



Influence of Some Culinary Plants on the Suppression of Human Low Density Lipoprotein (LDL) Peroxidation (In-vitro)

Faten K. Abd El-Hady*, Zeinab A. El-Shahid

Chemistry of Natural Products Department, National Research Center, Egypt.

*Corresponding author's E-mail: fatenkamal@hotmail.com

Accepted on: 01-03-2014; Finalized on: 30-04-2014.

ABSTRACT

Cardiovascular disease is one of the most reasons of mortality in world. Oxidation of low-density lipoprotein (LDL) has been strongly implicated in the pathogenesis of atherosclerosis. The use of some natural antioxidant and herbs may lead to the inhibition of production of oxidized LDL and may decrease both the development and the progression of atherosclerosis. The antioxidant activities using different assays (DPPH, Xanthine Oxidase system, LDL oxidation and protein modification) in fifteen different plant parts used in culinary and herbal medicine were evaluated. Seeds of (dill, fennel, basil, turnip) and leaves of (basil, olive, jew's mallow) showed the highest activity against inhibition of DPPH radical. Most plant extracts show relatively high activity against superoxide anion in Xanthine-XOD system. Seeds of (dill, fennel, jew's mallow), leaves of (dill, basil, salad rocket, jew's mallow) and thyme plant have the highest antioxidant activity against Copper-induced oxidation of LDL. Leaves of dill, basil, olive, salad rocket, jew's mallow and seeds of salad rocket, jew's mallow, beside de-oiled seeds of olive can decrease the level of protein modification. It could be concluded that culinary and medicinal herbal extracts investigated in the present study may play a role in scavenging the free radicals and increase the inhibitory effect not only against LDL lipid peroxidation, but also against modification of the protein moiety in LDL protein core, which are the early manifestations of atherosclerosis.

Keywords: Antioxidant, Edible plants, LDL peroxidation, Protein modification.

INTRODUCTION

Cardiovascular disease is one of the most reasons of mortality in world.¹ Although an increased concentration of plasma low density lipoprotein (LDL) is believed to be a major risk factor in this regard the underlying mechanisms remain unclear and needs more investigations.^{1,2} Several reports have indicated the role of oxidized LDL (Ox-LDL) in the pathogenesis of atherosclerosis.^{3,4} uptake of Ox-LDL by macrophages results in the formation of foam cells and Ox-LDL accumulated in vascular endothelial cells, and promotes the development of the characteristic fatty streaks found in atherosclerotic lesions.^{5,6} Also Ox-LDL has been detected in human and animal atherosclerotic lesions. Oxidized LDL has also been reported to compromise endothelial integrity, a silent feature of atherosclerosis.⁷

Oxidative stress describes a set of intracellular or extracellular conditions that lead to the chemical or metabolic generation of reactive oxygen/nitrogen species, including superoxide radicals, hydrogen peroxide (H₂O₂), hydroxyl radicals, singlet oxygen, lipid hydroperoxides, peroxyxynitrite, and related species.⁸ Reactive oxygen/nitrogen species can cause oxidative damage to essential cellular constituents, such as membrane lipids, proteins and DNA, which may ultimately result in cell death.⁹

Aerobic organisms attempt to protect themselves against oxidative damage with exogenous antioxidants (e.g., vitamin E, ascorbic acid, and carotenoids) obtained through the diet as well as endogenous antioxidants (e.g., glutathione, glutathione peroxidase, catalase, and superoxide dismutase). Furthermore, oxidative damage

mediated by the reactive oxygen species listed above is known to contribute to the aging process and the pathogenesis of cancer, cardiovascular disease, and other degenerative diseases.^{10,11}

Polyphenolic compounds are commonly found in both edible and inedible plants, they have multiple applications in food, cosmetic and pharmaceutical industries.¹² The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. In addition to their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, antimicrobial, anti-thrombotic, cardio-protective and vasodilator effects.¹³ Phytochemicals, including phenolics are suggested to be the major bioactive compounds contributing to the health benefits of vegetables and fruits.^{14,15} It was shown that the health properties of these natural products depend on the contents of bioactive compounds, mainly phenolic compounds, and partly on dietary fibers.^{16,17}

Researchers are recently interested in natural antioxidants from medicinal herbs to replace synthetic antioxidants. Therefore, the research into the determination of the natural antioxidant source is very important to promote public health.¹⁸ Recently, natural food and food-derived antioxidants such as phenolic phytochemicals have received considerable attention, because they are as chemo-preventive agents against oxidative damages.^{19,20} The quest for plants with medicinal properties continues to receive attention for a



complete range of biological activities. Currently, large and ever expanding global population prefers the use of natural products in treating and preventing medical problems.²¹

The aim of this study was to investigate some medicinal and culinary herbs and spices which can be used in the production of phytochemicals or may be served as raw material in food and drug industry. The ethanol, 70% ethanol and water extracts were investigated for antioxidant activity by DPPH, superoxide anion radical generated in Xanthine-Xanthine Oxidase system, inhibition of LDL peroxidation and protein modification *in-vitro*.

