Innovative Uses of Ancient Egyptian Medicinal Plants Based on Antioxidative and Antimicrobial Activities

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ABSTRACT

Egyptian medicinal plants are being used as pharmaceutical ingredients, and in other innovative ways as in food or feed supplementation. Therefore, the aim of the present study was carried out to screen the bioactive compounds in selected Egyptian medicinal plants (coriander, cumin, garlic, ginger, rosemary, sage and thyme) and to evaluate the antioxidant and antimicrobial activities of plant extracts in model system and in different kind of Egyptian food. Phenolic compounds of methanol extracts were separated and identified using HPLC. The antioxidant activity of plant extracts was tested in β-carotene-methyl linoleate system and also evaluated in sunflower oil at ambient temperature and during frying of potato chips. The antimicrobial activities of extracts were determined by disc diffusion with Gram-positive and Gram-negative species and also evaluated in salted fish. The results showed that individual rosemary, sage, thyme and garlic extracts (600 ppm each) were the most active natural antioxidants in model system and in sunflower oil during storage in the dark at ambient temperature. Combination of these four extracts (100 ppm each) showed the strongest antioxidant activity in sunflower oil during frying. Garlic, thyme and ginger extracts (1200 ppm) found to be active against all tested microorganisms. Combination of extracts (300 ppm each) prevents the growth of staphylococcal bacteria in salted sardine. It could be concluded that the combination of tested plants showed promising antioxidant and antimicrobial activities, thus justifying their traditional and innovation uses.

INTRODUCTION

There are some serious problems concerning the toxicity of synthetic antioxidants as BHA, BHT and TBHQ (Tappel, 1995). The main concern about the safety of such compounds is related to their metabolism and possible absorption and accumulation in body organs and tissues. Beside, BHA and BHT are quite volatile and decompose easily at high temperatures (Branen, 1975). Therefore, the search for preparations of natural additives as antioxidants and antimicrobial agents are highly recommended to achieve sufficiently a long shelf life of foods and a high degree of safety with respect to food borne pathogenic microorganisms.

Several medicinal and aromatic plants have been reported to exhibit antioxidant and antimicrobial activity (Abdalla and Roozen, 2001, Salem and Arafat, 2007 and Abdalla, 2007). It has been reported that antioxidant and antimicrobial activity of plant extracts might be due to their phenolic

compounds. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides (Peyrat-Millard *et al.*, 2003). Polyphenols are well documented to have microbicide activities against a huge number of pathogenic bacteria (Karou *et al.*, 2005).

The ancient Egyptian culture clearly documented their use of medicinal and aromatic plants in their hieroglyphic records. The government is now giving great attention to increase the cultivation area of different medicinal and aromatic plants. Therefore, the aim of the present study was carried out to screen the bioactive compounds in seven selected ancient Egyptian medicinal plants and to evaluate the antioxidant and antimicrobial activities of plant extracts in model systems and different kind of foods.

MATERIALS AND METHODS

1. Materials

Seven selected ancient Egyptian medicinal plants (coriander, *Coriander sativum L.*, cumin, *Cuminum cyminum L.*, garlic, *Allium sativum L.*, ginger, *Zingiber officinalis L.*, rosemary, *Rosmarinus officinalis L.*, sage, *Salvia officinalis L.* and thyme, *Thymus vulgaris L.*) were obtained from Agricultural Research Institute, Ministry of Agriculture, Egypt.

Fresh refined, bleached and deodorized sunflower oil containing 510 ppm α-tocopherol, and without addition of any synthetic antioxidants was obtained from Alexandria Oil and Soap Company, Alexandria, Egypt. Initial quality was determined as peroxide value (0.8 meq.O_2 per kg oil) and conjugated diene hydroperoxides (0.045 at 234 nm). Fatty acid profile was 6.5 % $C_{16:0}$, 2.8 % $C_{18:0}$, 24.6 % $C_{18:1}$ and 66.1 % $C_{18:2}$. Fresh sardine was salted and stored as described by Abdalla *et al.*(1994).

All chemicals in this study were of analytical grade (Merck). Folin-Ciocalteau reagent and butylated hydroxytoulene (BHT) were purchased from Fluka Chemical Company, Amsterdam, The Netherlands.

