The Antioxidative Action of Monoterpene From *Loranthus europaeus L.seeds*

Adnan J. M. Al-Fartosy
Dept. of Chemistry
Coll. of Science, Univ. of Basrah

Ali K. J. Al-Rikaby
Dept. of Food Science
Coll. of Agriculture Univ. of Basrah

Basrah – Iraq

SUMMARY

The Oils of *Loranthus europaeus L.seeds* had been extracted with n-hexane. A number of qualitative chemical analysis were carried to the extract using different techniques such as: Thin Layer Chromatography (TLC), UV/ visible and IR. The results revealed that the isolated oily extract is monoterpenoid fraction consist of five glucoside compounds.

In an attempt to assess the possible antioxidant activity for monoterpene extract of *Loranthus europaeus L.seed*, several assays were conducted. We found that the extract had the most potent antioxidative effect toward linoleic acid model system. The extract possessed strong reducing power ability and exhibited ferrous ion chelating capability. The extract act as retardation agent effective of corn oil oxidation.

Introduction

Consumption of significant amounts of polyunsaturated fatty acids has increased.The importance and use of the antioxidants to prevent the autooxidation. The use of antioxidants is a method of increasing the shelf life, especially of lipids foods [8]. Furthermore, antioxidant supplements, or food containing antioxidants may be used to help the human body to reduce the oxidative damage related to diseases, such as atherosclerosis, cancer and cirrhosis. Although almost all organisms possess antioxidant defence and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely [12].

Sythetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years (Romero *et al.*,2004).

Antioxidants such as tocopherols, carotenoids, terpenoids, ascorbic acid and phenoilic compounds, including tannins and flavonoids, are introduced into the human body in a form of food components [1].

The present work aims to isolate the monoterpene compounds were from *loranthus europaeus L.seeds* and assess their possible antioxidant activities.

Materials and Methods

2-1: Plant materials.

Loranthus europaeus L.seeds were supplied locally. The plant was botanically authenticated and voucher specimens were deposited in the Herbarium of BASRAH (Basrah, Iraq, College of Science, University of Basrah). The seeds were ground by hand mill and kept in polyethylene bags until time of use.

2-2: Instruments.

1- Rotary evaporator, Buchi Rota Vapor-RE.SP3 Pye Unicam.
2- Infra-red spectrophotometer (IR) model-sp3-300 pye unicam, UK.
3- UV/Visible spectrophotometer, model-sp8-100 pye unicams, UK.
4-Differential Scanning Calorimetry (DSC)Du-pont thermoanalyser model-990 was calibrated with indium metal (99.999%) and planometer model A. OTT. No. 114672.

2-3: Procedure.

2-3-1: Monoterpene extract of *Loranthus europaeus L.seeds*

100g of seeds were ground by hand mill and extracted continuously by soxhelet with (400ml) of n-hexane for (24h). the n-hexane extract was evaporated under reducing pressure to obtain a viscous oil (31g). the viscous oil was dissolved in (300ml) cold acetone, then filtered, the greenish-yellow acetone filtrate was concentrated to 10ml. To this solution 1.25g of silica gel HCl-washed was added, then evaporated to dryness. The solid residue was then applied on top of column filled with HCl-washed silica gel (66.1g). the column was first eluted with $CHCl_3$ (300ml), then $CHCl_3$–MeOH (97:3) (300ml), $CHCl_3$–MeOH (4:1) (300ml), and MeOH (300ml)were eluted successively. Collecting 100ml fraction. 18 fractions were combined the solvent was removed in vacuum to give a yellow residue (0.37g) [4].

2-3-2: Qualitative analysis

2-3-2-1: Preliminary tests:

Preliminary qualitative chemical tests were carried out on the foregoing prepared monoterpene extract of *Loranthus europaeus L.seeds*, as mentioned in sections (3-1). These tests were done in order to identify the chemical nature of this extract as shown in table (1).

2-3-2-2: Thin layer chromatography (TLC).