MATERIALS AND METHODS

Plants processing

Raw medicinal plants used locally in Egypt, fruits, seeds, vegetables, spices were selected. Common names, scientific names and parts used are shown in Table 1. One gram of powdered sample from each plant part was extracted with ethanol, 70% ethanol and water for 24 hours to give extract 1, 2 and 3 respectively. Extracts were filtered and stored at 0°C until used.

Determination of DPPH radical Scavenging activity

The DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity was determined according to the method of Matsushige et al.²² The absorbance was measured at 520 nm. Samples and DPPH were dissolved in methanol. Values are expressed as mean \pm SD, n = 3 at a concentration of (100 μ g/ml for all tested extracts).

Determination of superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity by generating superoxide anion free radical in Xanthine-Xanthine Oxidase system was measured following the method of Matsushige, et al.²² The color obtained was measured at 560 nm. Values are expressed as mean \pm SD, n = 3 at a concentration of (100 μ g/ml for all tested extracts).

Measurement of Copper-Induced LDL oxidation in-vitro

Isolation of LDL

LDL was isolated according to the method of Gugliucci and Menini.²³ LDL (1.019-1.055 g/ml) was separated by sequential ultra-centrifugation using TL-100 Ultracentrifuge (Beckman, U.S.A.) from plasma previously added with EDTA (0.1%). LDL then extensively dialyzed against three changes of phosphate- Buffered Saline (PBS) [10 mM Sodium phosphate buffer, PH 7.2, Containing 150 mM NaCl] Containing 0.01% EDTA at 4°C. Samples were stored at 4°C in the dark and used within 24hrs. Protein Content was determined according to Lowry's method.²⁴ LDL was oxidized using 5 μ M/ml CuSO₄ (freshly prepared). Incubation was carried out at 37°C for 1hr with the antioxidant (plant extract) before the addition of CuSO₄.

Oxidation of LDL was monitored in the presence or the absence of antioxidant by measuring the TBARS and protein modification.

Thiobarbituric Acid Reactive Substance (TBARS) assay

LDL was oxidized using 5 μ M CuSO₄.²⁵ Oxidation of LDL was monitored in the presence or absence of plant samples by measuring the absorbance of thiobarbituric acid reactive substances (TBARSs) at 534 nm using a UV spectrophotometer (UNICAM UV300). Malondialdehyde-bis-(dimethylacetal), which yields malondialdehyde (MDA) by acid treatment, was used as a standard. Values are expressed as mean \pm SD, n = 3 at a concentration of (100 μ g/ml for all tested extracts).

Protein Modification analysis

It was done according to the method of Visioliet al.²⁶ LDL (200 μ g protein /ml) was incubated with 5 μ M CuSO₄ at 37°C. After 2 hours incubation was stopped. 4-hydroxynonenal-lysine and malondialdehyde-lysine adducts formation was analyzed by measuring fluorescence at E_x360-E_m430 and E_x354- E_m410 respectively using Spectrofluorimeter (FP-777 Jasco, Japan). Values are expressed as mean \pm SD, n = 3 at a concentration of (100 μ g/ml for all tested extracts).

RESULTS

Fifteen different medicinal herbal and edible plant parts were extracted with three different systems (ethanol absolute, 70% ethanol and water). These extracts were assessed with four different procedures for antioxidant activity by measuring the DPPH radical scavenging assay, the Xanthine-Xanthine Oxidase system, inhibition of LDL peroxidation and protein modification *in-vitro*.

DPPH-free radical scavenging activity

The free radical scavenging activity (FRSA) of the whole plants extracts were estimated by reactivity with DPPH. From Table 1, it could be observed that: with ethanol extraction only two extracts give the highest activity; B1 (63.7%) and O1 (69.2%). Three extracts (F1, H1, J1) show moderate activity ranging from (48.6 -50.9%). With 70% ethanol extraction, six plants (A2, E2, B2, F2, D2, J2) show high FRSA ranging from (71.1 – 85.9%). Five extracts show moderate activity (C2, G2, I2, L2, O2) ranging from (43.9 – 59.1%). While with water extraction, eight plants (A3, C3, D3, E3, F3, G3, J3, O3) show high FRSA ranging from (62.4 -82.6%) and only two extracts (B3 and I3) show moderate activity (51.6 and 50.9%).