2. Methods

2.1. Extraction procedure

Fresh plants (used parts as indicated in Table 1) were dried at 30 ± 2 °C. Dried plants were ground (0.3 – 0.4 mm) and 500 g of each plant material was extracted with individual one of each solvent; ethanol, acetone or methanol (1:2, w/v) for 24h at room temperature. The extracts were filtered and filtrate was evaporated in a rotary evaporator at 40 °C and

weighed. On the other hand, essential oils were extracted by steam distillation at Agricultural Research Institute, Ministry of Agriculture, Cairo, Egypt.

2.2. Chemical and microbiological analysis

Total polyphenols were determined and phenolic compounds of different methanolic plant extracts were identified by HPLC as described by Abdalla *et al.* (2007).

Antioxidant activity of methanolic plant extracts was tested in β-carotene-methyl linoleate model system and also evaluated in sunflower oil during storage at ambient temperature and during deep-frying (Abdalla, 1999).

The antimicrobial activity of 30 mg of methanolic extract of each medicinal plant was investigated by disc-diffusion method on agar as described by Milosevic and Solujic (2006) and also evaluated in salted sardine fish (Abdalla *et al.*, 1994).

RESULTS AND DISCUSSION

1. Total polyphenols and phenolic compounds in plant extracts

Highest amount of total phenols was obtained by methanol extraction. The content of total phenols of different methanolic plants that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent varied from 613.5 to 335.2 mg/g dry weight (Table 1). Rosemary extract had the highest amount of polyphenols followed by sage, thyme and garlic extracts. Coriander and cumin extracts had the lowest amount of polyphenols. These variations in total phenol contents could be due to the specific nature of the plant type.

Major phenolic compounds of different plant extracts were identified using HPLC. There were great variations among the identified compounds as indicated in Table 1.

2. Antioxidative activity of plant extracts

Antioxidative activity in β-carotene-methyl linoleate model system was highest for mixtures of rosemary, sage, thyme and garlic extracts than individual extract and was more effective than BHT (Table 1).

A good correlation was found between polyphenol content and antioxidant activities in all screened plants showing that antioxidants activities present in rosemary, sage, thyme and garlic extracts and their combination essentially due to phenolic compounds. These results are in agreement with data of Abdalla (2004).

The effect of individual and mixtures of four selected plant extracts (garlic, rosemary, sage and thyme) on the oxidative stability of sunflower oil during storage at ambient temperature and during deep-frying were evaluated by measuring the primary oxidation products (conjugated diene hydroperoxides) and generation of secondary oxidation products (hexanal) as shown in Figures 1&2. In general, individual and mixtures of rosemary, sage, thyme or garlic extracts inhibited the primary and secondary oxidation products during storage of sunflower at ambient temperature in the dark for 25 month.

On the other hand, the stability of control sunflower oil without additives dramatically decreased during 10 days of intermittent deep frying for 80 frying procedures of potato chips. Similar results were obtained by Abdalla (1999). In this study the mixtures of four extracts (100 ppm each of rosemary, sage, thyme or garlic) showed the highest antioxidative activities and were more effective than BHT (Figure 1 B).

3. Antimicrobial activity of plant extracts

The results indicated that the methanolic extract of garlic followed by ginger and thyme had high antibacterial activity towards the E.coli and S.aureus (Table 2). The highest inhibition was measured as 18.8 mm with 30 mg extract for *S. aureus* and 16.2 for *E. coli.*

Accordingly, three methanolic extracts of individual or mixture of garlic, ginger and thyme were investigated in salted fish (Table 3). Total bacterial counts were declined in salted fish with natural additives than fish samples without additives. Eviscerated sardine fish samples salted with natural additives as garlic, ginger and thyme and stored at low temperature were found in a good hygienic salted and safe at consumption times of ripening and storage (Abdalla *et al.*, 1994).

CONCLUSION

The results of the present study showed that rosemary, sage, thyme and garlic extracts which contain high amount of phenolic contents with various phenolic compounds, exhibited the greatest antioxidant activity. Moreover, mixture of these four extracts in low levels showed the highest antioxidative activities and was more effective in sunflower oil during deep frying than BHT, indicating that such natural plant extracts were more stable at high temperatures than synthetic antioxidants. On the other hand, individual or mixture of garlic, ginger and thyme extracts, showed the greatest antimicrobial activity.

However, those components responsible for the antioxidative and antimicrobial activities of these plant extracts and their mixtures are currently unclear. Therefore, it will be of interest to study synergistic effects of such extract mixtures.