The foregoing prepared monoterpene extract of Loranthus europaeus L.seeds were tested for thin layer chromatography (TLC). A known volume

(two drops) of this extract (1mg/1ml) were spotted on thin layer chromatography (TLC) silica gel plate (2 × 10)cm using $CHCl_3$-Benzene (1:1) as a solvent system. After 30 second, the plates were dried using air drier and examined under long wavelength 366nm (UV). The plate were also stained with ethanolic rodamine-B solution, $SbCl_3$ in HCl (10%), phosphmolybidic acid (5%). [3].The plates were also developed using perchloric acid (2%), vanilin/sulfuric acid [2]. The plates were also developed using liebermann-Burchard, aqueous $KMnO_4$ (0.2%), Conc. H_2SO_4, p-anisaldehyde. and by using 2, 4-dinitrophenylhydrazine, iodine vapor, Bendict solution, Dragendroff reagent (Trease and Evane,1973) as shown in Table (2), Fig. (1).

2-3-3: Infrared spectroscopy (IR).

The IR absorption spectra of the monoterpene extract of *Loranthus europaeus L.seeds* were recorded from (4000-400)cm^{-1} region, as KBr disk. The absorption bands observed in the spectra are shown in Fig. (2), and Table (3).

2-3-4: Ultraviolet/visible spectroscopy (UV/Visible).

The absorption spectra of the monoterpene extract of *Loranthus europaeus L.seeds* was measured in petroleum ether (40-60C^o) at(200-800)nm and shows in Fig (3.

2-3-5: Determination of Antioxidatire activity.

20mg/ml of monoterpene extract or BHT was dissolved in 4ml of 95% ethanol and mixed with linoleic acid (2.5% in ethanol absolute) (4.1ml), 0.05M phosphate buffer pH 7.0 (8ml), and distilled water (3.9ml), and kept in screwcap containers at 40 C^o/24 hr in the dark. A 0.1ml of 30% ammonium thiocyanate,added at precisely 3 minute after the addition of 0.1m of 20mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500nm of the resulting red solution was measured. [13]. The percent inhibition of linoleic acid peroxidation was calculated as:

$$\text{Antioxidative activity (\%inhibition)} = \frac{\text{(absorbance of the sample)}}{\text{(absorbance of the control)}} \times 100$$

3-5-6: Measurment of the reducing power.

The monoterpene extract (20, 40, 60, 80 100 and 120mg/ml) or BHT mixed with an equal volume of 0.2M phosphate buffer (pH 6.0) and 1% potassium ferricyanide. The mixture was incubated at 50C^o for 20 min. Then an equal volume of 1% trichloroacetic acid was added to the mixture, and centrifuged at 6000 rpm for 10min. The upper layer of the mixture was

mixed with distilled water and 0.1% $FeCl_3$ with a ratio of (1:1:2), and measured the absorbance at 700nm. [14].

2-3-7: Ferrous Ion Chlating effect.

Reaction mixtures containing 0.1ml of the monoterpene extract in different concentrations (١, ٢, ٣, ٤, ٥, ٦mg/ml), 0.2ml of 0.5mM ferrous chloride and 0.2ml of 5mM ferrozine were incubated at 37 C^o for 10min. A 1.5ml of deionized water was added to the mixture, the absorbence at 562nm was measured [2].

3-2-8: Retardation of corn oil autooxidation.

0.5g of corn oil was dissolved in 24ml of chloroform – methanol mixture (1:2) and 1ml of the monoterpene extract was added in various concentrations (2, 4, 6, 8 and 10mg/ml). The homogenous mixture was incubated at 45C^o, and peroxide value was determined periodically [9].

Results and Discussion

3-1: Qualitative analysis for monoterpene extract of Loranthus europaeus L.seeds.

The results presented in Tables (1), (2) and Fig. (1) were shown that the monoterpene extract of *Loranthus europaeus L.seed* contains five components. These components are relate to the monoterpenes family because they give a positive test with $KMnO_4$, Conc. H_2SO_4 and sulfuric acid/vanillin reagents. The results revealed that all the components have ketone groups because they give a positive test with 2,4-dinitrophenydrazine reagent and negative test with tollene reagent. On the other hand, these components are sugar compounds as glycosides because they indicate a positive test with p-anisaldehyde reagen

Fig. (1): Thin layer chromatography for monoterpene extract of Loranthus europaeus L.seeds

Table (1): Qualitativ analysis for monoterpene extract

Test	Reagent	Observation
Alkaloids	Dragendroff,wagner, mayer	-
Amino acid	Ninhydrin	-
Carbohydrate	Molish test	+
Flavonoids	Alcoholic KOH (5N)+ Magnesium turning	-
Proteins	Biuret	-
Saponins	(5%) $HgCl_2$+$AgNo_3$	-
Phenols	(1% $FeCl_3$) + Ammonia vapor	-
Coumarins	(5%) NaOH + UV light	-
Glycosides	Bendict test	+
Terpenoids	Salkowski	+
Aldehyde and Ketone	2,4-dinitro phenylhydrazine	+
Aldehyde	Tollene test	-

