Minia J. of Agric. Res. & Develop.
Vol. (٣٠) No. ٢ pp ٢٤٣ -٢٥٧ ,
٢٠١٠

FACULTY OF AGRICULTURE

ANTIOXIDANT ACTIVITY, PHENOLIC COMPONENTS AND NUTRITIONAL EFFECT OF SOME AROMATIC PLANTS

Magda A.A. Seleim
Food Sci. and Tech. Dept., Fac. of Agric. Assiut University, Assiut.

Received ٦ Dec. ٢٠١٠ Accepted ١٢ Dec. ٢٠١٠

ABSTRACT

In the present study, essential oils were obtained from five selected aromatic plants, namely: fennel, rosemary, ginger, thyme and cinnamon. Their contents of total polyphenols were qualitatively and quantitatively determined using HPLC analysis. Antioxidant activities were determined with a Rancimat apparatus comparing with synthetic antioxidant (BHT). The nutritional effect on rats serum lipids was also studied.

Aromatic plant extracts could be a potential source of natural antioxidants and can be added to foods to replace synthetic antioxidants, minimizing oil peroxidation.

Generally, the obtained results showed that the studied aromatic plants were rich in phenolic components but rosemary had the highest level and thyme contained the lowest. These phenolic compounds demonstrated good antioxidant activity and the plants, rich in phenolic acids and flavonoids could be considered as a good source of natural antioxidants. Also, examined aromatic plants could be regarded as a good treatment for decreasing serum total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides, as well as increasing HDL-cholesterol level in rat serum lipids.

INTRODUCTION

Oxidative degradation of lipids is a major factor limiting the shelf life of foods. The free-radical reaction of lipid peroxidation is generally responsible for the deterioration of lipid-containing foods. It decreases nutritional and sensory properties of foods since it involves the loss of essential fatty acids and vitamins, the generation of toxic compounds, causing additionally, flavor, texture and color deterioration (Morrissey *et al*., ١٩٩٨). The use of antioxidants during the manufacturing process can minimize the extent of lipid peroxidation (Shahidi and Wanasundara, ١٩٩٢).

Recently, there is an increasing interest both at industry and scientific levels for the use of spices and aromatic plants because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. These properties could be due to the presence of many substances, including some vitamins, flavonoids, terpenoids, carotenoids, etc., which, render spices and some aromatic plants or their antioxidant components as preservative agents in food (Calucci *et al*., ٢٠٠٣).

Polyphenolic compounds are commonly found in both edible and non-edible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kahkonen *et al*., ١٩٩٩). Aromatic plants are used in many domains, including nutrition, flavoring, or as beverages (Djeridane *et al*., ٢٠٠٦).

Many aromatic plants have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, antimicrobial, hypolipidemic and anticarcinogenic potential (Luo *et al*., ٢٠٠٤).

Crude extracts of aromatic plants and other plant materials rich in phenolic compounds are of increasing interest in food industry because they can retard oxidative degradation of lipids and thereby improve the quality and nutritional value of foods.

The aim of this study was to evaluate and compare the antioxidant activities of some aromatic plant oils namely: fennel, rosemary, ginger, thyme and cinnamon using the Rancimat method. Total phenolic content, qualitative and quantitative analysis of the major phenolics were determined using HPLC analysis.

The nutritional effect of the studied aromatic plants on rat serum lipids (triglycerides, total cholesterol, HDL, LDL and VLDL-cholesterols) was also investigated.

MATERIALS AND METHODS

Materials:

Five selected aromatic plants, namely: fennel, rosemary, ginger, thyme and cinnamon were obtained from local market. Sunflower oil was donated from El-Nile Company for oils and soaps, Assuit, Egypt.

Experimental animals:

Fourty two male of albino rats were obtained from Animal House, Faculty of Medicine, Assiut University and were randomly divided into seven groups (each group consisted of six rats) of similar total weight. The rats in each group were assigned to the corresponding experimental diet and were housed individually in cages in a controlled environment. Diets and water were supplied ad libtum throughout the study.

Diets:

Basal Diet (BD): Normal diet provided from animal house.

Rich cholesterol diet (RCD): Basal diet+١ g brain (gavage) according to Al-Sharjabi (٢٠٠٥).

