

# Using of Egyptian herbs extracts in food processing and therapeutic nutrition applications

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استخدام مستخلصات الأعشاب المصرية في تطبيقات تجهيز الأغذية والتغذية العلاجية

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### المستخلص

هذه الدراسة ، سنحاول فتح طريق جديد لاستخدام مستخلص الأعشاب المختار في مختلف تقنيات الغذاء والتغذية العلاجية. تم تخفيض العديد من الخصائص الكيميائية للدهون المستخرجة من كرات اللحم الممزوجة مع مستخلصات الأعشاب المختبرة بما في ذلك قيمة الحمض (AV) ، وقيمة البيروكسيد (PV)، ومحتوى malonaldehyde (MDA) بالمقارنة مع تلك السيطرة بنسبة ٨.٩٩ - ٣٩.٦٨ ، ١٣.٢٩ - ٢٩.٠٧ و ١٠.٦٣ - ٣٧.٤١ % ، على التوالي. في حين تم تسجيل عدد اليود من هذه العينات في الاتجاه المعاكس أي زيادة بنسبة تتراوح بين ٧.٦٢ - ٢١.٥٨ % . العمل المثبط لكل مستخلصات الأعشاب المختبرة ومزيجها ضد أكسدة LDL المستحثة بـ CuSO4 ، كما يتضح من خلال تقليل إنتاج densees مترافق بطريقة تعتمد على الجرعة. بالمقارنة مع مستخلصات الأعشاب المختبرة، فإن النعناع ، البردقوش ، الزنجبيل ومزيجها تصرف بشكل أكثر دراماتيكية في حماية LDL ضد الأكسدة، مما يشير إلى إمكانية أن تكون هذه المستخلصات واعدة أكثر في الوقاية من تصلب الشرايين عن طريق تثبيط أكسدة LDL

الكلمات الرئيسية:

كرات اللحم، النعناع، المردقوش، والزنجبيل، والثوابت الدهنية، وتثبيط أكسدة LDL.

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## Antioxidant activities and total phenolics content in some Egyptian herbs

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**Abstract:** The present study, we will try to open a new avenue for the using of such selected herbs extract in different food technology and therapeutic nutrition applications. Many chemical characteristics of extracted fat of meatballs mixed with the tested herbs extracts including acid value (AV), peroxide value (PV), and malonaldehyde (MDA) content were decreased when compared with the control ones by the rate of ranged 8.99 – 39.68, 13.29 – 29.07 and 10.63 – 37.41%, respectively. While, the iodine number of those samples were recorded the opposite direction i.e. increased by the rate of ranged 7.62 – 21.58 %. The inhibitive action of the all tested herbs extracts and their mixture against CuSO<sub>4</sub>-induced LDL oxidation, as evidenced by decreased conjugated dienes production in a dose-dependent fashion. As compared to the tested herbs extracts, the spearmint, marjoram, ginger and their mixture acted more dramatically in protecting LDL against oxidation, indicating a possibility those extracts may be more promising in the prevention of atherosclerosis by inhibiting LDL oxidation.

