Bull. Fac. Agric., Cairo Univ.,54 (2003):77-92.

INHIBITORY EFFECTS OF SOME ESSENTIAL OILS ON CU^{2+}-INDUCED OXIDATIVE MODIFICATION OF HUMAN LOW DENSITY LIPOPROTEINS

(Received: 8. 5.2002)

By
Kh. F. El-massry

Flavour and Aromatic Department, National Research Center, Giza

ABSTRACT

Essential oils are natural products extracted from plant materials, which can be used as antibacterial, antifungal, antioxidant and anticarcinogenic agents or to preserve and give specific flavours to foods. The activities of five essential oils isolated from clove, marjoram, *Artemisia judaica* (Shih balady), ginger and eucalyptus (fruits) in inhibiting the copper- catalysed oxidation of human low-density lipoproteins (LDL) were determined *in vitro*. The lag time in the conjugated-diene formation was dose-dependently prolonged by addition of the tested essential oils. Clove (mainly eugenol) prolonged the lag time of conjugated-diene formation more than 367 min, followed by the marjoram, ginger, eucalyptus (fruits) and *Artemisia judaica* , within 700 min under the experimental conditions. Total phenolic contents of these essential oils gave a correlation with LDL antioxidant activity and could play a role in protecting LDL against oxidation if absorbed by the body. Prevention of both the formation and action of reactive products by antioxidants as present in these essential oils is of great public health importance in decreasing the risk of major diseases, which is the optimal approach to disease control and also as an effective route to lower costs of medical care.

Key words : *coronary heart disease(chd),essential oils, ldl oxidation, conjugated-diene,phenolic compounds.*

1. INTRODUCTION

The oxidation of human low-density lipoproteins (LDL) is now recognized to be the key event in the initiation and progression of atherosclerosis (Steinberg,1992). The atherogenecity of oxidized LDL is due to its high concentration of polyunsaturated fatty acids (PUFAs), which are highly susceptible to free radical oxidation (Esterbauer *et al.*,1992 and Hertog *et al.*,1997). With a continued high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to the generation of foam cells and plaques; the symptoms of atherosclerosis.

Therefore, inhibiting LDL oxidation is known to reduce the formation of atherosclerotic lesions.

Most chronic diseases, including coronary heart disease (CHD) and many types of cancer depend on the *in vivo* conversion of cellular macromolecules or of carcinogens to specific reactive, oxidized forms. For that reason, health promoting nutrition involves the daily intake of vegetables and fruits, fruit juices and tea which are rich sources of micronutrients with antioxidant properties. Diets high in fruits and vegetables have also been associated with lower chronic disease rates CHD and cancer (Bailey and Williams, 1994). Dietary antioxidants have been suggested to play a potentially important protective role against atherosclerosis and CHD by inhibiting the oxidation of LDL (Kearney and Frei, 1994). Many studies have implicated the consumption of phenolic antioxidants in food products, as factor responsible for reduced disease(Rimm *et al.*,1993).

Many aromatic plants and their essential oils are used as flavouring agents in a wide range of food, beverage, and confectionery products and fragrance applications. They are known to support various biological activities such as antimicrobial and antioxidant properties (Frankel *et al.*,1996 and Griffin *et al.*,1999).

Antioxidants rein the free radicals by offering their own electrons. Free radicals can be produced from a number of sources such as cigarette smoke, pollutants, pesticides, herbicides and from exposure to sun light. Free radicals are responsible for inflammation, compromised immune systems and degenerative and other age-related diseases. Many synthetic antioxidants have shown toxic and mutagenic

effects, which have directed most of the attention on the naturally occurring antioxidants. Their use has mainly centered around prevention and maintenance of health. In fact, essential oils can play a significant role in the scavenging effect as well (Fejes *et al.*,1998).

Herbs, spices, fruits, nuts and leafy vegetables have been widely used not only for food but also as medicine in minor aliment. The flavour-imparting essential oils of the spices, herbs and leafy vegetables are important and can represent more than 5% of their fresh mass. As a consequence essential oils are enjoying a surge of public interest (Hadly and Petry, 1999). Gould (1997), reported that the terpenoids such as carveol, limonene, sobreol and perillyl alcohol, which are found in plant essential oils are effective in the treatment of breast,liver and/or other cancers. These facts support the idea that some essential oils will act against damaging free radicals and may be natural protectors against such diseases. Besides, essential oils are natural products extracted from plants and play different roles. They are antibacterial, antifungal and also they preserve and give specific flavours when added to foods; in addition, they are used in cosmetology for their aromatic and antioxidant properties. Moreover, essential oils could fulfill many biological functions in the producing organism. Pharmaceutical activities (hepatoprotective and anti-carcinogenic) of specific essential oils or active principle of clove (eugenol) were investigated and the protective activities against hepatotoxicity in animal models were shown (Krishnaswany and Raghuramuhu, 1998). Dietary isoprenoid constituents from essential oils were found to suppress tumor growth in animal models (Elson and Yu, 1994).

Radical scavengers from essential oils and other natural sources as eugenol, isoeugenol, coniferaldehyde,which could be used as raw materials for cosmetics, have been shown effective for their hydroxyl radical($OH^{?}$) scavenging ability. They can be used as antioxidants for skin change caused by ($OH^{?}$) generated by UV light (Clarys and Barel, 1998). These compounds can become important in the search for "natural" replacement for "synthetic" antioxidants food additives (Aeschbach *et al.*, 1994 and Knekt *et al.*,1996).

Essential oils are used as natural additives in many foods, and they are being consumed every day, but their antioxidant activities are not well described. Although essential oils have been used for

remedies and food more than a thousand years, most people used them according to their experiences without knowledge about relationships between constituents and their activities.

In the present study, we examined the effect of some essential oils on copper induced low density lipoprotein oxidation *in vitro* and related it to that of the total phenolic contents with antioxidant properties. The conjugated diene method is one of the most frequently used assays to investigate the influence of test substances upon LDL-oxidation.The susceptibility of LDL to *in vitro* oxidation, as compared with other oxidation parameters (autoantibodies to malondialdehyde modified LDL, oxidized LDL, and thiobarbituric acid- reactive substances) showed the highest significance in correlation with coronary artery disease. Therefore, this method seems to be valid for measuring the potency of antioxidants (Halevy *et al.*, 1997).

The chemical compositions as well as the antioxidant activities have been reported for some essential oils; however, no literature data are available concerning the activities of these essential oils in inhibiting the copper- catalyzed oxidation of human low- density lipoproteins (LDL) *in vitro*.

This study evaluates the antioxidant activity by inhibiting human LDL oxidation *in vitro* and this activity was related to the total phenolic compounds.

2. MATERIALS AND METHODS

2.1. Essential oils

The essential oils of clove (*Eugenia caryophyllus*) and ginger (*Zingiber officinale*) [local market], marjoram (*Marjorana hortensis M.*), Eucalyptus (*Eucalyptus camaladulensis var. brevirostris*) fruits [Nile Delta, Egypt] and artemisia (*Artemisia judaica L.*) [North coast, Egypt] , used in this study were isolated by hydrodistillation using the British Pharmacopoeia essential oil distillation apparatus (Deans,1991). Serial dilutions of the oils were made with absolute alcohol.

2.2. Determination of total phenolic compounds

The concentration of phenolic compounds in the essential oils of clove, marjoram, eucalyptus fruits, artemisia judaica and ginger was

determined according to the method of Jayaprakasha *et al.*(2001), and the results were expressed as gallic acid equivalents. The essential oils were dissolved in a mixture of methanol and water (6 :4v/v). Samples (0.2 ml) were mixed with 1.0 ml of 10-fold-diluted Folin- Ciocalteu reagent at 0.8 ml of 7.5% sodium carbonate solution. After the mixture had been allowed to stand for 30 min at room temperature, the absorbance was measured at 765 nm using spectrophotometer (SHIMADZU UV-1601 PC, JAPAN). The estimation of phenolic compounds in tested samples was carried out in triplicate and the results are averaged.