Rat groups were fed during experimental period as follows:

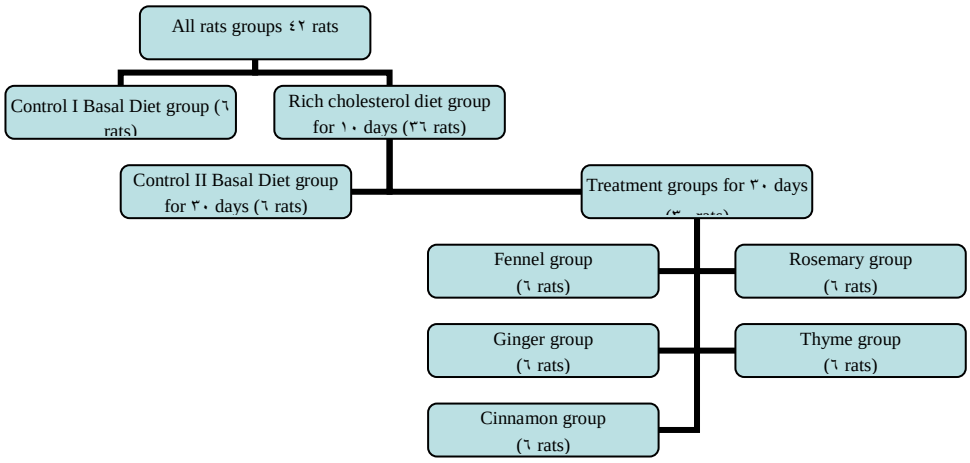

Fig. ١: Flow sheet diagram of different rat groups during nutritional period.

Methods:

Aromatic plant oil extraction:

The aromatic plant oils were extracted using the water distillation method described by Ravindran et al. (٢٠٠٧).

Antioxidant activity:

Antioxidant activities of the studied aromatic plant oils compared with synthetic antioxidant (BHT) were determined with a Rancimat apparatus (Metrohm, Herisau, Switzerland) by measuring the induction period of oils containing the antioxidant, according to the method described by Hasenhuettl and wan (١٩٩٢).

The antioxidant index was calculated as:

$$\text{Antioxidan t index} = \frac{\text{Induction period of oil with extract}}{\text{Induction period of oil alone}}$$

Total polyphenols content:

Total polyphenol content was demonstrated using Folin-Ciocalteu colorimetric method as described by Huang *et al*. (٢٠٠٧).

The absorbance of the resulting blue color was measured at ٧٦٥ nm with a shimadzu UV spectrophotometer.

Quantification was done with respect to the standard curve of gallic acid. The results were expressed as mg gallic acid/١٠٠ g dry weight.

Phenolic content:

Qualitative and quantitative analysis of major phenolics were determined by using HPLC analysis as described by Aaby *et al*. (٢٠٠٤).

Blood analysis:

Blood sample was taken from each group of rat for the determination of:

Serum total cholesterol level (mg/dL).

Serum high density lipoprotein HDL level (mg/dL).

Serum triglyceride level (mg/dL).

Total cholesterol content:

Total cholesterol content were determined colorimetrically with commercially available kits (Cholesterol C-test, ELITECH diagnostics, French) according to Allain *et al*. (١٩٧٤).

HDL cholesterol content:

HDL cholesterol content were determined colorimetrically with commercially available kits (HDL-cholesterol test, ELITECH diagnostics, French). The quantitative estimation of HDL cholesterol was made using HDL cholesterol precipitating reagent in combination with enzymatic colorimetric assay kit for total cholesterol, where chylomicrons, very low density lipoprotein (VLDL) cholesterol, and low density lipoprotein (LDL) cholesterol fractions were precipitated from serum or plasma by means of phosphotungstic acid and magnesium ions, according to Lopes-Virella *et al*. (١٩٧٧). After centrifugation, high density lipoprotein (HDL) cholesterol was then determined in the supernatant using a cholesterol reagent and the derived dilution factor in the calculation.

Triglycerides content:

Triglyceride concentrations were determined colorimetrically at ٥٤٦ nm with commercially available kits (Triglycerides test, ELITECH diagnostics, French), according to Bucolo and David (١٩٧٣).