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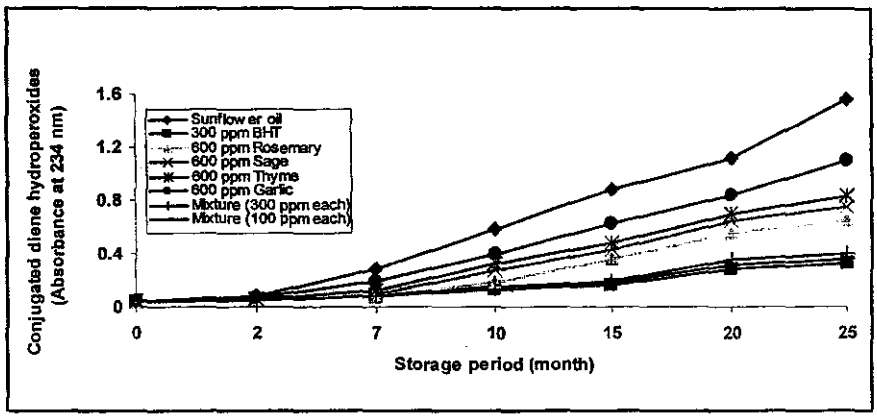
Table 1. Total phenolic contents, major phenolic compounds and antioxidative activity of Egyptian medicinal plants.

Medicinal plant	Egyptian common name	Part used	Phenolic content (mg/g dry weight)	Major phenolic compounds (mg/g)	Antioxidative activity*
Coriander (*Coriander sativum L.*)	Kosbara	Seed	455.4	γ-linalool(41), α-pinene(19), β-pinene(15), genariol(9), coumarins(7)	6.9
Cumin (*Cuminum cyminum L.*)	Kamoun	Seed	335.2	cuminaldehyde(36), 1,4-p-menthadiene(33), β-pinene(13), p-cymene(6)	8.8
Garlic (*Allium sativum L.*)	Toom	Bulb	562.8	diallyldisulfide(51), diallyltrisulfide(19), allinin(17),allicin(11)	16.5
Ginger (*Zingiber officinalis L.*)	Zangabil	Rhizomes	464.4	linalool(24), cineole(11), zingiberol(9)	11.8
Rosemary (*Rosmarinus officinalis L.*)	Hasaleban	Leaves	613.5	rosmanol(22), rosemaric acid(14), cineole(8), epirosmanol(6), carnosol(5)	18.8
Sage (*Salvia officinalis L.*)	Maryamia	Leaves	601.4	terpinene(31), sabinene(19), p-cymene(7), rosemarinic acid (9), limonene(6),myrcene(6)	17.9
Thyme (*Thymus vulgaris L.*)	Zaatar	Leaves	546.7	thymol(61), carvacrol(16), carvone(11), linalool(9), β-pinene(7), cymene(5)	17.5

* Antioxidative activities of plant extracts were tested in β-carotene-methyl linoleate model system. Antioxidative activity of butylated hydroxytoluene (BHT) and mixtures of rosemary, sage, thyme and garlic extracts (300 ppm each and 100 ppm each) were 19.2, 20.2 and 22.1, respectively. Values are mean of five determinations.

A



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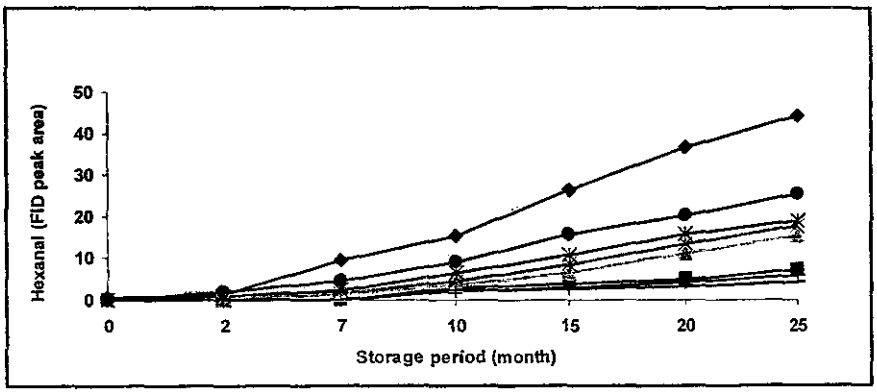

Figure 1. Effect of some Egyptian medicinal plant extracts on the formation of conjugated diene hydroperoxides (A) and generation of hexanal (B) in sunflower oil during storage in the dark at 20 ± 2 °C.

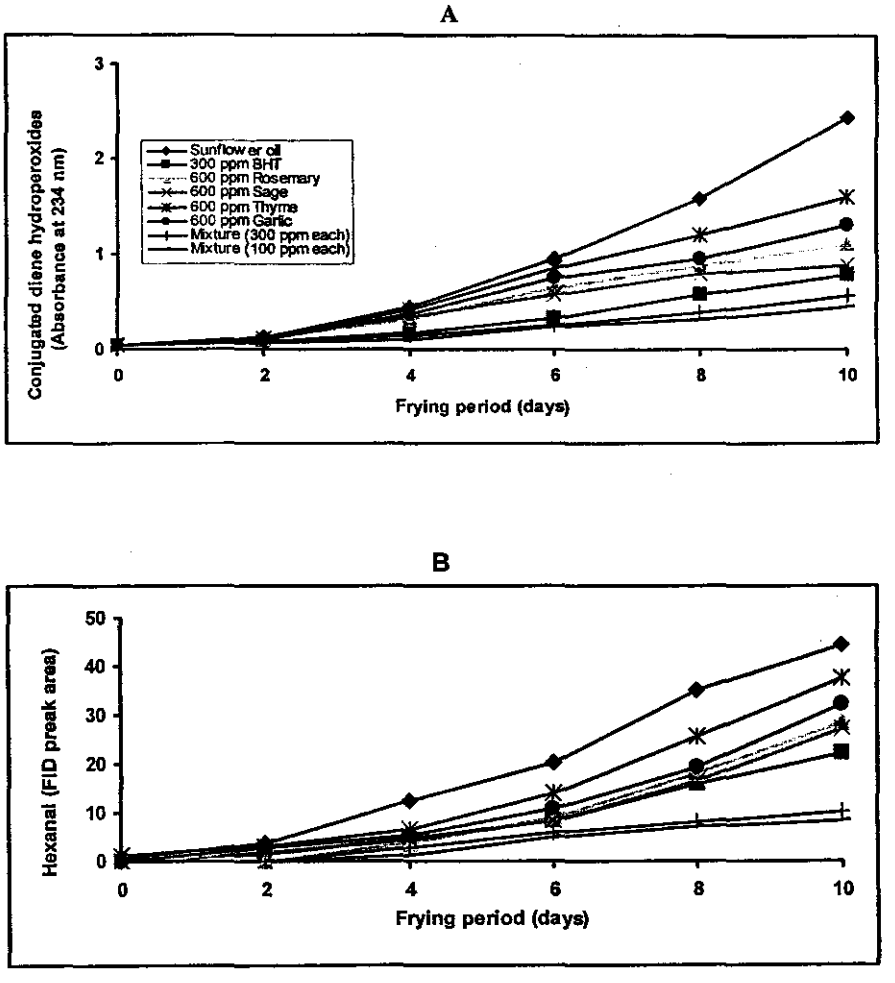

Figure 2. Effect of some Egyptian medicinal plant extracts on the formation of conjugated diene hydroperoxides (A) and generation of hexanal (B) in sunflower oil during frying at 180 °C for consecutive 10 days.

Table 2. Antimicrobial activity of Egyptian medicinal plant extracts expressed as inhibition zone diameter (mm).

	Diameter zone (mm)	
	E.. coli (G-)	S. aureus (G+)
Coriander	7.4	8.8
Cumin	5.6	5.2
Garlic	16.2	18.8
Ginger	9.7	8.9
Rosemary	7.8	6.5
Sage	9.9	11.7
Thyme	11.8	12.6