**Keywords:** Meatballs, Spearmint, marjoram, ginger, fat constants, inhibiting LDL oxidation.

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## Introduction

Herbs are dried parts of aromatic plants including the seeds, flowers, leaves, bark or roots. From the dawn of civilization thousands of years before Christ, from Egypt, China, India, Arabia, Persia and Greece, documented records exist to witness the sophisticated knowledge and traditions connected with the cultivation and use of many herbs (Loewenfeld and Back, 1979). In Egypt, there are several hundreds of herbal species distributed in the spacious deserts. For example, ginger (*Zingiber officinale*), marjoram (*Majorana hortensis*) and spearmint (*Mentha spicata* L.) which are chosen for the present study for many reasons including: 1) it is a very common herb among several hundreds of herbal species distributed in the spacious deserts of Egypt, 2) it is well known as herb in world's herbal medicine, 3) this herb exhibit a wide range of therapeutic effects include stimulant, carminative, diaphoretic and mildly tonic, a useful emmenagogue, aching tooth frequently relieve the pain, warm infusion, valuable in spasms, colic, to give relief from pain in dyspeptic complaints, dried leaves and tops may be applied in bags as a hot fomentation to painful swellings and rheumatism, as well as for colic, and an infusion made from the fresh plant will relieve nervous headache (reviewed in Chevallier, 1996, Charai *et al.*, 1996, Vera and Chane, 1999, Duke *et al.*, 2002, Novak *et al.*, 2002 and Miguel *et al.*, 2003), and 4) all of these therapeutic effects principally attributed to the key constituents belong to phytochemicals i.e. phenolics, flavonoids, volatile oils and terpenes which present in the standardized extract of herb (Vera and Chane, 1999, Novak *et al.*, 2002 and Miguel *et al.*, 2003). Therefore, in the present study, we will try to open a new avenue for the using of such selected herbs extract in different food technology and therapeutic nutrition applications.

## 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.

### 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

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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.

## Using of the phenolic extracts of the selected plant parts in food industry and therapeutic nutrition applications

### Meat-balls manufactured

Red Beef meat and fat were purchased from the local market. They were minced, mixed (90 % red meat, 9.8 % fat and 0.2% of tested phenolics extract either singular or mixture) and used for meat-ball manufacture (25 g per each). Meat-balls were grilled without anything added over charcoal briquettes on an outdoor grill until well done.

### Acid value, peroxide value and iodine number

Acid value (mg KOH /g oil), peroxide value (meq O<sub>2</sub> / kg oil) and iodine value (g iodine/100 gm fat), were determined using the methods described in the AOAC, (1985).

### Malonaldehyde content

Thiobarbituric acid (TBA, malonaldehyde content) was determined as described by Pearson (1970) as follows: 10 g of the sample were distilled with (47.5 ml of distilled water + 2.5 ml HCl, 4N) for 10 min. A 5 ml of the distilled water were added to 5 ml of TBA reagent (0.2883 gm TBA/100 ml of 90% glacial acetic acid) into stoppered tube, and then heated in a boiling water bath 35 min. After cooling, absorbance was measured at 538 nm using Labo-med., Inc., spectrophotometer. The TBA value was calculated by multiplying the absorbance (ABS) by the factor (7.8). The results represented as mg malonaldehyde/kg sample.

### Inhibition of low density lipoprotein (LDL) oxidation

Inhibition of LDL oxidation was determined according to the method of Princen *et al.*, (1992). Adult male white albino rat, Sprague Dawley strain, serum was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Quantities of 5.0 ml diluted serum were mixed with 10 µl DMSO or 10 µl



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DMSO containing various concentrations of the all tested vegetables processing by-product extracts. A 20 µl of CuSO<sub>4</sub> solution (2.5 mM) was added to initiate the reaction and the absorbance at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final result was expressed by calculation the net area under the curve.

## Statistical Analysis

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

## Results and Discussion

### Food processing applications (Raising the quality parameters of meatballs)

As we know, antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Long time ago, synthetic antioxidants such as butyl hydroxyanisole (BHA) and butyl hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. But, many restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Branen, 1975 and Ito *et al.*, 1983). Thus, the interest in natural antioxidants has increased considerably (Loliger, 1991). The ability of some phenolic compounds to act as antioxidants has been demonstrated in the present study and others.