Estimation of LDL and VLDL cholesterol in serum:

The concentration of LDL cholesterol was calculated according to the equation of Friedewald *et al*. (١٩٧٢) as follows:

[LDL-chol] = [Total chol] – [HDL-chol] – [TG]/٥

All concentrations were in mg/dL.

The quotient [TG]/٥ were used as a measure of VLDL-cholesterol concentration. It was assumed first, that virtually all of the plasma TG was carried on VLDL, and second, that the TG: cholesterol ratio of VLDL was constant at about ٥:١ (Friedewald *et al.*, ١٩٧٢).

RESULTS AND DISCUSSION

Antioxidant activity of aromatic oils plants:

Antioxidant activities of studied aromatic plant extracts are presented in Table ١. ; BHT was presented for comparison. With the exception of ginger and thyme, the extracts showed higher antioxidant activity in sunflower oil. Aromatic plant extracts contained phenolic structure which were capable of minimizing oil , protecting sunflower oil against autoxidation (Arouma *et al*., ١٩٩٢).

Table ١: Antioxidant activities of aromatic plant extracts.

Plant extract	Antioxidant index in sunflower oil
Fennel	٢.٤
Rosemary	٢.٦
Ginger	١.٦
Thyme	١.٥
Cinnamon	٢.٣
BHT	٢.٥

Total phenolics content:

The amount of total phenolics content, estimated by Folin-Ciocalteu method of the studied aromatic plant samples are presented in Table ٢. The amount of total phenolics varied widely between selected aromatic plants, ranged from ٢.٤٨ to ١٧.٦٠ mg gallic acid/١٠٠g of dry weight. The highest level of total phenolics was found in rosemary, while thyme contained the lowest level. Phenolics contents can be arranged in the decreasing order as follow: rosemary > fennel > cinnamon > ginger > thyme. The obtained results showed that aromatic plants had relatively high level of polyphenols, and the

differences between the results could be due to genotypic or and environmental differences within species (Shan *et al*., ٢٠٠٥).

Table ٢: Total phenolic content (mg gallic acid/١٠٠ g dry weight) of aromatic plants.

Aromatic plant	Total phenolic content
Fennel	١٣.٦٣
Rosemary	١٧.٦٠
Ginger	٣.٩١
Thyme	٢.٤٨
Cinnamon	١١.٤٢

Identification of phenolic components:

The major types and the representative components of phenolic compounds in the samples were analyzed using HPLC method compared with authentic phenolic standard (Table ٣). Caffeic acid, p-coumaric acid, ferulic acid and neochlorogenic acid were identified as the major phenolic acids present in the studied samples, while, luteolin, apigenin, kaempferol and isorhamnetin were identified as the major flavonoids.

Table ٣: Quantitative analysis of the major phenolic compounds of aromatic plants (mg/١٠٠g dw).

Aromatic plants	Phenolic acids				Flavonoids			
	Caffeic acid	p-coumaric acid	Ferulic acid	Neochlorogenic acid	Luteolin	Apigenin	Kaempferol	Isorhamnetin
Fennel	٦٤٠	٤١.٠	٢٢.٦	٨.٠	٣٢١.٠	٢٤.٠	١٩.٦	٣٦.٧
Rosemary	٨٧٢	٣٦.٢	٢٩.٦	١٢.٨	٦٢٠.٠	٣٩.٠	١٨.٦	١٢.٨
Ginger	١٩٣	٤١.٦	١٦.٨	١٠.٢	٨٢.٠	٣٠.٢	٤١.٥	٢٢.٣
Thyme	١٦٠	٩٠.٢	٣٤.٥	٢٠.٣	٦٦.٣	٢١.٠	٣٢.٧	٢٠.٢
Cinnamon	٥١٢	٤٢.٦	٢٠.٨	٩.٦	١٠٢.٠	١٦.٢	٤٥.٦	١١.٩

Considerable variation was found in the phenolic compounds of the different aromatic plants. The main phenolic acids in these plants were caffeic acid and p-coumaric acid. However, ferulic acid and neochlorogenic acid occurred in minor quantities. These results are in agreement with those reported by Luo *et al.* (٢٠٠٤) and Shan *et al.* (٢٠٠٥).