Table (2): Thin layer chromatography (TLC) for monoterpene extract

Phenols test	Rf values	Vanillin Conc. HCl
Phenols test	0.92 0.81 0.74 0.37 0.07	2,4-dinitro phenylhydrazin
Ketone group test	-	Bendict
Flavonoids test	0.92 0.81 0.74 0.37 0.07	Conc. H_2SO_4
Mono or di terpenoids test	-	Liberman-Burchard
Steroids or Triterpenoids test	0.92 0.81 0.74 0.37 0.07	p-anisaldehyde

Carbohydrat test	-	Dragendroff
Alkaloids test	0.92 0.81 0.74 0.37 0.07	Vanilline-H_2SO_4
Mono terpinoids	-	0.5% Rodamin B
Lipids test	0.92 0.7	I_2
Organic compounds test	0.92 0.81 0.74 0.37 0.07	0.2% aqueous $KmnO_4$
Mono or di terpenoids test	-	Perchloric acid
Steroids test	0.92 0.81 0.74 0.37 0.07	0.5% Phospho molybidic
Terpenoids test	-	$SbCl_2$ Conc. HCl
Saponis test	0.92	UV366

The above available results were confirmed by the IR spectrum, Fig. (2) and Table (3) indicate that the appeared broad peak a (3300 cm^{-1}) related to the vibrational stretching for (-OH) group and the band at (1090 cm^{-1}) for stretching vibration of (-C-O-C) group which indicate the presence of sugar compounds as glycosides. The band at (1650 cm^{-1}) is related to the vibrational stretching for (C = C) bond in alkenes which indicate the presence of conjugated double bond system.

The bands at (2910, 2810 cm^{-1}) are related to the vibrational asymmetrical and symmetrical successively for (C-H) bond in CH_2 and CH_3 groups which indicate the presence of aliphatic compound. The bond at (1710 cm^{-1}) is related to the vibrational stretching for (C = O) group which indicate the presence of ketone compound [10,5].

Table (3): The locations of important bands of IR spectra for monoterpene extract of Loranthus europaeos seeds.

Frequency intensity (cm^{-1})	Group or Class	Assignment of Remark
3300 (br)	OH	OH stretching
2910	CH3 or CH2 in aliphatic compound	CH stretching, Asymmetrical
2810		CH stretching, symmetrical
1710 (sh,s)	C = O	C = O stretching
1650 (sh,s)	C = C	C = C stretching
1090 (w)	C-O-C	C-O-C stretching

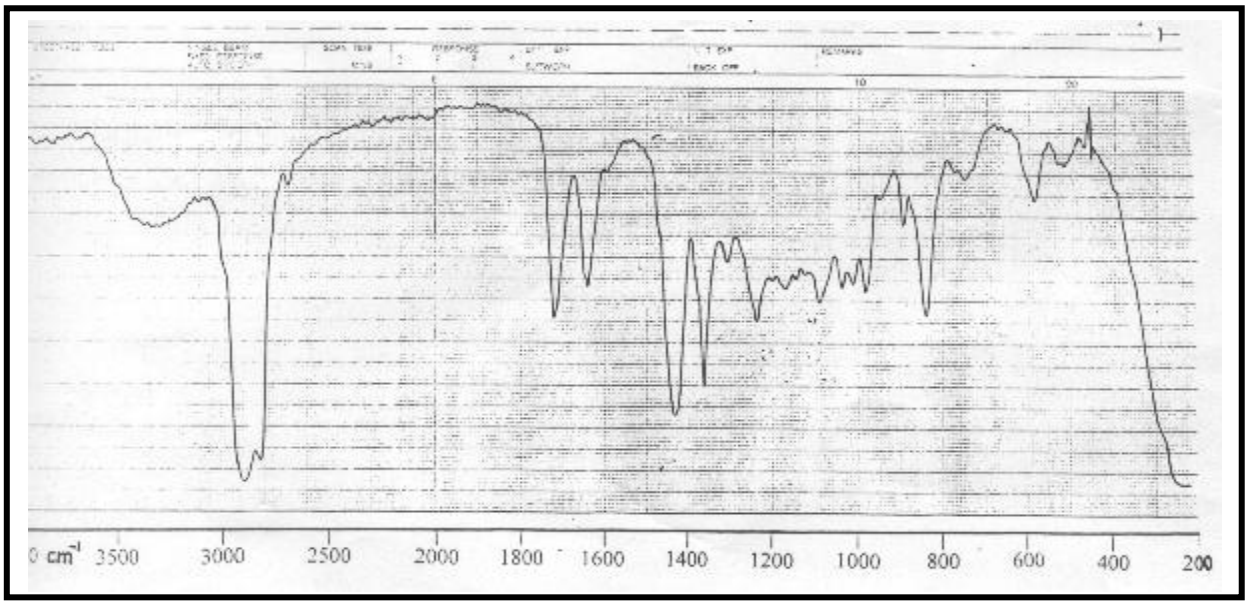


Fig (2): IR spectrum for monoterpene extract

The obtained data are confirmed by the UV-visible spectrum results Fig (3) which show three peaks. The peak at wave length (340nm) is related to the electronic transition for the double bond system such as (C =

O, C = S and N = O) groups. takes place due to the presence of non-cooperative electrons on O, S, N atoms. The other two peaks can be related to the conjugated double bond system with (C = O) group. In system like this two transition were observed: the first at high wavelength (660nm) which is related to transition and the second at low wavelength (400nm) which is relates to transition. In Fig (3), we noticed that the absorbance is displaced toward high wavelength. The reason behind this is that the monoterpene extract of *Loranthus europaeus seeds* has a yellow colour due to the conjugated double bond system. The conjugated double bond systems creates an intermixing in the molecular orbitals and this produces now orbitals. The new orbitals are very near to each other and this causes shift in absorbance.

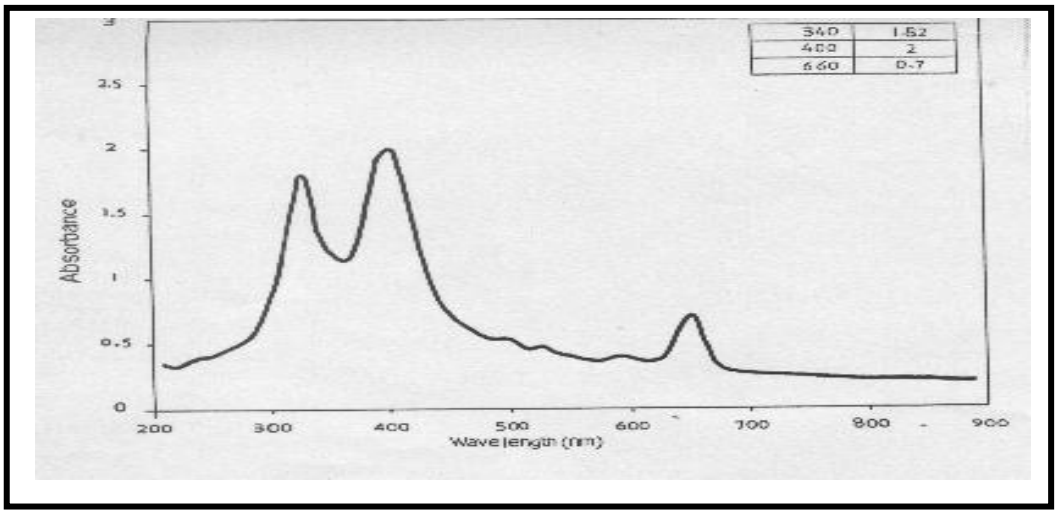


Fig (3): UV/Visible spectrum of monoterpene extract

3-2: Antioxidative activity properties of Monoterpene.

The most commonly used method for determination antioxidative activity is to measure the inhibitory degree of autoxidation of linoleic acid, but the analysis takes 5-6 days [6].Instead, we employed a modified rapid photometric assay to evaluate the antioxidant activity of monoterpene extract [7]. As shown Fig. (4), the antioxidant activity are dosedependent and reached a plateu when the concentration of monoterpene extract exceeded 2.0mg/ml.

The antioxidant activity may have a reciprocal correlation with their reducing power. [8].The reducing power of monoterpene extract was shown in Fig. (5). The reducing increased as the extract concentration increased, indicating some compounds in monoterpene were both electron donors and could react with free redicals to convert them into more stable products ant

to terminate redical chain reactions. The concentrations to attain one absorbance unit at 700nm were 0.2mg/ml for BHT and 0.4mg/ml for the monoterpene extract. This result indicated that the reducing power muchless than BHT

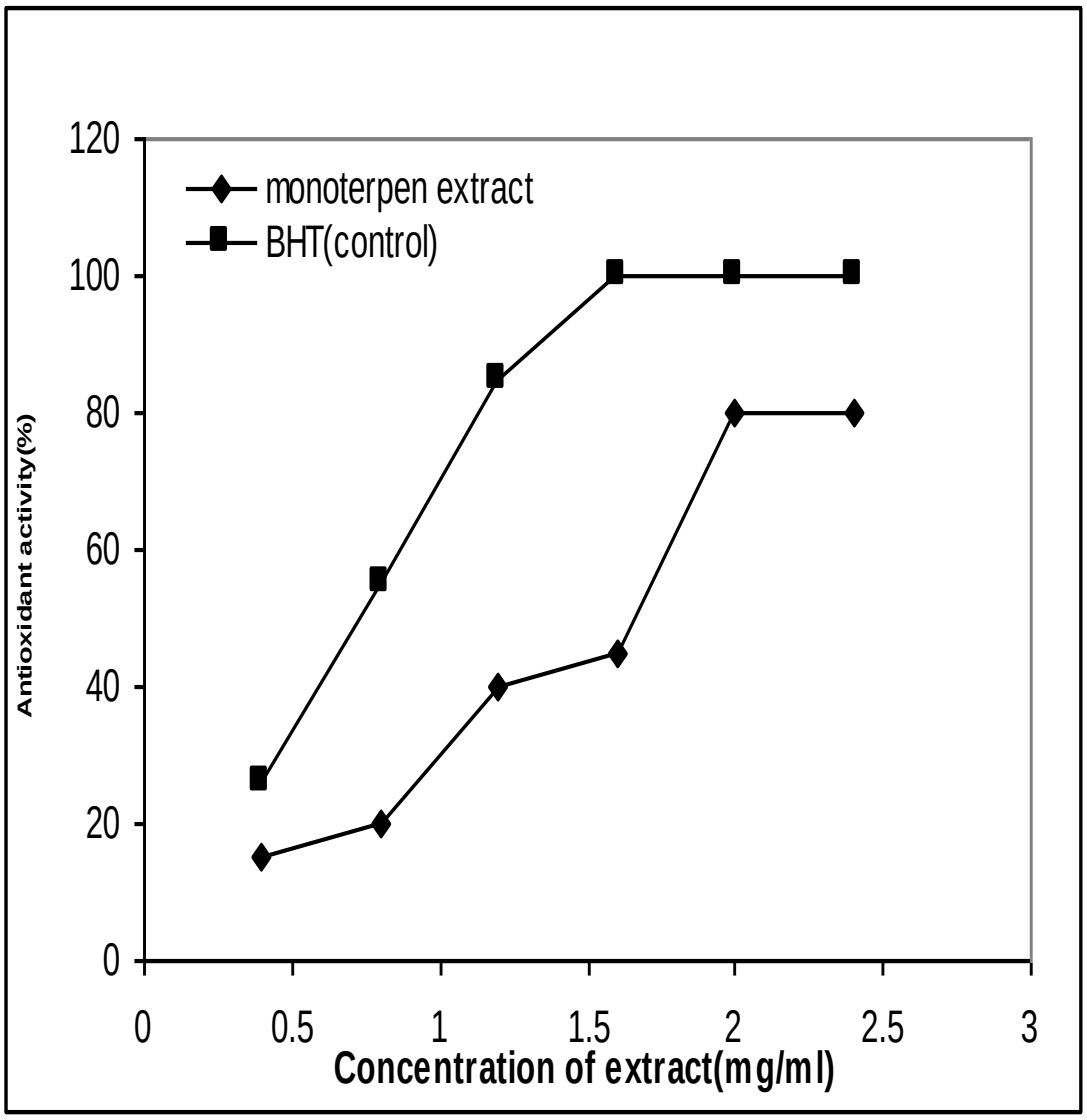


Fig.(4):Antioxidant activity of monoterpene extract

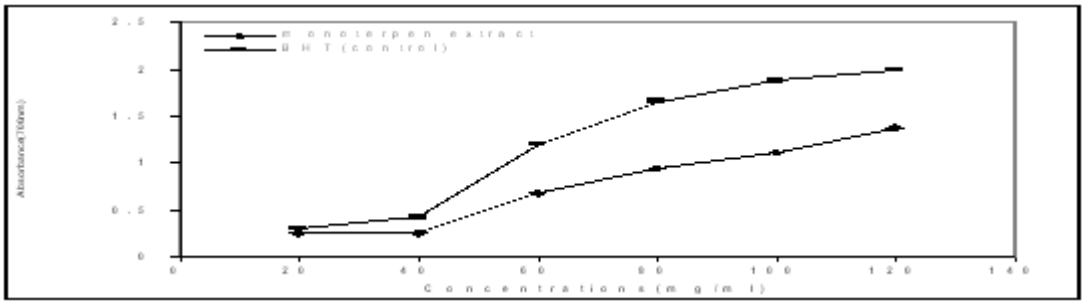


Fig.(5):Reducing power of monoterpen extract

The further tested with ferrous ion chelating activity of monoterpene extract. We found that extract possessed noticeable chelating activity of ferrous ion. (Fig.6). Changes in peroxide values of corn oil during the storage at 45C^{o} are shown in Fig. (7). The increases of peroxide values were delayed proportionally to the amount of the extract added, suggesting that extract also retard the oxidation of corn oil. However, their antioxidative effect was inferior to BHT, which completely inhibited the oxidation of corn oil during the storage at 45 ^{o}C. The objective of this study was to obtain information on the antioxidant potentials of monoterpene extract. A modified linoleic acid peroxidation was first performed to evaluate the potential of antioxidative activity of the extract. The degree of peroxidation was measured by ferric thiocyanate assay, which is based on the complex of ferric ion with thiocyanate and xylenol orange. It has been shown to be an easy, rapid and sensitive method of measuring peroxides in lipids. [9].The antioxidative activity expontially increased as a function of the development of the reducing power, suggesting that the antioxidative properties can be associated with the development of the reducing power [11]. Therefor, the antioxidative activity of monoterpene extract may be related to it reducing

power. in conclusion, reducing power ferrous ion chelating ability may account for antioxidant ability of monoterpene extract.

The results indicated that extract can work to some extent as peroxides destroyers which react with hydroperoxides to give stable products by nonradical processes besides antioxidant breaker which interfere with the free radical chain reaction [7].

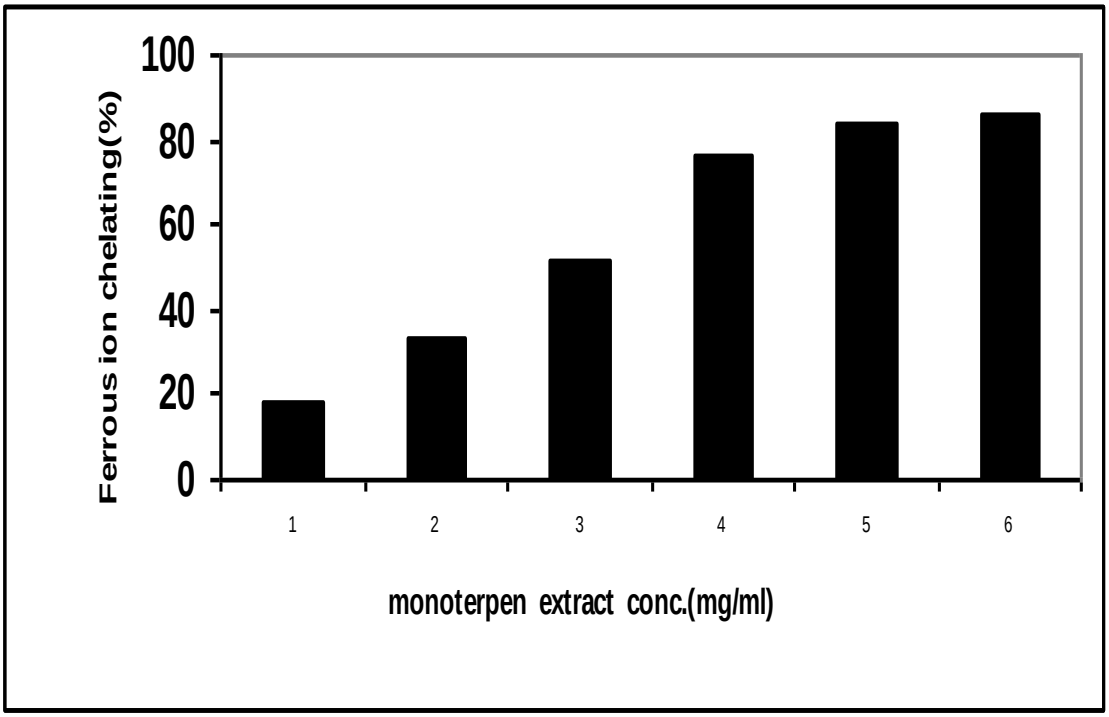
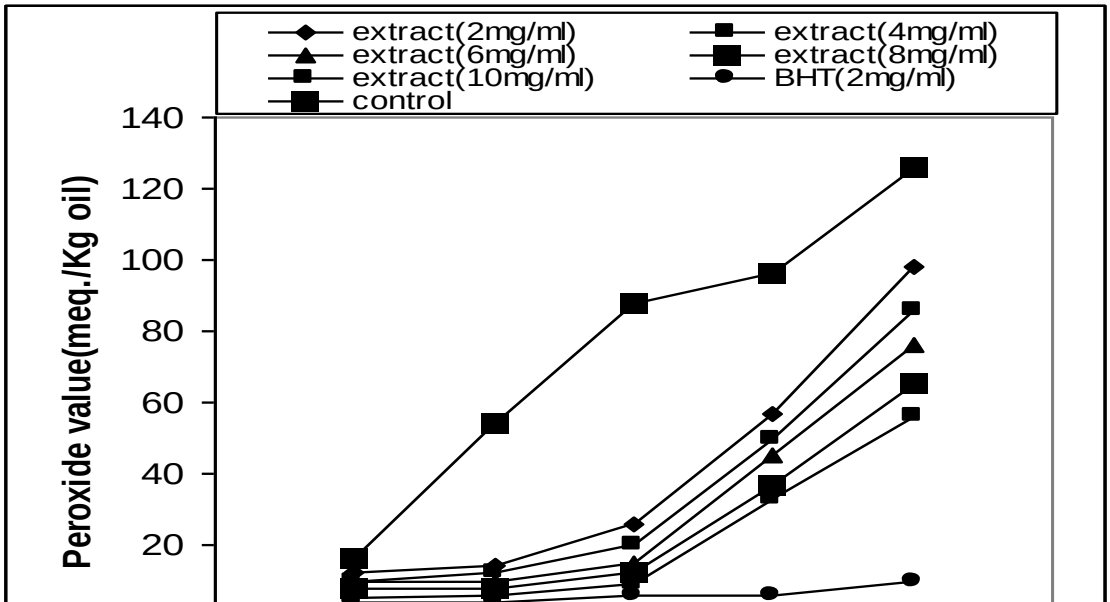


Fig.(6):Ferrous ion chelating effect of monoterpen extract



References

1- Atoui, A. K.; Mansouri, A.; Boskou, G. and Kefalos, P. (2005). Tea and herbal infusions: Their antioxidative activity and phenolic profile. Food chemistry, 89: 27-34.

2- Gibbons, A. and Gray, I. A. (1998). "Isolation by planer chromatography". In: Natural product isolation (Cannel, P.J.R.) Human press, New Jersy. 7, 209.

3- Harborne, J. B. (1984). Phytochemical methods. Champman and Hill , New York, U. S. A. 2th ed.

4- Huang, D. J.; Lin, C. D.; Chen, H. J. and Lin, Y. H. (2004). Antioxidative and antiproliferative activities of sweet potato constituent. Bot. Bull. Acad. Sin. 45: 179-186.

5- Lambert, J. B.; Shurvel, H. F.; Lightner, D. A. and Cooks, R. G. (1987). Introduction to organic spectroscopy. Macmillan pub. Comp., New York, U.S.A.

6- Mitsuda, H.; Yuasumoto, K. and Iwami, K. (1996). Antioxidative action of indole compounds during the autooxidation of linoleic acid Food chemistry, 80: 210-214.

7- Moreno, C. S.; Larrauri, J. A. and Calixto, F. S. (1999). Free radical scavenging capacity and inhibition of lipid oxidation. Food Res. Int., 32: 407-412.

8- Romero, A. M.; Doval, M. M.; Sturla, M. A. and Judis, M. A. (2004). Antioxidant properties of polyphenol-containing extract from soybean fermented with *Saccharomyccs cerevisiae*. Eur. J. Lipid Sci. