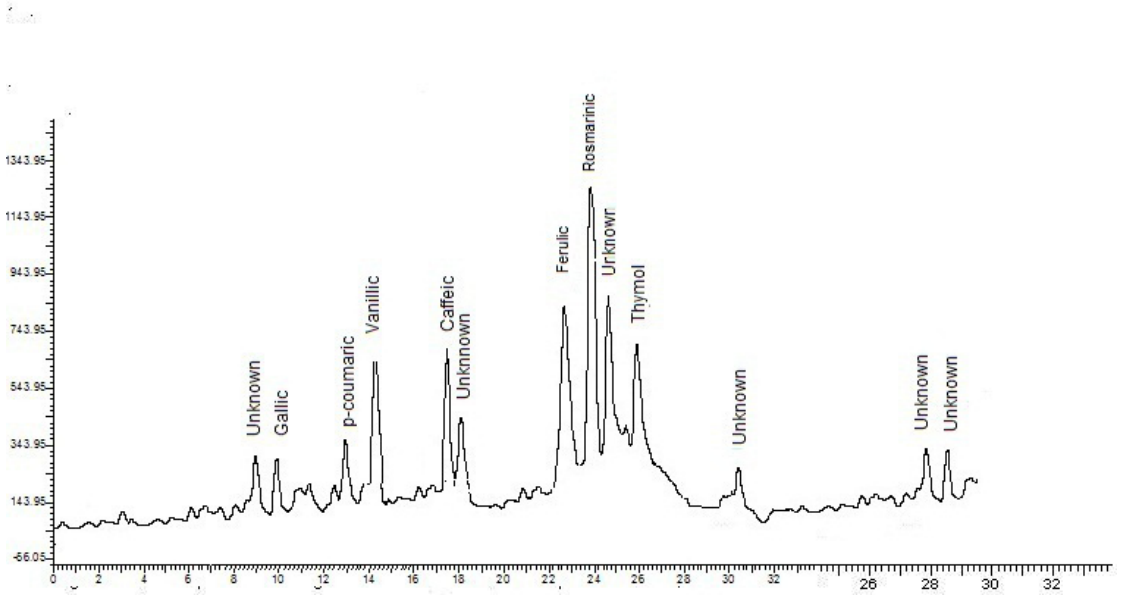

Fig. 1: HPLC profile of thyme methanol extract

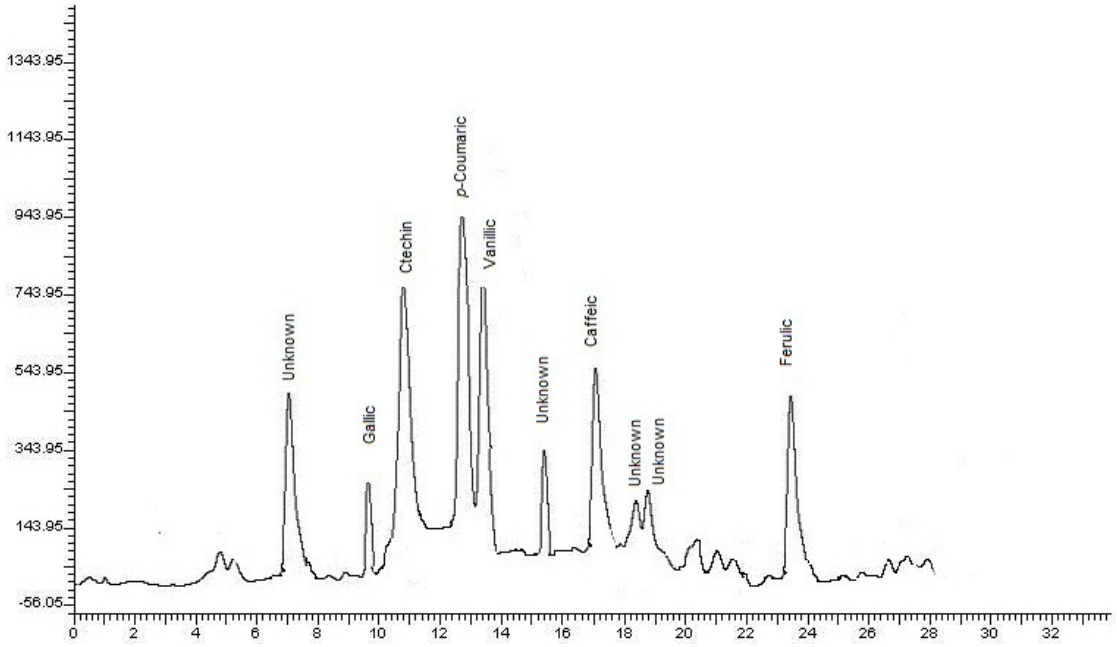

Fig. 2: HPLC profile of cinnamon methanol extract

could be identified in cinnamon extract as follows: gallic acid, catechin, *p*-coumaric acid, vanillic acid, caffeic acid and ferulic acid. Shan *et al.* (2005) reported that gallic acid, caffeic acid,*p*- coumaric acid, rosmarinic acid and thymol were identified in HPLC analysis of thyme extract . Moreover, Suhaj (2006) reported that caffeic-acid, ferulic-acid, gallic-acid, *p*-coumaric-acid, rosmarinic-acid thymol and vanillic-acid were detected in Thyme (*Thymus vulgaris*). Muchuweti *et al.* (2007) found that the phenolic acids identified in cinnamon were vanillic acid, caffeic, and ferulic acid. Meanwhile, Junli *et al.* (2012) detected catechin, gallic acid, vanillic acid, and *p*-coumaric acid in cinnamon.

Effect of boiling on phenolic compounds content and antioxidant activity of thyme and cinnamon extracts: Data in Table (3) represent the effect of boiling for (5,10,15 and 20 min) on the phenolic compounds and antioxidant activity of thyme and cinnamon extracts .It can be noticed that, phenolic content decreased significantly as the period of boiling was elongated. Moreover, antioxidant activity decreased with phenolics reduction , this means that phenolic compounds are responsible for antioxidant activity of both thyme and cinnamon extracts. It is worth to mention that the reduction in phenolics content amounted to be 43.45% and 36.14%, meanwhile reduction in antioxidant activity

Table 3: Effect of boiling on phenolic compounds content and antioxidant activity of thyme and cinnamon

Time	Thyme				Cinnamon			
(min)	*Polyphenols mg gallic acid /g	Reduction (%)	Antioxidant activity (%)	Reduction (%)	*Polyphynols mg gallic acid /g	Reduction (%)	Antioxidant activity(%)	Reduction (%)
0	$21.22^{a}\pm0.9$	---	$83.70^{a}\pm1.3$	---	$16.13^{a}\pm0.7$	---	$73.60^{a}\pm1.6$	---
5	$19.16^{b}\pm0.3$	9.70	$80.90^{a}\pm1.0$	3.30	$15.70^{a}\pm0.5$	2.66	$72.70^{a}\pm1.5$	1.20
10	$16.50^{c}\pm0.7$	22.24	$73.00^{b}\pm2.0$	12.80	$14.50^{a}\pm0.35$	10.10	$53.00^{b}\pm2.4$	27.90
15	$15.60^{c}\pm0.3$	26.48	$64.80^{c}\pm1.6$	27.60	$11.50^{b}\pm0.7$	28.70	$51.00^{b}\pm1.2$	30.70
20	$12.00^{d}\pm0.7$	43.45	$57.50^{d}\pm2.5$	31.30	$10.30^{b}\pm0.81$	36.14	$44.60^{c}\pm2.2$	39.40

* On dry weight basis

Values are means of triplicates ± standard deviations .Means in a column not sharing the same superscript are significantly different at $P \leq 0.05$

reached to 31.30% and 39.40% after 20 min of boiling for thyme and cinnamon extracts, respectively. These data are in agreement with other authors who confirmed the negative effect of heating on phenolic compounds and antioxidant activity of both herbs extracts under study, whereas data of Mansour & Khalil (2000) indicated that elongating the period of boiling resulted in a significant decrease in the antioxidant activity of the freeze-dried of some plant extracts. Furthermore, Shan *et al.* (2005) found that the phenolic compounds in the spices were responsible for their antioxidant capacity and results emphasized the importance of phenolic compounds in the antioxidant behaviour of spice extracts and also indicated that the phenolic compounds contributed significantly to the total antioxidant capacity. Meanwhile, El- Badawey *et al.* (2010) concluded that elongating the boiling period resulted in a significant decrease in the antioxidant activity of all plant material extracts.

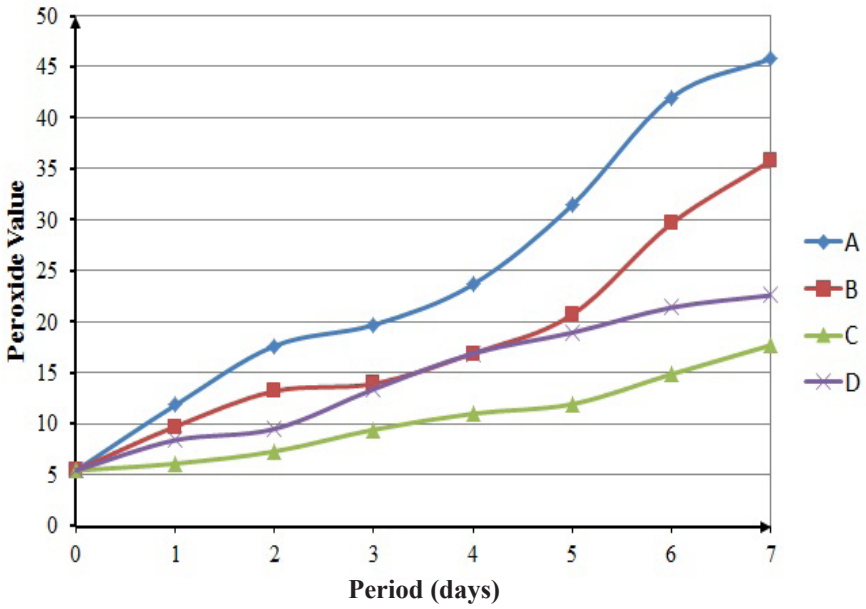

Fig. 3: Peroxide value of sunflower oil containing BHT, thyme and cinnamon extracts stored at 60ºC for 7 days

A = Sunflower oil (Control sample). B = Sunflower oil + 400 ppm BHT.
C = Sunflower oil + 400 ppm thyme extract.
D = Sunflower oil + 400 ppm cinnamon extract.

Oxidative stability of sunflower oil: Figure (3) shows, the inhibition effect on the peroxide values of sunflower oil stored at 60ºC for 7 days. The oil contained 400 ppm of each methanolic extract of thyme or cinnamon compared with a sample containing 400 ppm BHT as a synthetic antioxidant along with the control. The highest inhibition effect of peroxide formation was obtained in the case of using the thyme methanolic extract followed by cinnamon extract, then sample with BHT while the control was tailed behind . The results also revealed that the higher phenolic content had the higher inhibition effect on PV. This finding is in agreement with the conclusion obtained by Zheng & Wang (2001) who stated that the biphenyls, dimmers of thymol, and flavonoids isolated from thyme showed antioxidant activity as strong as BHT.

Antimicrobial activity of thyme and cinnamon extracts: Data presented in Table (4) shows the antimicrobial effect of thyme and cinnamon extracts on growth of two strains of bacteria (*Bacillus subtilis* B505. and *Escherichia coli* D H5x), one strain of yeast (*Saccharomyces cerevisiae* ATCC 4126) and one strain of fungi (*Aspergillus niger* (wild type)). The results show that there were clear inhibition zones around each of the two studied extracts, the diameter of the inhibition zones for thyme and cinnamon extracts were , 14.00 and 21.50 mm for *Bacillus subtilis* , 8.00 and 14.50 mm for *Escherichia coli* , 18.50 and 32.00 mm for *Saccharomyces cereviviae* and finally 20.00 and 28.00 mm for *Aspergillus niger* . From the previous data, it could be concluded that the cinnamon extract was

Table 4: Antimicrobial activity of thyme and cinnamon extracts

Microorganism	Diameter of inhibition zone (mm)*	
	Thyme	Cinnamon
Bacteria		
Bacillus subtilis	14.0	21.5
Escherichia coli	8.0	14.5
Yeast		
Saccharomyces cerevisiae	18.5	32.0
Mold		
Aspergillus niger	20.0	28.0

*Data are average of duplicate determinations.

more effective in preventing microbial growth of the studied bacteria, yeast and fungi strains as compared with the thyme extract because it exhibited wider diameter zones of inhibition. Meanwhile, each of both extracts had a noticeable antimicrobial effect on growth of the investigated strains. In accordance, Nanasombat & Wimuttigosol (2011) revealed that cinnamon had strong antifungal effect.

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النشاط المضادة للأكسدة ولنمو الميكروبات لمستخلصات الزعتر و القرفة

ماجدة سالم شرارة

قسم علوم وتقنية الأغذية – كلية الزراعة- جامعة الإسكندرية- الشاطبى- الرقم البريدى ٢١٥٤٥- مصر

أجريت هذه الدراسة على مستخلصي عشبي الزعتر و القرفة و ذلك للتعرف على كمية وماهية المركبات الفينولية و النشاط المضاد للأكسدة فضلا عن النشاط المضاد للميكروبات مع تقدير التركيب الكيماوي. تبين أن المركبات الفينولية تتواجد بتركيز ٢١,٢٢ و ١٦,١٣ مجم حامض جاليك /جم فى كل من الزعتر و القرفة على الترتيب. و أوضحت كروماتوجرامات الفصل بواسطة HPLC ثلاث عشرة قمة فى حالة مستخلص الزعتر مقابل عشر قمم فى مستخلص القرفة. وجدت الأحماض الفينولية التالية فى كلا المستخلصين : الجاليك – الباراكيوماريك – الفانيليك – الكافييك – الفيريوليك بالإضافة إلى الروزمارينيك و الثيمول فى الزعتر و الكاتيكين فى القرفة . و اتضح أن لمستخلصي الزعتر و القرفة نشاطا عاليا كمضادات أكسدة (٨٣,٧٪ ، ٧٣,٦٪ على الترتيب) و الذى تم قياسه بطريقة DMPD كشارد كابح لمضاد الأكسدة . أوضحت الدراسة أن غلى مستخلص الزعتر و القرفة لمدة ٢٠ دقيقة قد أدى إلى حدوث انخفاض جوهرى فى محتوى المستخلص من المركبات الفينولية، و كان ذلك مصحوبا بانخفاض النشاط المضاد للأكسدة للمستخلصين موضع الدراسة. تبين أن إضافة مستخلص الزعتر بتركيز ٤٠٠ جزء فى المليون إلى زيت دوار الشمس أعطى أقل قيم للبيروكسيد خلال تخزين الزيت لمدة ٧أيام على درجة حرارة ٦٠ م° متبوعا بالزيت المضاف إليه نفس التركيز من مستخلص القرفة ثم مضاد الأكسدة الصناعى BHT وذلك مقارنة بالعينة الحاكمة (الكونترول). أظهرت مستخلصات كل من الزعتر و القرفة نشاطا ملحوظا مضادا للميكروبات التالية (*Bacillus subtilis, Escherichia coli, Sacch romyces cerevisiae, Aspergillus niger*). وكان مستخلص القرفة هو الأكثر فاعلية كمضاد ميكروبي مقارنة بمستخلص الزعتر.