Generally, results showed that the aromatic plants were rich in phenolic components and demonstrated good antioxidant activity. These plants, rich in phenolic acids and flavonoids could be a good source of natural antioxidants. Therefore, the qualitative and quantitative analysis of major individual phenolics in the aromatic plants could be helpful for explaining the relationships between the total antioxidant capacity and the total phenolic contents in the aromatic plants.

Nutritional effect of aromatic plants on rat serum lipids:

Rats were divided into seven groups and fed during experimental period (٤٠ days) as shown in Fig. ١. At the first ten days, except group ١ which left as control all groups fed Rich cholesterol diet to raised serum lipids level. Table ٤ shows that the higher increase was in LDL-cholesterol (٧٦.٣٪), triglycerides and VLDL-cholesterol (٥٢.٢٪) then total cholesterol (٣٨.٦٪), while, HDL-cholesterol decreased by ٢٣.٣٪.

Table ٤: Changes in rat serum lipids during experimental without any addition.

Rat serum lipid	Group	Means	% changes
Triglyceride	Control I at zero time	١٣٥.٧٦	٥٢.٢↑
	Control I at end time	١٣٧.٢٤	
	Control II at zero time	٢٠٦.٥	
	Control II at end time	١٩٥.٢٢	
Total cholesterol	Control I at zero time	١١٧.٢٥	٣٨.٦↑
	Control I at end time	١١٩.١٠	
	Control II at zero time	١٦٢.٦	

	Control II at end time	١٥٧.٩٤	
HDL-cholesterol	**Control I at zero time**	٣٧.٦٦	٢٣.٣↑
	Control I at end time	٣٨.٤٧	
	Control II at zero time	٢٨.٩	
	Control II at end time	٣٢.٢٤	
LDL-cholesterol	**Control I at zero time**	٥٢.٤٤	٧٦.٣↑
	Control I at end time	٥٣.١٩	
	Control II at zero time	٩٢.٤	
	Control II at end time	٨٦.٦١	
VLDL-cholesterol	**Control I at zero time**	٢٧.١٥	٥٢.٢↑
	Control I at end time	٢٧.٤٤	
	Control II at zero time	٤١.٣	
	Control II at end time	٣٩.٠٥	

The data presented in Figures (٢-٦) show the effect of adding aromatic plants to rat diets on serum lipids.