2.3. LDL Lipid peroxidation

Blood samples were collected after an overnight fast of ≥12 hr from healthy male volunteers. Plasma was separated by centrifugation (600Xg for 10 min at 4°C). After separation of fresh plasma, LDL samples were isolated immediately using single-step ultracentrifugation. Plasma (2ml) was adjusted to a density of 1.21 g/ml with KBr and layered under 10 ml saline containing 0.01 % EDTA in 12 ml quick-seal tubes. The tubes were centrifuged at 65000 rpm for 6hr at 4°C using a Ti 75 fixed-angle-rotor in a Beckman L5-75 ultracentrifuge (Beckman Instruments, Munich, Germany). The yellow LDL-band was removed through the side of the tube with a needle and syringe. Immediately prior to the oxidation incubations, LDL was separated from EDTA by gel filtration using Econo-Pac 10 DG columns (BIO RAD GmbH, Munich, Germany)(Mc Dowell *et al.*,1995). LDL concentration was determined by measuring total cholesterol using CHOD-PAP-method since more than 98 % purity of LDL- solution after LDL preparation could be obtained (Siedel *et al.*,1983). The LDL-oxidation was initiated by addition of a freshly prepared aqueous $CuSO_4$ solution.

The LDL-solution was diluted with oxygen-saturated phosphate buffer solution (PBS) pH 7.4. In all experiments, the final conditions were: temperature 30 °C, 0.08g/l lDl-cholesterol , and 5 umol/l $CuSO_4$, final test volume1 mL. The lDl-solution was supplemented with 0.2, 0.4 and 1umol/L of the tested substances and vitamin C prior to the initiation of oxidation of lDl by copper ions.

The kinetics of the oxidation of lDl were determined by monitoring the change of conjugated diene formation at 234 nm

absorbance by Perkin Elmer Lambda 2 ultraviolet / vis spectrophotometer equipped with 8-position automatic sample changer (Ueberligen, Germany). The change in absorbance was recorded at 5 min intervals at 37 °C. The absorbance curve (at 234 nm) was divided into three phases; lag time, propagation and decomposition phases. The lag time (the oxidation resistance marker) was defined as the interval between the intercept of the tangent of the slope of the curve in the propagation phase with the base line, and was expressed in minutes (Hirano *et al.*,1997).

3. RESULTS AND DISCUSSION

There has been an increasing interest in the interaction of naturally occurring antioxidants with LDL since the oxidation of LDL leading to uptake *via* the microphage scavenger receptors in the arterial wall is considered to be an important event in atherosclerosis. Also much attention has been focused on identifying dietary factors capable of inhibiting oxiditative modification of LDL.

The concentrations of total phenols as determined by the Foiln-Ciocalteu method (Table 1) varied from 3 to 75 %. The total phenolic concentration is the highest for clove (contains mainly eugenol) followed by ginger, marjoram, eucalyptus fruits and artemisia judaica (75, 13, 10, 7 and 3 %, respectively). Total phenolic content in the essential oils is directly related to the plant variety and location, growing factors in the environment, extracting techniques and the aging process.

The variation in the total phenolic concentration could be ascribed to the chemical compositions of each oil (Table 3). Some phenolic compounds are probably more active than others, and it is important to analyze and correlate the major phenolic compounds found in essential oils with the inhibition of LDL oxidation results. Structural, physical and chemical properties for each phenolic compound are probably extremely important to explain their antioxidant activities, and if each one is absorbed, it may prevent LDL oxidation *in vivo*.

It has been documented that Cu^{2+}-induced oxidized LDL exhibits biological and immunological properties similar to those *in vivo* (Chen, *et al.*,1999). Cu^{2+}- induced oxidized LDL is recognizable by scavenger receptors and causes cholesterol ester accumulation in macrophages. Vitamin C was used as a positive control in this study.

Antioxidant activities of essential oils were evaluated by measuring the inhibition of dieneformation during copper- catalyzed human LDL oxidation *in vitro*. The antioxidant effects of the tested essential oils; clove, ginger, marjoram, eucalyptus fruits and artemisia judaica were investigated individually at three different levels and as expected, large differences in antioxidation activity were observed between the tested essential oils (Table 2 and Figure 1).