Scavenging ability for superoxide anion radical

The FRSA activity of different plant extracts on superoxide anion generated by Xanthine-Xanthine oxidase enzymatic method was evaluated. It could be observed that: with ethanol extraction, twelve extracts show high FRSA ranging from (60.9%-79.9%). In 70% ethanol extraction, also twelve extracts exhibit high FRSA ranging from (65.3%-84.7%). With water extraction, thirteen extracts (A3, B3, C3, D3, E3, F3, H3, I3, J3, L3, M3, N3, O3) show



high FRSA that ranges between (61.9 - 81.3%). It is clear that most of the extracts exhibit high FRSA against superoxide anion in Xanthine-XOD system.

De-oiled seeds of turnip (H) showed higher FRS activity in DPPH and XOD assays than that of its seeds (G), while that is in contrary with De-oiled seeds of Salad rocket (K) and its seeds (L).

Table 1: Assessment of Antioxidant activity through inhibition of DPPH and Superoxide anion radicals

Botanical name	Common name	Part used	% inhibition of DPPH radical			% inhibition of superoxide anion radical		
			Extract (1)	Extract (2)	Extract (3)	Extract (1)	Extract (2)	Extract (3)
<i>Anethum graveolens</i>	Dill	A – (Seed)	34.9 ± 0.002	85.9 ± 0.001	82.6 ± 0.002	78.9 ± 0.002	76.5 ± 0.003	79.5 ± 0.003
		B – (Leaves)	63.7 ± 0.002	75.3 ± 0.002	50.9 ± 0.001	50.7 ± 0.003	8.8 ± 0.002	73.1 ± 0.004
<i>Foeniculum vulgare</i>	fennel	C – (Seed)	0.60 ± 0.003	44.1 ± 0.002	77.1 ± 0.003	73.9 ± 0.003	74.8 ± 0.003	79.6 ± 0.004
<i>Ocimum Basilicum</i> L.	Basil	D – (Seed)	31.8 ± 0.002	78.6 ± 0.002	73.6 ± 0.002	41.2 ± 0.003	1.5 ± 0.003	73.8 ± 0.003
		E – (Leaves)	37.3 ± 0.002	71.1 ± 0.001	68.7 ± 0.004	70.1 ± 0.004	78.6 ± 0.003	72.7 ± 0.001
<i>Thymus vulgaris</i>	Thyme	F – (Plant)	50.9 ± 0.002	77.2 ± 0.002	71.8 ± 0.004	65.6 ± 0.003	70.1 ± 0.002	68.7 ± 0.002
<i>Brassica rapa</i>	Turnip	G – (Seed)	4.7 ± 0.004	55.8 ± 0.002	62.6 ± 0.002	67.0 ± 0.002	70.7 ± 0.001	55.8 ± 0.004
		H – (De-oiled seeds)	48.6 ± 0.002	37.2 ± 0.003	33.7 ± 0.003	72.4 ± 0.001	84.7 ± 0.001	78.9 ± 0.001
<i>Oleauro Paea</i> L.	olives	I – (De-oiled seeds)	28.7 ± 0.001	45.5 ± 0.003	51.6 ± 0.003	79.9 ± 0.001	77.6 ± 0.001	81.3 ± 0.001
		J – (Leaves)	49.9 ± 0.001	75.9 ± 0.002	62.4 ± 0.004	69.0 ± 0.003	74.5 ± 0.004	61.9 ± 0.002
<i>Eruca sativa</i>	Salad rocket	K – (De-oiled seeds)	16.0 ± 0.003	38.2 ± 0.003	35.4 ± 0.003	60.9 ± 0.003	65.3 ± 0.002	44.6 ± 0.003
		L – (Seed)	9.1 ± 0.002	43.9 ± 0.001	33.1 ± 0.004	70.7 ± 0.001	70.6 ± 0.003	75.2 ± 0.001
		M – (Leaves)	30.4 ± 0.002	26.5 ± 0.002	28.1 ± 0.002	77.7 ± 0.002	79.3 ± 0.001	73.5 ± 0.001
<i>Corchorus olitorius</i> L.	Jew's mallow	N – (Seed)	16.8 ± 0.001	25.8 ± 0.002	18.5 ± 0.002	67.3 ± 0.001	82.3 ± 0.001	77.6 ± 0.001
		O – (Leaves)	69.2 ± 0.002	59.1 ± 0.002	62.6 ± 0.001	33.1 ± 0.003	40.8 ± 0.004	72.8 ± 0.003

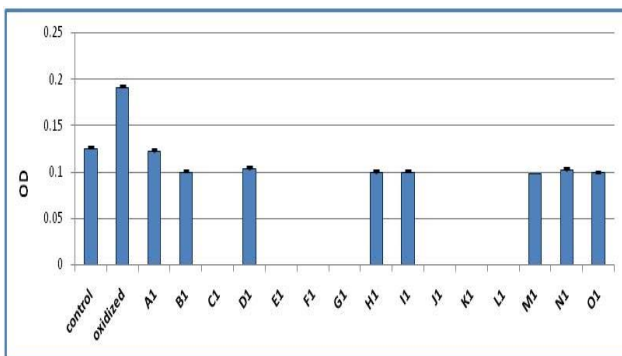


Figure 1: Antioxidant activity of plants (extract 1) on copper-induced human LDL peroxidation in-vitro. Values are expressed as mean ± SD, n = 3 at a concentration of (100 µg/ml for all tested extracts).

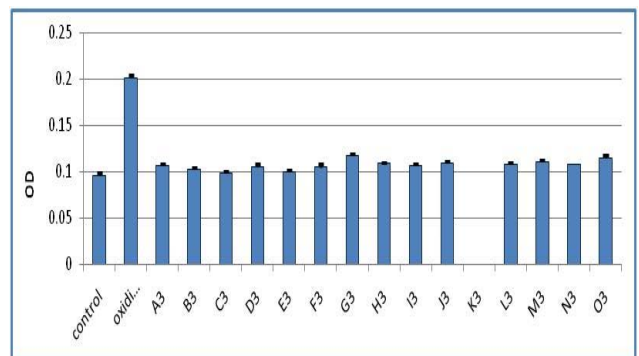


Figure 3: Antioxidant activity of plants (extract 3) on copper-induced human LDL peroxidation in-vitro. Values are expressed as mean ± SD, n = 3 at a concentration of (100 µg/ml for all tested extracts).

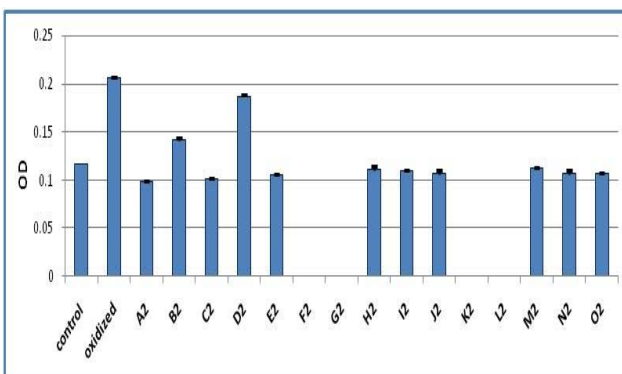


Figure 2: Antioxidant activity of plants (extract 2) on copper-induced human LDL peroxidation in-vitro. Values are expressed as mean ± SD, n = 3 at a concentration of (100 µg/ml for all tested extracts).

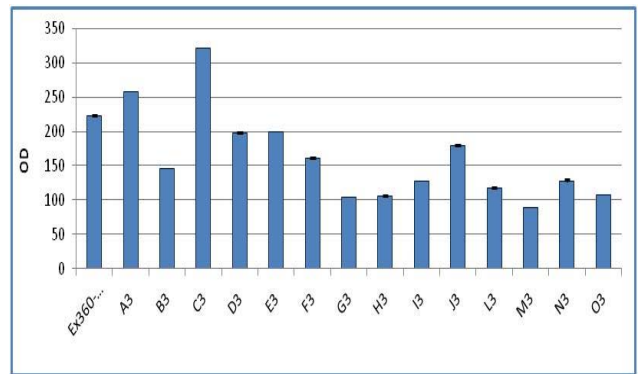


Figure 4: Effect of different plant extracts on protein modification (inhibition of 4-hydroxynonenal-lysine adduct formation was analyzed by measuring fluorescence at E_x360-E_m430).

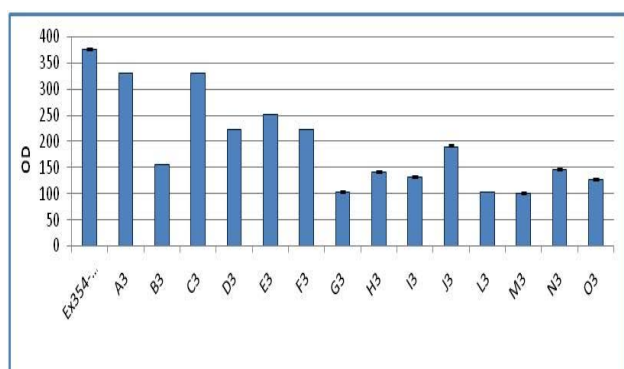


Figure 5: Effect of different plant extracts on protein modification (inhibition of malondialdehyde-lysine adduct formation was analyzed by measuring fluorescence at Ex354-Em410)

Effect of different plant extracts on Copper-induced oxidation of human LDL

Plant extracts showed the highest FRS activity against DPPH radical and/or Xanthine –XOD system were assessed by measuring the inhibition of human LDL oxidation in-vitro.

Effect of different plant extracts on TBARS formation

LDL oxidation was evaluated by measuring the inhibition of Thiobarbituric acid Reactive Substances (TBARS) formation. Figures 1, 2 and 3 illustrate the effect of different extracts on LDL oxidation in-vitro. In Figure 1 most of plant extracts showed relatively high protective effect against LDL oxidation in regard to oxidized LDL. Although, we cannot neglect the effect of alcohol, which causes the oxidation of normal LDL to some extent, Figure 1 showed that these plant extracts are capable to prevent oxidation of LDL due to the effect of both alcohol and CuSO₄, (A1, D1, H1, I1, M1, N1, O1).

Figure 2, shows the effect of 70% ethanol extraction on LDL oxidation. With regard to oxidized LDL, most extracts exhibit high protective effect against oxidation. These extracts are (A2, C2, E2, H2, I2, J2, M2, N2, O2). Figure 3, illustrates the effect of water extraction on LDL oxidation. Comparing to oxidized LDL, all the plant extracts show protective effect against LDL oxidation.

From data shown in Figures 1, 2 and 3, it was observed that the water extract of seeds of (dill, fennel, jew's mallow) and leaves of (dill, basil, salad rocket, jew's mallow) have the highest antioxidant activity against Copper-induced oxidation of LDL.

Effect of different plant extracts on protein modification

Values of fluorescence at Ex360 – Em430 and Ex354 – Em410 increased 120 min after the addition of CuSO₄ to LDL samples indicating the formation of 4-HNE-lysine and MDA-lysine adducts, respectively. Pre-incubation of LDL with each plant extract resulted in marked reduction of the protein modification levels. Figures 4 and 5 showed that some plant extracts (G3, H3, I3, L3, M3, N3, O3) exerts marked reduction of fluorescence value (more

than 50 %) less than the value of oxidized LDL. Other plant extracts (B3, D3, E3, F3, and J3) exert around 40% reduction in fluorescence values. The other plant extracts have no significant effect. De-oiled seeds of turnip (H) and its seeds (G), have high antioxidant activity against Copper – induced oxidation of LDL and protein modification.

DISCUSSION

Cardiovascular disease is one of the leading causes of mortality in our society.²⁷ To date, considerable evidence supports a role for oxidatively modified LDL in the pathogenesis of atherosclerosis.²⁸ The uptake of oxidized LDL (Ox-LDL) by macrophages results in the formation of foam cells and cellular cholesterol accumulated in vascular endothelial cells, and promotes the development of the characteristic fatty streaks found in atherosclerotic lesions.^{20,27-29} There is an experimental evidence indicating that different antioxidant compounds given at high pharmacological doses are effective in decreasing both atherogenesis and LDL oxidation in animals.¹⁸ Natural antioxidants are healthier and more beneficial and have fewer side effects than synthetic antioxidants. Recently, it has become widely accepted that diet may play an important role in health promotion and disease prevention.³⁰ Polyphenolic compounds in the diet enhance the stability of low-density lipoprotein (LDL) to oxidation, and evidence exists that LDL oxidation plays a significant role in atherosclerosis and coronary heart disease.³ Laranjinha et al. suggested possible explanations for the protecting effects of compounds and extracts on LDL: "(i) Scavenging of various radical species in the aqueous phase, (ii) Interaction with peroxy radicals at the LDL surface, (iii) Partitioning into the LDL particle and terminating chain- reactions of lipid peroxidation by scavenging lipid radicals, and (iv) Regenerating endogenous α -tocopherol back to its active anti oxidative form".³¹

For that, the antioxidant effects of plant products must be evaluated by different in-vitro assays to get relevant data. DPPH, Xanthine Oxidase system, LDL oxidation and protein modification are methods that commonly used for determining in-vitro antioxidant activity. DPPH measures sample's free radical scavenging by combining both a hydrogen atom transfer and a single electron transfer reaction.³² The free radical scavenging activity of the whole plants extracts which were estimated by reactivity with DPPH, revealed that, seeds of (dill, fennel, basil, turnip) and leaves of (basil, olive, jew's mallow) showed the highest activity against inhibition of DPPH radical. Our data are in agreement with data obtained from a previous study.³³

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Endogenously, superoxides could be produced in large amounts by various metabolic and physiological processes.^{34,35} The formation of the

superoxide radical leads to a cascade formation of other reactive oxygen species in the cell, such as hydrogen peroxide, hydroxyl radical, peroxy nitrite, or singlet oxygen in living systems.³⁶ Superoxide radical decreases the activity of other antioxidant defense enzymes such as catalase and glutathione peroxidase.

In the present study the effects of different plant extracts on superoxide radical were determined by the Xanthine - Xanthine oxidase system and the results are shown in Table 1. Most plant extracts show relatively high FRSA against superoxide anion in Xanthine-XOD system. These data are in agreement with data obtained from a previous study.³³

The antioxidant activity of the plant extracts was also determined by the ability to inhibit LDL peroxidation. The lipid peroxidation products in un-oxidized LDL, oxidized LDL with Cu²⁺ in the presence or absence of extracts were assayed as thiobarbituric acid reactive substances (TBARS) and as protein modification products (fluorescence). TBARS, a marker for lipid peroxidation was measured. It was observed that TBARS levels were undetectable in control LDL, slightly rising only after 3hrs.of incubation. Incubation of LDL with Cupper Sulfate resulted in a marked elevation of the level of TBARS. After 24hrs.of incubation in the presence of Cu²⁺, level of TBARS did not further increase significantly (data not shown). Pre-incubation of LDL with any of the plant extracts resulted in marked inhibition of the formation of TBARS, where seeds of (dill, fennel, jew's mallow), leaves of (dill, basil, salad rocket, jew's mallow) and thyme plant have the highest antioxidant activity against Cupper-induced oxidation of LDL. These data are in agreement with data obtained from previous studies.^{20,26,33,37}

The LDL particle contains large amounts of polyunsaturated fatty acids which make this lipoprotein more prone to the oxidative degradation even in the absence of prooxidants. The in-vitro oxidation of LDL by metal ions (Cu and Fe) occurs in three phases: an initial lag phase (consumption of endogenous antioxidant) a propagation phase (rapid oxidation of unsaturated fatty acid to lipid hydroperoxides) and a decomposition phase (hydroperoxides are converted to reactive aldehydes like malondialdehyde, 4-hydroxynonenal). These aldehydes react with lysine residues in apoB-100, resulting in oxidized LDL.^{38,39} The effect of different plant extracts on LDL resistance to oxidative modification was tested using the classical copper-catalyzed oxidation systems. The Cu-catalyzed LDL oxidation depends on the reduction of the metal ion probably through the reaction with endogenous lipid hydroperoxides, resulting in the production of lipid hydroperoxyl radicals.^{37,40-42} The reduced copper in its turn decomposes preexisting peroxides, producing alkoxyl radicals. Therefore, the inhibition of the Cu-catalyzed oxidation represents the association of metal ions, chelation and scavenging of different free radicals.

Among the amino acid located mainly on protein surfaces is lysine, which is a primary target for ROS mediated

oxidation with their readily hydrogen bonding properties.⁴³ Side-chain amine groups of this amino acids react readily with carbonyl compounds derived from lipid oxidation and form Schiff bases, Michael adducts, and their cyclic products. Therefore, this amino acid is first modified during oxidation, and can remain reactive through propagation and termination stages.

One of the accepted procedures for measuring LDL resistance to in-vitro oxidation is to monitor fluorescence development (FD) during LDL oxidation. The fluorescence spectra of native LDL displays a band centered at 332nm. In our Experiment, the fluorescence developed during LDL oxidation was caused by reaction of protein amino groups (mainly lysine as discussed above) with aldehydes generated during decomposition of Peroxidized polyunsaturated fatty acids (PUFA). Detection of these aldehydes fluorescence is considered a marker for oxidation at the protein core of LDL. It represents the late stage of the oxidation process.⁴⁴ Plants used in this study [dill, fennel, basil, thyme, turnip, olive, salad rocket, jew's mallow] are highly effective against LDL peroxidation , while some of them can decrease the level of protein modification [leaves of dill, basil, olive, salad rocket, jew's mallow and seeds of salad rocket, jew's mallow] beside de-oiled seeds of olive (Fig., 4 and 5), the data obtained in these study is in agreement with that of Visioli et al and Portella et al.^{26,44}

CONCLUSION

It could be concluded that culinary and medicinal herbs extracts used may play a role in scavenging the free radicals and increase the inhibitory effect not only against LDL lipid peroxidation, but also against modification of the protein moiety in LDL protein core. So, they could be useful in early manifestation of atherosclerosis.

Our study provides (for the first time) primary evidence suggesting that these culinary and medicinal herbs in further in-vivo studies could play an important role in inhibiting lipid peroxidation and protein modification in biological systems through their antioxidant, metal chelating and free radical scavenging activities.

Acknowledgement: The authors are grateful for the financial support by the National Research Center of Egypt (Contract 1/48/ 5).

REFERENCES

1. Willcox BJ, Curb JD, Rodriguez BL, "Antioxidants in cardiovascular health and disease: Key lessons from epidemiologic studies", *Am. J. Cardiol.*, 101(10A), 2008, 75D-86D.
2. Lobbes MB, Lutgens E, Heeneman S, Cleutjens KB, KooiME, van Engelshoven JM, Daemen MJ, Nelemans PJ, "Is there more than C-reactive protein and fibrinogen, The prognostic value of soluble CD40 ligand, interleukin-6 and oxidized low-density lipoprotein with respect to coronary and cerebral vascular disease", *Atherosclerosis*, 187, 2006, 18-25.



3. Steinberg D, "Low density lipoprotein oxidation and its pathobiological significance", J. Biol. Chem., 272, 1997, 20963-20966.
4. Yekini I, Hammoudi F, Martin-Nizard F, Yous S, Lebegue N, Berthelot P, Carato P, "Antioxidant activity of benzoxazolinonic and benzothiazolinonic derivatives in the LDL oxidation model", Bioorg. Med. Chem., 17, 2009, 7823-7830.
5. Ani M, Moshtaghi AA, Ahmadvand H, "Comparative effects of copper, iron, vanadium and titanium on low density lipoprotein oxidation in-vitro", Iran Biomed. J., 11, 2007, 113-118.
6. Witztum JL, Steinberg D, "Role of oxidized low density lipoprotein in atherogenesis", J. Clin. Invest., 88, 1991, 1785-1792.
7. Leopold JA, Loscalzo J, Oxidative risk for atherothrombotic cardiovascular disease, Free Radic. Biol. Med., 47, 2009, 1673-1706.
8. Saltman B, "Oxidative stress: a radical review", Semin Hematol, 26, 1989, 249-256.
9. Squadrito GL, Pryor WA, "Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide", Free Radic Biol Med., 25, 1998, 392-403.
10. Sies H, "Oxidative stress: oxidants and antioxidants", Exp Physiol., 82, 1997, 291-295.
11. Kim JY, Park JY, Kang HJ, Kim OY, Lee JH, "Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants: a randomized, double-blind, placebo-controlled trial", Nutrition Journal, 11, 2012, 1-9.
12. Kahkonen MP, Hopia A, Vuorela H, Rauha J, Pihlaja K, Kujala TS, "Antioxidant activity of plant extracts containing phenolic compounds", J. Agric. Food Chem, 47, 1999, 3954-3962.
13. Balasundram N, Sundram K, Sammar S, "Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses", Food Chem, 68, 2006, 191-203.
14. Sinelli N, Spinardi A, Di Egidio V, Mignani I, Casiraghi E, "Evaluation of quality and nutraceutical content of blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy", Postharvest Biol Technol., 50, 2008, 31-36.
15. Chun OK, Kim DO, Smith N, Schroeder D, Han JT, Lee CY "Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet", J. Sci. Food Agric., 85, 2005, 1715-1724.
16. Salawu SO, Sanni DM, Akindahunsi AA, "HPLC/DAD/MS phenolic profile, antioxidant activities and inhibitory action of *Struthium sparganophora* (Linn) and *Telfairia occidentalis* (Hook. F) against low density lipoprotein oxidation", African Journal of Food Science and Technology, 4, 2013, 1-8.
17. Bagheri S, Ahmadvand H, Khosrowbeygi A, Ghazanfari F, Jafari N, Nazem H, Hosseini RH, "Antioxidant properties and inhibitory effects of *Satureja khuzestanica* essential oil on LDL oxidation induced-CuSO₄ in-vitro", Asian Pac J Trop Biomed., 3, 2013, 22-27.
18. Lee OH, Lee BY, Lee J, Lee HB, Son JY, Park CS, Shetty K, Kim YC, "Assessment of phenolics enriched extract and fractions of olive leaves and their antioxidant activities". Bioresour. Technol., 100, 2009, 6107-6113.
19. Ahmadvand H, Bagheri SA, Boshtam M, Abdolhahpour F, "Effects of olive leaves extract on LDL oxidation induced-CuSO₄ in-vitro", Pak J Pharm Sci., 25, 2012, 571-575.
20. Barku VYA, Opoku-Boahen Y, Owusu-Ansah E, Dayie NTKD, Mensah FE, "In-Vitro Assessment of Antioxidant and Antimicrobial Activities of Methanol Extracts of Six Wound Healing Medicinal Plants", Journal of Natural Sciences Research, 3, 2013, 74.
21. Matsushige K, Basnet P, Kadota S, Namba T, Potent Free radical scavenging activity of dicaffeoylquinic acid derivatives from propolis, Journal of Traditional Medicines, 13, 1996, 217-228.
22. Gugliucci A, Menini T, Three different pathways for human LDL oxidation are inhibited in vitro by water extracts of the medicinal herb *Achyrocline satureoides*, Life Sciences, 71, 2002, 693-705.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin-phenol reagent, J. Biol. Chem., 193, 1951, 265-275.
24. Masaki N, Kyle ME, Faber JL, Tert-Butyl Hydroperoxides kills cultured hepatocytes by peroxidizing membrane lipids, Arch. Biochem. Biophys, 269, 1989, 390-399.
25. Visioli F, Bellomo G, Montedoro GF, Galli G, Low density lipoprotein oxidation is inhibited *in vitro* by olive oil Constituents, Atherosclerosis, 117, 1995, 25-32.
26. Soriano JB, Lamprecht B, "Chronic obstructive pulmonary disease: a worldwide problem", Med Clin North Am., 96, 2012, 671-680.
27. Castela JE, Gago-Dominguez M, "Risk factors for cardiovascular disease in women: relationship to lipid peroxidation and oxidative stress", Med Hypotheses, 71, 2008, 39-44.
28. Miller YI, Choi SH, Fang L, Tsimikas S, "Lipoprotein modification and macrophage uptake: role of pathologic cholesterol transport in atherogenesis", Subcell Biochem., 51, 2010, 229-251.
29. Hajhashemi V, Zolfaghari B, Yousefi A, "Anti nociceptive and anti-inflammatory activities of *Satureja hortensis* seed essential oil, hydroalcoholic and polyphenolic extracts in animal models", Med Princ Pract., 21, 2012, 178-182.
30. Laranjinha JA, Almeida LM, Madeira VM, "Reactivity of dietary phenolic acids with peroxy radicals: Antioxidant activity upon low density lipoprotein peroxidation", Biochem. Pharmacol., 48, 1994, 487-494.
31. Prior RL, Wu X, Schaich, "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements", J. Agric. Food Chemistry, 53, 2005, 4290-4302.
32. Abd El-Hady FK, Hegazi AG, Mahdy EM, Shousha WG, El-Shahid ZA, Inhibitory Effect of Some Local Medicinal Plants on In-Vitro Oxidative Modification of Low-Density Lipoprotein, Egyptian Pharmaceutical Journal, 11, 2012, 38-41.

33. Blaszczyk J, Kedziora J, Luciak M, Sibinska E, Trznadel K, Pawlicki L, "Effect of morphine and naloxone on oxidative metabolism during experimental renal ischemia and reperfusion", *Experimental Nephrology*, 2, 1994, 364-370.
34. Bedard L, Young MJ, Hall D, Paul T, Ingold KU, "Quantitative study on the peroxidation of human low-density lipoprotein initiated by superoxide and by charged and neutral alkylperoxyl radicals", *Journal of the American chemistry society*, 123, 2001, 12439-12448.
35. Lee J, Koo N, Min DB, "Reactive oxygen species, aging and antioxidative nutraceuticals", *Comprehensive Reviews in Food science and food safety*, 3, 2004, 21-33.
36. Kuate D, Etoundi BCO, Soukontoua YB, Ngondi JL, Oben JE "Comparative study of the antioxidant, free radical scavenging activity and human LDL oxidation inhibition of three extracts from seeds of a Cameroonian spice, *Xylopiaparviflora* (A.Rich) Benth (*Annonaceae*)", *I.J.Biomed. Pharmaceutical Sci.*, 5, 2011, 18-30.
37. Esterbauer H, Gebiki J, Puhl H, Jurgens G, "The role of lipid peroxidation and antioxidants in oxidative oxidative modification of LDL", *Free Radical Biology and medicine*, 13, 1992, 341-390.
38. Mertens A, Holvoet P, "Oxidized LDL and HDL: Antagonists in atherothrombosis", *The Federation of American Societies for Experimental Biology journal*, 15, 2001, 2073-2084.
39. Gebicki JM, Jurgens G, Esterbauer H, "Oxidation of low – density lipoprotein in-vitro. In :Sies H(Ed) *Oxidative stress: Oxidants and Antioxidants*", Academic Press, London, 1991, 371-397.
40. Thomas CE, Jackson RL, "Lipid hydroperoxides involvement in copper-dependent and independent oxidation of low density lipoprotein", *The Journal of Pharmacology and Experimental Therapeutics*, 256, 1991, 1182-1188.
41. Patel RP, Svistunenko D, Wilson MT, Darley_Usmar VM "Reduction of Cu (II) by lipid hydroperoxides: implications for the copper-dependent oxidation of low-density lipoprotein", *Biochemical Journal*, 322, 1997, 425-433.
42. Schaich KM, Co-oxidation of proteins by oxidizing lipids. In: *Lipid Oxidation Pathways*. Kamal-Eldin, A., Min, D. B., Eds., AOCS Press, Urbana, IL, 2, 2008, 181-272.
43. Portella RL, Barcelos RP, da Rosa EJF, Ribeiro EE, da Cruz I B M, Suleiman L, Soares FAA, Guaraná (PaulliniacupanaKunth) effects on LDL oxidation in elderly people: an in-vitro and in-vivo study, *Lipids in Health and Disease*, 12, 2013, 2-9.

Source of Support: Nil, **Conflict of Interest:** None.