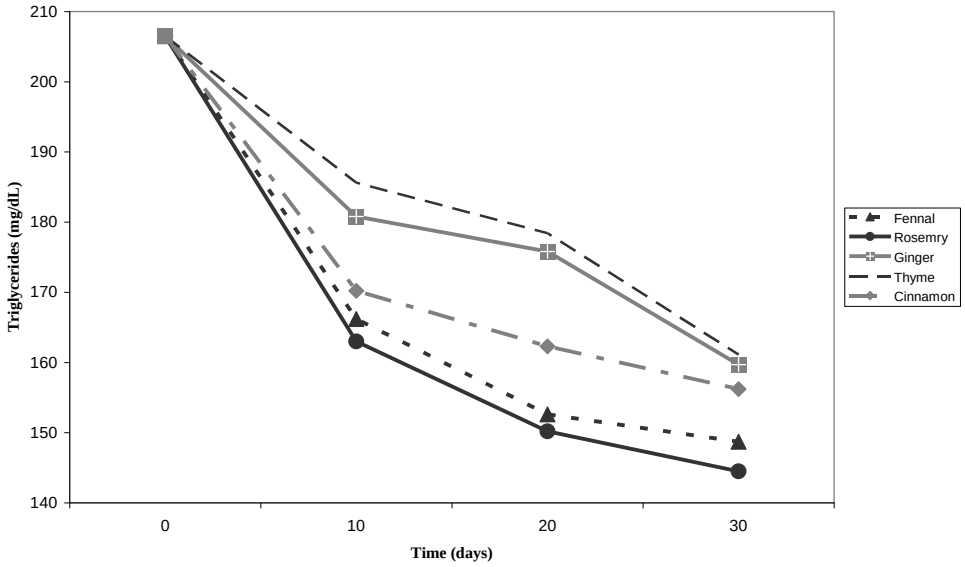

Fig. ٢: Effect of adding aromatic plants on rat serum Triglycerides

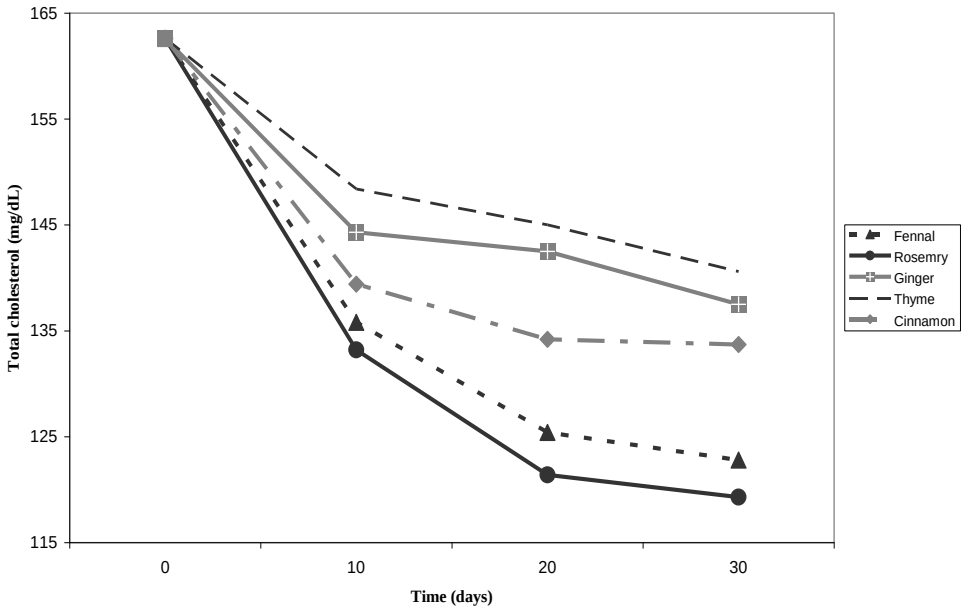

Fig.٣: Effect of adding aromatic plants on rat serum total cholesterol.

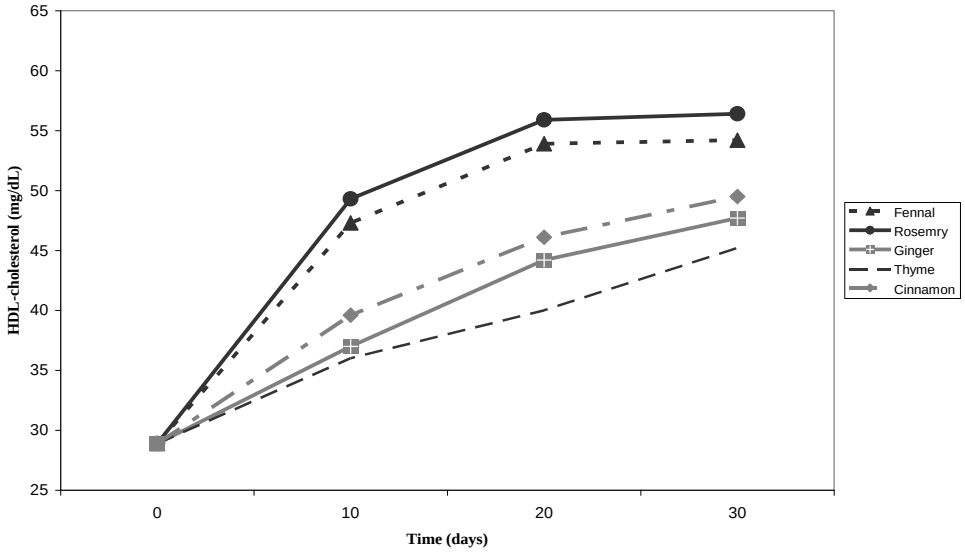

Fig.٤: Effect of adding aromatic plants on rat serum HDL-cholesterol.

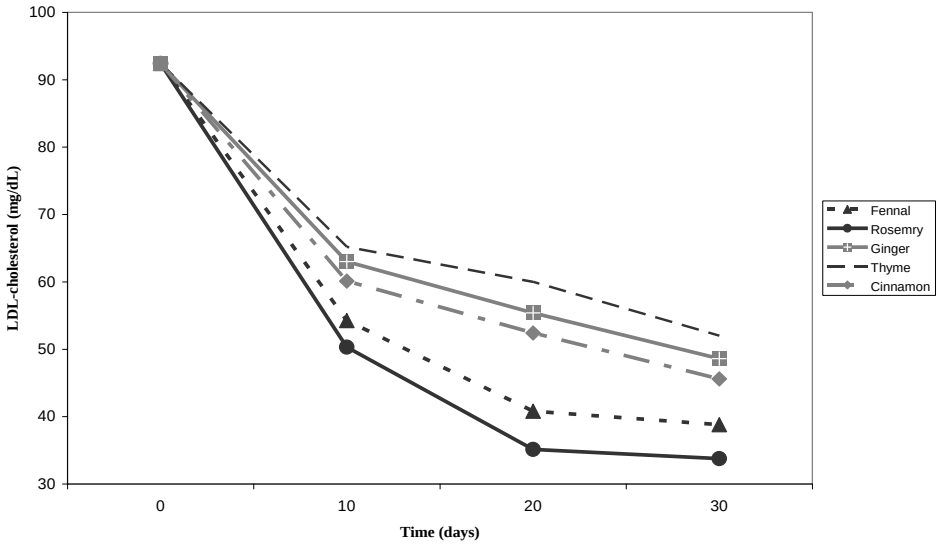

Fig.٥: Effect of adding aromatic plants on rat serum LDL-cholesterol

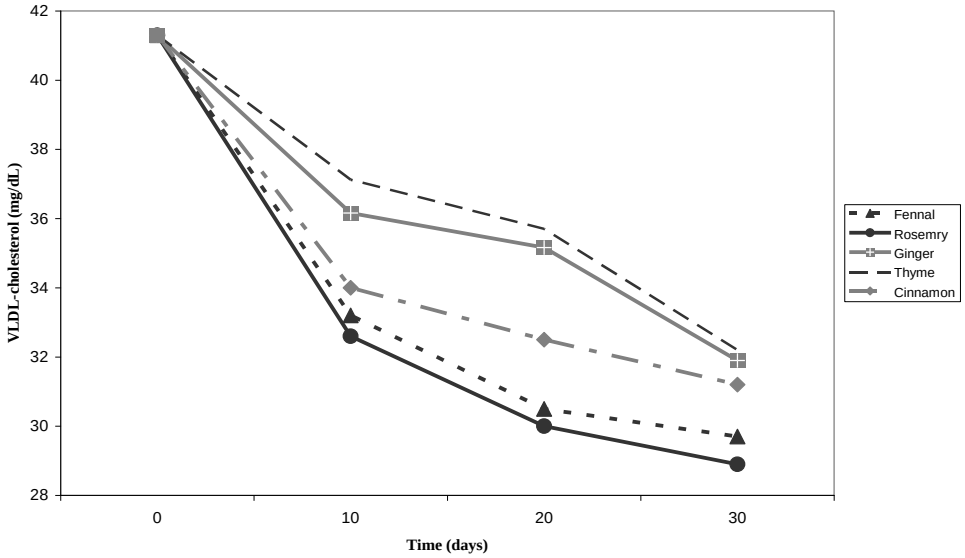

Fig.٦: Effect of adding aromatic plants on rat serum VLDL-cholesterol.

Fig. ٢ shows that the highest decrease in rat serum triglycerides was in groups fed on rosemary specially after ٣٠ days of treatment, followed by fennel, cinnamon, ginger and thyme in the descending order. This could be due to inhibition of hepatic triglyceride synthesis and stimulation of hepatic peroxisomal β-oxidation (Ruiz-Gutierrez *et al*., ١٩٩٩).

Fig. ٣ shows that the highest decrease in rat serum total cholesterol was in rats fed cinnamon, possibly due to inhibiting absorption and synthesis of cholesterol. Whereas there was a decrease in lymphatic absorption of cholesterol accompanying an increase in fecal excretion of neutral, but not acidic sterioids, particularly when cholesterol-enriched diet was given (Hirose *et al*., ١٩٩١).