In a trial to extend the using of natural antioxidants, the phenolic extracts of the selected herbs will be used for raising the quality control parameters in manufactured food products. Meat-balls represent one of the most common meat products distributed in local and international restaurants. Because of the raising of fat content, this meat product is much susceptible to oxidation and rancidity. All of these chemical changes could be responsible for degradation in quality control parameters as well as probably inducing the toxic effects. In the present study, many extracts of the selected herbs were added to meat-balls during its manufacture. Data in Table (1) indicated the effect of the tested herbs extracts on the chemical characteristics of fat extracted from broiled meat-balls in compared with the control samples. From such data it could be noticed that many chemical characteristics of the extracted fat samples including acid value (AV), peroxide value (PV), and malonaldehyde (MDA) content were decreased when compared with the control ones by the rate of ranged 8.99 – 39.68, 13.29 – 29.07 and 10.63 – 37.41%, respectively. While, the iodine number of those samples were recorded the opposite direction i.e. increased by the rate of ranged 7.62 – 21.58 %.



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In similar studies, 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.*, (2003) found that the washing of oils used in deep-fat frying of tamia and potato for 12 h with water containing phenolic acids leads to significant improvement in all chemical and physical properties of that oil samples. Additionally, 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. Also, Mohamed (2005) found that the treatment of smoked fish with 10 ppm *p*-coumaric acid during smoking process leads to decrease the PAH formation by the rate of 8.24 %. That rate was increased with the increasing of the *p*-coumaric concentration and recorded 31.53 % with 50 ppm. The same behaviour was recorded with ellagic acid. Resently, **Shehata and Radwan, (2017)** confirmed that the beneficial effects of plant part (onion skin extracts) rich in phenolics content such found in the tested herbs extracts addition to some foods, like edible oils to maintain oxidative stability and charcoal broiled meat to minimize the formation of some toxic and carcinogenic compounds.

## Therapeutic nutrition applications [Inhibition of low density lipoprotein (LDL) oxidation]

Dose-dependent inhibition of CuSO<sub>4</sub>-induced LDL oxidation *in vitro* by tested herbs extracts is shown in Figure (1). From such data it could be noticed that

**Table 1.** Effect of methanolic extracts of tested herbs on the Chemical characteristics of fat extracted from broiled meat-balls

Parameter	Control Mean ±SD	Plant product							
		Spearmint		Marjoram		Ginger		Herbs mixture*	
		Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change
Acid value (mg KOH/gm oil)	1.89 ± 0.25	1.58 ± 0.29	-16.40	1.49 ± 0.11	-21.16	1.72 ± 0.22	-8.99	1.14 ± 0.09	-39.68
Peroxide value (meq/kg oil)	6.02 ± 0.85	5.11 ± 1.01	-15.12	4.84 ± 0.39	-19.60	5.22 ± 0.59	-13.29	4.27 ± 0.38	-29.07
Iodine number (g)	70.90 ± 2.75	77.90 ± 3.86	9.87	80.39 ± 4.51	13.39	76.30 ± 2.98	7.62	86.20 ± 3.01	21.58
Malonaldehyde (mg/kg)	11.20 ± 1.37	9.64 ± 0.84	-13.93	8.96 ± 1.06	-20.00	10.01 ± 0.67	-10.63	7.01 ± 1.14	-37.41

\* Each value represents mean ±SD. \*\* Herbs mixture consists of spearmint, marjoram and ginger by the equal ratios.

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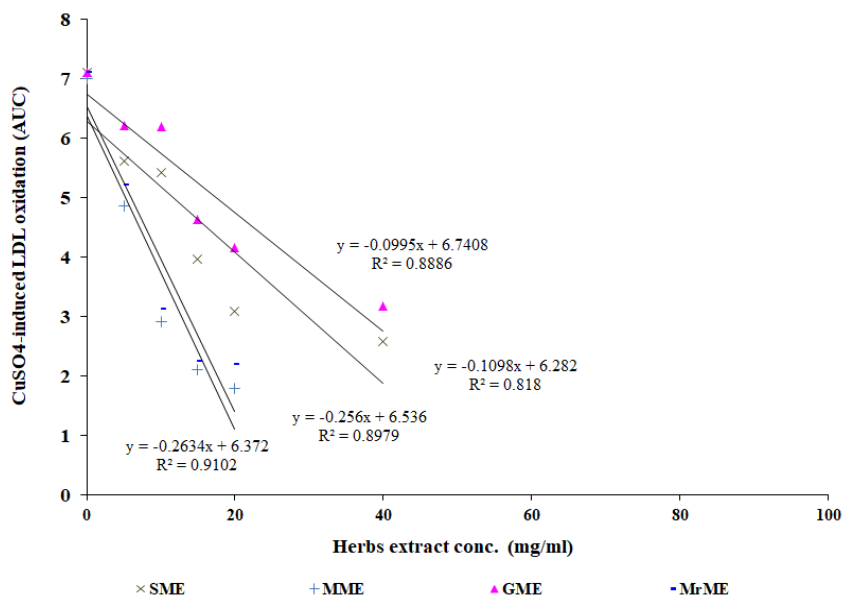
the inhibitive action of the all tested herbs extracts and their mixture against CuSO<sub>4</sub>-induced LDL oxidation, as evidenced by decreased conjugated dienes production in a dose-dependent fashion. As compared to the tested herbs extracts, the spearmint, marjoram, ginger and their mixture acted more dramatically in protecting LDL against oxidation, indicating a possibility those extracts may be more promising in the prevention of atherosclerosis by inhibiting LDL oxidation. Such effect could be attributed to the different bioactive compounds as antioxidants (phenolics, vitamins, volatile oil components etc) contained in such tested herbs extracts. Such data are in accordance with that obtained by Aviram *et al.*, (2000) who found that pomegranate juice effectively protect LDL against oxidation *in vitro*, which was attributed to the high levels of polyphenols and ascorbic acid (including the tested herbs extracts) contained in the juice. Also, Li *et al.*, (2006) confirmed the inhibitive action of pomegranate pulp and peel extracts against LDL oxidation for the same reasons. They also found that the peel extract acted more efficiency as compared to the pulp extract, in protecting LDL against oxidation due to its higher content of polyphenolic compounds. Such mechanisms of actions, protecting LDL against oxidation by phenolic compounds, could be included increased the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in liver and lungs as well as increase in inhibition of NADPH-dependent lipid peroxidation (Majid *et al.*, 1991). Furthermore, phenolic acids exhibited a complex reaction with peroxy radicals and inhibition of the LDL oxidation (Laranjinha *et al.*, 1994). In the same context, Shalaby, (2015) reported that 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. Also, Aly *et al.*, (2017) reported that vegetables processing by-product extract roles in the alleviation of health complications caused by diabetes in rats. From first principles, it is easy to see that antioxidants might protect a target by: 1) scavenging oxygen-derived species, either by using protein catalysts (enzymes) or by direct chemical reaction proceeds); 2) minimizing the formation of oxygen-derived species; 3) binding metal ions needed to convert poorly reactive species (such as O<sub>2</sub> - and H<sub>2</sub>O<sub>2</sub> ) into nasty ones (such as OH); 4)





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**Figure 2.** Dose-dependent inhibition of CuSO<sub>4</sub>-induced LDL oxidation *in vitro* by VPBE. The conjugated dienes formation was monitored kinetically as the absorbance at 234 nm and the result is expressed as the area under the curve (AUC). SME, spearmint methanol extract; GME, ginger methanol extract; MrME, marjoram methanol extract and MME, mixture methanol extract of SME, GME and MrME by equal parts.

repairing damage to the target; and 5) destroying badly damaged target molecules and replacing them with new ones (reviewed in Symons and Gutteridge, 1998)

On the other side, many studies reported that the “oxidative modification of lipoproteins” hypothesis proposes that LDL oxidation plays a key role in early atherosclerosis (Chisolm and Steinberg, 2000). The oxidized LDL is atherogenic due to its cytotoxic toward arterial cells and stimulates the monocytes to be adhesive to the endothelium which leads to the development of atheromatous plaques (Hong and Cam, 2015). Also, data of the present study proved that the tested herbs extracts and their mixture could be used successfully as a promising tool in the prevention of atherosclerosis through inhibiting LDL oxidation process.

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