The essential oils of clove, ginger, marjoram, eucalyptus fruits and *artemisia judaica* effectively prolonged the lag time of the conjugateddiene formation in the Cu^{2+}- induced oxidation of LDL (Table 1). The lag time was 28 min in the absence of the tested extracts (control), but was prolonged in a dose dependent manner by the addition of each essential oil. Clove (mainly eugenol) showed high antioxidant activity and appreciably prolonged the lag time (t = 367min), compared with Vitamin C (t = 142 min). Other essential oils were less effective in protecting LDL from oxidation by diene formation within 700 min under the experimental conditions.

Table (1): Effect of different essential oils on the LDL oxidation lag time (min) as monitored by the UV absorption of dienes.

Essential oils	Inhibition LDL oxidation (lag time min)	Total phenolic content (%) mg/100mg gallic acid
Control	28	-
Vitamin C	142	-
Eugenia caryophyllus (Clove)	367	75
Marjorana hortensis M. (Marjoram)	230	10
Zingiber officinale (Ginger root)	173	13
Eucalyptus camaldulensis var. brevirostris (fruits)	117	7
Artemisia judaica L. (Shih Balady)	69	3

Results are expressed as mean SD of three determinations % : mg/100mg gallic acid.

Table (2): Effect of different concentrations of tested essential oils on the LDL oxidation lag time (min) as monitored by the UV absorption of dienes.

Essential oil Conc.	Clove	Ginger	Marjoram	Eucalyptus (fruits)	Ar temisia judaica	Vit.C
0.2μmol/l	367	173	230	117	69	142
0.4μmol/l	413	224	299	158	96	198
1 μmol/l	567	318	373	264	173	355

Results are expressed as mean ± SD of three determinations.

Table (3) :Major compounds of tested essential oils .

Essential oils	Principal substances
Eugenia caryophyllus **(Clove)**	**eugenol, eugenyl acetate, methyl eugenol, caryophylene**
Marjorana hortensis M. **(Marjoram)**	**α-pinene, careen, γ-tepinene, thujanol, terpinolene, sabinene hydrate, sabinene hydrate acetate, terpin-4-ol,thymol, linalool**
Zingiber officinale **(Ginger root)**	**Terpinolene, borneol, isoborneol, zingiberene, farnesene, zingiberenol, paradol, shogaol, gingerol**
Eucalyptus camaldulensis var. brevirostris **(fruits)**	**α-pinene, p-cymenene, isoterpinolene, 2-methyl-6-ethyl phenol, hydroxy cumin, thymol, p-cymen-7-ol, guryunene, drimmenol**
Artemisia judaica L. **(Shih Balady)**	**Thujone, camphor, 2-(4-phenoxyl)ethanol, piperitone, ethyl cinnamate (trans), spathulenol**

El-massry *et al.*,(2002)and El-Ghorab *et al.*,(2002)(Personal communication).

Our results are in accordance with those of Teissedre and Waterhous (2000), who studied the inhibition of oxidation of human LDL by 23 essential oils, not including our tested oils, and reported

their antioxidant activity average levels. This study showed that when eugenol is the major component as in clove essential oil, the inhibition of LDL oxidation is between 68-100 %. Also the nature and concentrations of phenolic compounds in the different types of essential oils are to be considered important to understand their potential health and therapeutic effects.

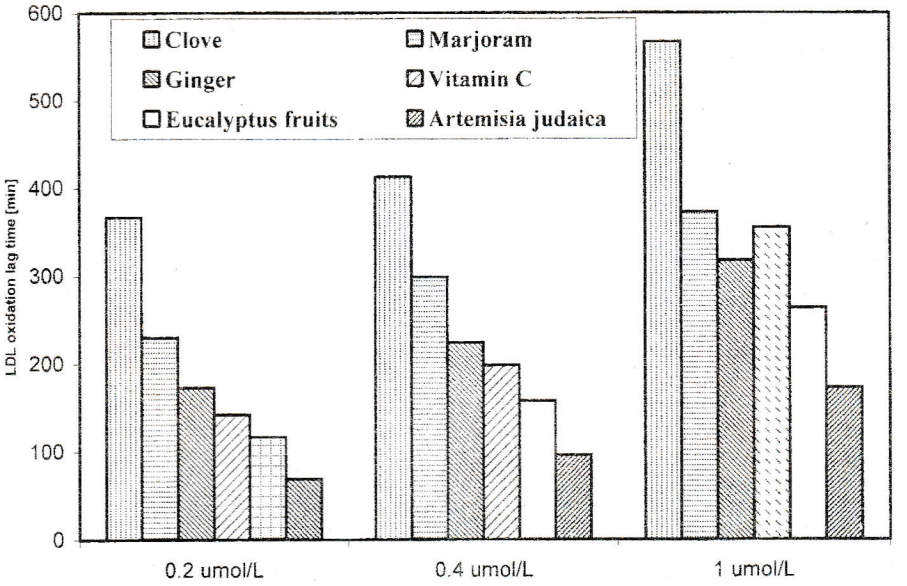

Fig. (1): Effect of different concentrations of tested essential oils on the LDL oxidation lag time [min] as monitored by the UV absorption of dienes

It is noteworthy to mention that marjoram essential oil exerts higher antioxidant activity than that of ginger oil in spite of containing lower phenolic content than the later (10%). This might be explained on the basis that besides phenolics, it has other active compounds such as terpin -4-ol , γ- terpinene , sabinene hydrate and sabinene hydrate

acetate which possess antioxidant activities by scavenging radicals (Choi *et al.* ,2000).

With regard to ginger; it was reported that dietary consumption of ginger extract significantly attenuates the development of atherosclerotic lesions. This atherogenic effect is associated with a significant reduction in plasma and LDL cholesterol levels and a significant reduction in the LDL basal oxidative state, as well as their susceptibility to oxidation and aggregation (Fuhrman *et al.*, 2000).

The variations observed in inhibition of LDL oxidation for the different varieties can also be explained by other factors than the extraction process during essential oils isolation. First, a difference in the phenolic concentrations is related to plant maturity and can vary depending on the seasonal climate(humidity, rain, sun exposure) and the location. Second, there may be an oxidation of phenolic compounds during storage.

In our previous work, the composition of major monomer phenolic constituents for each oil varieties is summarized in Table(3) (El-massry *et al.*, 2002 and El-Ghorab *et al.*, 2002). These results indicate that the phenolic compounds found in essential oils at different levels are active in protecting LDL from oxidation.

These results support the concept that the inhibition of oxidation of LDL depends on the phenolic concentration of essential oils. The nature of the phenolics plays also an important role in the antioxidant activity that they confer to essential oils used in this study. Naturally occurring phenolic acids, such as caffeic and chlorogenic acids, were reported to have anti-lipid peroxidation activities to LDL oxidation induced by metmyoglobin/H2O2. Prooxidant and antioxidant activities depending on the LDL oxidation phase (Yamanaka *et al.*,1997). Also the differences in antioxidant activities toward LDL oxidation observed here could also be ascribed to other factors, including differences in solubilities and partitioning behaviour between aqueous and lipid phases in the LDL system. Thus, the physicochemical properties of antioxidants are known to affect their antioxidant efficacy in complex, multiphase systems (Frankel *et al.*, 1994). Furthermore, the copper- mediated oxidation of tryptophan residues in the LDL- apolipoprotein B was shown to play an important role in initiating lipid oxidation in LDL particles (Giessauf *et al.*, 1995). Structural features conferring differences in protein binding may

affect the antioxidant activity of phenolics in inhibiting oxidation of LDL. The wide mixture of phenolic antioxidants found in plant foods may interact to produce synergistic protection against LDL oxidation (Kinsella *et al.*, 1993).

In general, it is believed that a free radical attacks LDL, and abstracts one of the two hydrogen atoms on a bisallylic carbon atom of a polyunsaturated fatty acid in phospho-lipid, cholesterol ester, or triacylglycerol in the LDL particle. The unpaired electron left on this carbon atom is delocalized to form conjugated-diene.

The LDL oxidation is protected by endogenous antioxidants such as α-tocopherol, retinoids and carotinoids (Kim *et al.*,2000). The oxidation of LDL lipids could occur under circumstances when the LDL particle is depleted of its antioxidants.

In our present experiments, the lag time of conjugated-diene formation in the Cu^{2+}-induced LDL oxidation is shown to be prolonged dose-dependent by the addition of tested essential oils (Table2). These results show that the extracts may suppress the formation of free radicals which had been induced by Cu^{2+}, or protect exogenous antioxidants present in the LDL, maintaining their levels longer and delaying the start of lipid peroxidation.

Such a mechanism has been demonstrated *in vitro* to explain the compensating effect of ascorbic acid and α-tocopherol (Sato, *et al.*,1990).

On the other hand, Gugliucci *et al.*(1994),reported that the lipid peroxidation of plasma mainly represents LDL oxidation, and whole plasma oxidation is used to evaluate the relative roles of the different endogenous antioxidants.

From these results, we can suggest that the tested essential oils increase LDL resistance to oxidation, decreasing the consumption of endogenous antioxidants, and administration of them may be beneficial in the prevention of atherosclerosis and cardiovascular diseases. Also the data presented confirm that the free radical scavenging activities observed in each essential oil is due to the presence of specific phytochemicals which are known to possess reductive properties *e.g.*, the phenolic monoterpenes; thymol and carvacrol, and other compounds with a phenolic nucleus present in the active volatile oils . This supports the view that some essential oils

rich in phenolic compounds are highly bioavailable and may be active in the body as antioxidants and free radical scavengers.

CONCLUSION

The results of the present work indicate the efficiency of the tested essential oils in inhibiting the copper- catalyzed oxidation of LDL and this activity may be ascribed to their different phenolic compositions. Moreover, ingestion of these compounds may help to prevent *in vivo* oxidative damage, such as lipid peroxidation, which is associated with many diseases, including cancer, atherosclerosis, diabetes and immune deficiency.

Essential oils appear to be a good source of natural antioxidants in addition to its properties of contributing various flavour notes to food.

Acknowledgements

The author wishes to thank Dr. R. Dittrich (Division of Gynaecological Endocrinology and Reproductive Medicine, Department of Obstetrics and Gynaecology, University of Erlangen-Nuremberg, Erlangen, Germany) for laboratory facilities and Inge Hoffmann for technical assistance.

4. REFERENCES

Aeschbach R., Loliger J., Scott B.C., Murcia A., Butler j., Halliwell B. and Aruoma O.I. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem.Toxicol. 32 : 31-36.

Bailey G.S. and Williams D.E. (1994). Potential mechanisms for food related carcinogens and antimutagens. Food Technol. 47: 105-108.

Chen C.C., Chen H.Y., Shiao M.S., Lin Y.L., Kuo Y.H. and Ou J.C. (1999). Inhibition of low-density lipoprotein oxidation by tetrahydrofuran lignans from *Forsythia suspensa* and *Magnolia coco*. Planta Medica. 65 : 709-711.

Choi H.S., Song H.S.,Ukeda H.and Sawamura M. (2000). Radical-scavenging activities of essential oils and their components: Detection using 1,1-Diphenyl- 2-picrylhydrazyl. J. Agric. Food

Chem. 48 : 4156-4161.

Clarys P. and Barel A.(1998). Efficacy of tropical treatment of pigmentation skin disorders with plant hydroquinone glucosides as assessed by quantitative color analysis. J.Dermatol. 25 : 412-414.

Deans S.G.(1991). In:Modern methods of plant analysis, Vol.,2: Essential oils and waxes, p.309, ed.H.F.Linskens and J.F. Jackson, Springer-Verlog, Heidelberg.

El-Ghorab A.H., Hoda M. Fadel and Khaled F. El-massry (2002).The Egyptian *Eucalyptus camaldulensis* var. *brevirostris*: chemical compositions of fruits volatile oil and antioxidant activity. (Flavour and Fragrance Journal, in press).

El-massry K.F, El-Ghorab A.H. and Mansour A.F. (2002). Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. (Food Chemistry, in press).

Elson C.E. and Yu S.G.(1994). The chemoprevention of cancer by mevalonate-derived constituents of fruits and vegetables. J.Nutr. 124 : 607-614.

Esterbauer H., Streigl G., Puhl H. and Rotherender M. (1992). Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. Free Rad. Res. Comm. 6 : 67-75.

Fejes S., Kery A., Blazovics A., Lugasi A., Lemberkovics E., Petri G. and Szoke E.(1998). Investigation of the *in vitro* antioxidant effect of *Petroselinum crispum* (Mill.) Nym.ex.A.W.Hill. Acta Pharm.Hung. 68 : 150-156.

Frankel E.N., Huang S.W., Kanner J. and German J.B. (1994). Interfacial phenomena in the evaluation of antioxidants: bulk oils versus emulsions. J. Agric. Food Chem. 42 : 1054-1059.

Frankel E.N., Huang S.W., Prior E. and Aeschbach R.(1996). Evaluation of antioxidant activity of rosemary extracts carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. J.Sci.Food Agric. 72 : 201-208.

Fuhrman B., Rosenblat M., Hayek T., Coleman R. and Aviram M. (2000). Ginger extract consumption reduces plasma cholesterol, inhibits LDL attenuates development of atherosclerosis in atherosclerotic and apolipoprotein deficient mice. J.Nutr. 130 : 1124-1131.

Geissauf A., Steiner E. and Esterbauer H.(1995). Early destruction of tryptophan residues of apolpoprotein B is a vitamin E independent process during copper mediated oxidation of LDL. Biochem. Biophys. Acta, 125 : 221-232.

Gould M.N. (1997). Cancer chemoprevention and therapy by monoterpenes. Environ. Health. Perspect. 105 : 977-979

Griffin S.G., Wyllie S.G., Markhan J.L. and Leach D.N. (1999). The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour Fragrance J. 14 : 322-332.

Gugliucci A., Meninin T. and Stahl A.J. (1994). Susceptibility to copper mediated antioxidative of VLDL+LDL fractions from diabetic patients. Biochem. Mol. Biol. Int. 32 : 139-147.

Hadly S.K. and Petry J.J. (1999). Medicinal herbs: a primer for primary care. Hosp.Pract.(Off Ed), 15,34,6,105-106.

Halevy D.,Theiry J., Nagel D., Arnold S., Erdman E., Hofling B., Cremer P. and Siedel D.(1997). Increase oxidation of LDL in patients with coronary artery disease is independent from dietary vitamin E and C. Arterioscler.Thromb.Biol. 17 : 1432-1437.

Hertog M.G.L., Feskens E.J.M. and Kromhout D. (1997). Antioxidant flavonols and coronary heart disease risk. Lancet. 349 : 699.

Hirano R., kondo K., Iwamoto T., Igarashi O. and Itakura H. (1997). Effects of antioxidants on the oxidative susceptibility of low-density lipoprotein. J.Nutr.Sci.Vitaminol. 43 : 435-444.

Jayaprakasha G.K., Singh R.P. and Sakariah K.K. (2001). Antioxidant activity of grape seeds (*Vitis vinefera*) extracts on peroxidation models *in vitro*. Food Chem. 73 : 285-290.

Kearney J.F. and Frei B.(1994). Antioxidant protection of low-density lipoprotein and its role in the prevention of atherosclerosis vascular disease. In Natural antioxidants in human health. Frei B.Ed.; Academic Press:San Diego,CA;pp. 303-351.

Kim D.W., Yokozawa T., Hattori M., Kadota S. and Namba T. (2000). Inhibitory effects of an aqueous extract of *Apocynum venetum* leaves and its constituents on Cu^{2+}- induced oxidative modification of low density lipoprotein. Phytotherapy Research, 14 : 501-504.

Kinsella J.E, Frankel E., German B. and Kanner J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol. 47 : 85-89.

Knekt P., Javinen R., Reunanen A. and Maatela J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. Br. Med. J. 312 : 478-481.

Krishnaswany K. and Raghuramuhu N. (1998). Bioactive phytochemicals with emphasis on dietary practices. Indian J. Med. Res. 108 : 167-181.

Mc Dowell I.F., Mc Eneny J. and Trimble E.R. (1995). A rapid method for measurement of susceptibility to oxidation of low density lipoprotein. Ann. Clin. Biochem. 32 : 167-174.

Rimm E.B., Stampfer M.J., Ascherio A., Giovannucci G.A., Colditz G.A. and Willet W.C.(1993). Vitamin E consumption and the risk of coronary heart disease in men. New Englan. J. Med. 328 : 1450-1456.

Sato K., Niki E. and Shimasaki H.(1990). Free radical-mediated chain oxidation of low-density lipoprotein and its synergistic inhibition by vitamin E and vitamin C. Arch.Biochem.Biophys. 279 : 402-405.

Siedel J., Hagele E.O., Ziegenhorn J. and Wahlefed A.W. (1983). Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin. Chem. 29 : 1075-1080.

Steinberg D. (1992). Metabolism of lipoproteins and their role in the pathogenesis of atherosclerosis. Atherosclerosis Rev. 18 : 1-6.

Teissedre P.L. and Waterhouse A.L.(2000). Inhibition of oxidation of human low density lipoproteins by phenolic substances in different essential oils varieties. J. Agric. Food Chem. 48 : 3801-3805.

Yamanaka N., Oda O. and Nagao S. (1997). Prooxidant activity of caffeic acid, dietary-flavonoid phenolic acid, on Cu^{2+}-induced low density lipoprotein oxidation. FEBS Lett. 405 : 186-190.

دراسة التأثير المثبط لبعض الزيوت العطرية على أكسدة دهون الدم قليلة الكثافة (الليبوبروتينات) باستخدام طريقة تحفيز الأكسدة بأيونات النحاس

خالد فاروق سيد حسن المصري

قسم كيمياء مكسبات الطعم و الرائحة – المركز القومي للبحوث ، الجيزة

ملخص

تعتبر الزيوت العطرية إحدى النواتج الطبيعية المستخلصة من النباتات والخضراوات والتي يمكن استخدامها كمواد مكسبة للطعم والرائحة وكذلك الاستفادة منها كمضادات للميكروبات ومانعة للأكسدة ولبعض الأمراض مثل السرطان. تم تقدير نشاط خمسة زيوت عطرية تم استخلاصها من نباتات القرنفل ، البردقوش ، ثمار الكافور ،الزنجبيل و الشيح البلدي و ذلك فى تثبيط أكسدة دهون الدم قليلة الكثافة (الليبو بروتينات) باستخدام طريقة تحفيز الأكسدة بأيونات النحاس معمليا و كذلك المحتوى الفينولى لتلك الزيوت. وقد أظهرت النتائج أن إطالة الوقت اللازم لحدوث الأكسدة (lag-time) يعتمد أساسا على نوع و كمية الزيت العطري المستخدم. ووجد أن زيت القرنفل (المكون الأساسي له مادة Eugenol) أطال الوقت اللازم لحدوث الأكسدة لمدة ٣٦٧ دقيقة يليه زيوت البردقوش ، الزنجبيل ، ثمار الكافور ثم الشيح البلدي (٢٣٠ ، ١٧٣ ، ١١٧ ، ٦٩ دقيقة على التوالي مقارنة بفيتامين C (١٤٢ دقيقة) . كما أظهرت النتائج أن هناك علاقة بين المحتوى الفينولى الكلى لهذه الزيوت و درجة تثبيطها للأكسدة المحدثة بأيونات النحاس (إطالة وقت بداية الأكسدة) مما يمثل أهمية كبيرة لهذه الزيوت إذا ما استخدمت فى حماية دهون الدم قليلة الكثافة من الأكسدة

و تتفق تلك النتائج مع المحتوي الفينولي لتلك الزيوت العطرية و نشاطيتها كمثبطات لأكسدة دهون الدم قليلة الكثافة مما يشكل اتجاها هاما لاستخدام تلك الزيوت العطرية كمواد مانعة وواقية لحدوث بعض الأمراض خاصة أمراض تصلب الشرايين مما يمثل خطوة هامة للوقاية من الامراض و تقليل تكلفة العلاج.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٤) العدد الأول (يناير ٢٠٠٣):٧٧-٩٢.