However, adverse trend of rat serum HDL-cholesterol level (Fig. ٤) was shown in LDL-cholesterol. Fig. ٥ shows that the highest decrease in rat serum LDL-cholesterol was in rats fed cinnamon followed by those fed thyme.

The trend of serum VLDL-cholesterol level in rats during the experimental period is shown in Fig. ٦. They showed the same trend as of triglyceride. It was assumed that first all of the plasma triglyceride is carried on VLDL, and second, that the (triglyceride: cholesterol) ratio of VLDL is constant at about ٥:١ (Friedewald *et al*., ١٩٧٢).

Extracted natural antioxidants (aromatic plant extracts) could be considered as a good treatment for decreasing serum total cholesterol,

LDL-cholesterol, VLDL-cholesterol and triglycerides, but it increase HDL-cholesterol level of rats serum.

It could be concluded that the studied aromatic plant samples were rich in phenolic components and demonstrated good antioxidant activity. Moreover, aromatic plants may contain polar products which would be able to lower lipid concentrations in hyperlipidemia rats, and could be beneficial in preventing hyperlipidemia and related cardiovascular diseases.

REFERENCES

Aaby, K.; Hvattum, E. and Skrede, G. (٢٠٠٤). Analysis of flavonoids and other phenolic compounds using HPLC with color metric array detection. Relationship to antioxidant activity. Journal of the Agricultural and Food Chemistry, ٥٢, ٤٥٩٥-٤٦٠٣.

Al-Sharjabi , F.A. , (٢٠٠٥) : Biochemical and nutritional studies on some oil seeds . PH.D. Thesis, Food Sci.& Techn. Dept., Faculty of Agric., Assiut, University , Egypt .

Allain, C.C.; Poon, L.S.; Chan, C.S.G.; Richmond, W. and Fu, P.C. (١٩٧٤). Enzymatic determination of total serum cholesterol. Clin. Chem., ٢٠, ٤٧٠-٤٧٥.

Aruoma, O.I.; Halliwell, B.; Aeschbach, R. and Loliger, J. (١٩٩٢). Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. Xenobiotica ٢٢, ٢٥٧-٢٦٨.

Bucolo, G. and David, H. (١٩٧٣). Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem., ١٩, ٤٧٦-٤٨٢.

Calucci, L.; Pinzono, C.; Zandomeneghi, M., and Capocchi, A. (٢٠٠٣). Effect of gamma-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. Journal of the Agricultural and Food Chemistry, ٥١, ٩٢٧-٩٣٤.

Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P. and Vidal, N. (٢٠٠٦). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, ٩٧, ٦٥٤-٦٦٠.

Friedewald, W.T.; Levy, R.I. and Fredrickson, D.S. (١٩٧٢). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., ١٨, ٤٩٩-٥٠٢.

Hasenhuettl, G.L. and Wan, P.J. (١٩٩٢). Temperature effects on the determination of oxidative stability with the Metrohm Rancimat. J. Am. Oil Chem. Soc., ٦٩, ٥٢٥-٥٢٧.

Hirose, N.; Inoue, T.; Nishihara, K.; Sugano, M.; Akimoto, K.; Shimizu, S. and Yamada, H. (١٩٩١). Inhibition of cholesterol absorption and synthesis in rats. J. Lipid Res., ٣٢, ٦٢٩-٦٣٨.

Huang, W.Y.; Cai, Y.Z.; Xing, J.; Corke, H. and Sun, M. (٢٠٠٧). A potential antioxoidant resource: endophytic fungi isolated from traditional Chinese medicinal plants. Economic Botany, ٦١, ١٤٣٠.

Kahkonen, M.P.; Hopia, A.I.; Vuorela, H.J.; Rauha, J.P.; Pihlaja, K. and Kujala, T.S. (١٩٩٩). Antioxidant activity of plant extracts containing phenolic compounds. Journal

of the Agricultural and Food Chemistry, ٤٧, ٣٩٥٤-٣٩٦٢.

Lopes-Virella, M.F.; Stone, P.; Ellis, S. and Coiweil, J.A. (١٩٧٧). Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem., ٢٣, ٨٨٢-٨٨٤.

Luo, Y.; Cai, Q.; Sun, M. and Corke, H. (٢٠٠٤). Antioxidant activity and phenolic compounds of ١١٢ traditional Chinese medicinal plants associated with anticancer. Life Science, ٧٤, ٢١٥٧-٢١٨٤.

Morrissey, P.A.; Sheehy, P.J.A.; Galvin, K.; Kerry, J.P. and Buckley, D.J. (١٩٩٨). Lipid stability in meat and meat products. Meat Science, ٤٩, S٧٣-S٨٦.

Ravindran,P.N., Babu N.K. and Sivaraman K.(٢٠٠٧). Turmeric: The genus curcuma. CRC Press, New York .

Ruiz-Gutierrez, V.; Perez-Espinosa, A.; Vazquez, C.M. and Santa-Maria, C. (١٩٩٩). Effect of dietary fats on lipid composition and antioxidant enzymes in rat liver. British J. Nutr. ٨٢, ٢٣٣-٢٤١.

Shahidi, F. and Wanasundara, J.P.K.P.D. (١٩٩٢). Phenolic antioxidants. Critical Reviews in Food Science and Nutrition, ٣٢, ٦٧-١٠٣.

Shan, B.; Cai, Y.Z.; Sun, M. and Corke, H. (٢٠٠٥). Antioxidant capacity of ٢٦ spice extracts and characterization of their phenolic constituents. Journal of the Agricultural and Food Chemistry, ٥٣, ٧٧٤٩-٧٧٥٩.

Magda A.A. Seleim

النشاط المضاد للأكسدة ، المركبات الفينولية والتأثير الغذائي لبعض النباتات العطرية

ماجدة عبد الحميد أحمد سليم

قسم علوم وتكنولوجيا الأغذية – كلية الزراعة – جامعة أسيوط

فى هذه الدراسة تم الحصول علي الزيوت العطرية من خمسة أنواع من النباتات العطرية شائعة الاستخدام وهى: الشمر ، حصالبان ، الزنجبيل ، الزعتر ، القرفه ، حيث تم تقدير محتواها من المركبات الفينولية الكلية، بالإضافة إلي التقدير الوصفي والكمي للفينولات باستخدام جهاز الـ HPLC .

كذلك تم تقدير النشاط المضاد للأكسدة للزيوت المستخلصة باستخدام جهاز الرانسيمات مع مقارنتها بمضادات الأكسدة الصناعية BHT . بالإضافة إلي ذلك تم دراسة التأثير الغذائي للنباتات موضع الدراسة علي ليبيدات الدم في فئران التجارب .

وقد أوضحت النتائج المتحصل عليها ما يلي :

١- تعتبر الزيوت المستخلصة من النباتات العطرية موضع الدراسة ذات نشاط مضاد للأكسدة مرتفعاً مقارنة بمضادات الأكسدة الصناعية وذلك لمحتواها المرتفع من المواد الفينولية .

٢- تعتبر النباتات العطرية مصدراً جيداً لمضادات الأكسدة الطبيعية والتي يمكن استخدامها كبديل آمن لمضادات الأكسدة الصناعية لتقليل الأكسدة في الزيوت ، نظراً لمحتواها من الأحماض الفينولية والفلافونويدات .

٣- احتوي مستخلص حصالبان علي أعلي تركيز في المركبات الفينولية الكلية بينما كان أقل تركيز في مستخلص الزعتر .

٤- أدي استخدام النباتات العطرية موضع الدراسة إلي خفض محتوي ليبيدات الدم في فئران التجارب من الكوليسترول الكلي والجلسريدات الثلاثية ، وكذلك الكوليسترول منخفض الكثافة ، بينما حدث زيادة واضحة في الكوليسترول مرتفع الكثافة.

وبصفة عامة يمكن التوصية باستخدام النباتات العطرية ومستخلصاتها كمصدر جيد وآمن لمضادات الأكسدة الطبيعية لمنع التدهور الأكسيدي في الزيوت وكذلك لخفض محتويات ليبيدات الدم من الكوليسترول ومشتقاته .