Table (3). The microbial content of fresh, salted and stored *Sardinella aurita*

Treatments	Counts × 10^4 (CFU/g)											
	Total plate count			Coilform			*E. coli*			*Staph. aureus*		
	Fresh	Salted *	Stored**	Fresh	Salted	Stored	Fresh	Salted	Stored	Fresh	Salted	Stored
Whole fish												
No additives(control)	20.5	9.2	15.4	0.2	ND	ND	0.1	ND	ND	0.6	0.2	0.5
+ 1200 ppm Garlic	20.5	2.1	5.3	0.2	ND	ND	0.1	ND	ND	0.6	ND	ND
+ 1200 ppm Ginger	20.5	4.5	7.5	0.2	ND	ND	0.1	ND	ND	0.6	0.1	0.2
+ 1200 ppm Thyme	20.5	4.1	6.2	0.2	ND	ND	0.1	ND	ND	0.6	0.1	0.2
+ Mixture (600 ppm)	20.5	1.1	3.5	0.2	ND	ND	0.1	ND	ND	0.6	ND	ND
+ Mixture (300 ppm)	20.5	0.5	2.2	0.2	ND	ND	0.1	ND	ND	0.6	ND	ND
Eviscerated fish												
No additives(control)	20.5	7.4	14.9	0.2	ND	ND	0.1	ND	ND	0:5	0.2	0.3
+ 1200 ppm Garlic	20.5	1.8	4.8	0.2	ND	ND	0.1	ND	ND	0.5	ND	ND
+ 1200 ppm Ginger	20.5	2.9	5.5	0.2	ND	ND	0.1	ND	ND	0.5	ND	ND
+ 1200 ppm Thyme	20.5	2.5	4.9	0.2	ND	ND	0.1	ND	ND	0.5	0.1	0.1
+ Mixture (600 ppm)	20.5	0.6	2.6	0.2	ND	ND	0.1	ND	ND	0.5	ND	ND
+ Mixture (300 ppm)	20.5	0.2	1.6	0.2	ND	ND	0.1	ND	ND	0.5	ND	ND

*Salted fish at room temperature (20 ± 2 °C) for 6 weeks.
** Stored salted fish at room temperature for 12 weeks.
ND is not detected. Mixtures are combination of garlic, ginger and thymes extracts.

الملخص العربى

الإستخدامات الحديثة للنباتات الطبية المصرية القديمة تبعا للنشاط المضاد للأكسدة والنمو الميكروبى

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أستاذ الصناعات الغذائية – قسم علوم الأغذية – كلية الزراعة سابا باشا

تستخدم النباتات الطبية فى مصر منذ عدة قرون فى علاج العديد من الأمراض وحالياً يضاف العديد من تلك النباتات أو مستخلصاتها إلى الأغذية كمواد مضافة طبيعية. وبناء على الإستخدامات الحديثة لتلك النباتات فى العديد من الأغذية لذا فإن الهدف من هذة الدراسة هو التعرف على أهم المركبات الفعالة فى بعض النباتات الطبية المصرية Coriander, Cumin, Garlic, Ginger, Rosemary, (Sage and Thyme) وذلك وفقا للنشاط المضاد للأكسدة والمضاد للنمو الميكروبى لتلك النباتات فى بعض الأغذية. هذا وقد تم تقدير المحتوى الفينولى فى مستخلصات النباتات كما تم عزل والتعرف على المركبات الفينولية المختلفة فى كل مستخلص بواسطة HPLC وتم إختبار النشاط المضاد للأكسدة للمستخلصات فى نظام *B-carotene-methyl linoleate system* وأيضا فى زيت عباد الشمس كما تم إختبار النشاط المضاد للنمو الميكروبى للمستخلصات النباتية مع العديد من أنواع البكتيريا الموجبة والسالبة لجرام وأيضاً فى السردين المملح. وأوضحت النتائج أن أهم مستخلصات النباتات التى لها فعل ونشاط مضاد للأكسدة أفضل من مضادات الأكسدة الصناعية هى Rosemary, Sage, Thyme and Garlic وأن مخلوط هذة المستخلصات بنسب أقل عن إستخدامها كل على حدى كان له نشاط مضاد للأكسدة أعلى ولها درجة ثبات عالية فى درجات الحرارة المرتفعة. كما أوضحت النتائج أن أهم مستخلصات النباتات التى لها فعل ونشاط مضاد للنمو الميكروبى هى Garlic, Thyme and Ginger. ويتضح من ذلك أن إستخدام مخلوط مستخلصات هذة النباتات المشار إليها كمواد مضافة طبيعية للعديد من الأغذية له تأثير إيجابى على زيادة فترة صلاحية وسلامة الغذاء. وخلاصة نتائج هذا البحث توضح مدى أهمية مثل هذة النباتات الطبية ومدى أهمية إستخدامها كمواد مضافة طبيعية فى العديد من الأغذية والصناعات الغذائية الحديثة وإحلالها محل المواد الحافظة والمضافة الصناعية وذلك بعد إجراء التحاليل عليها والتعرف على المركبات الفعالة بها.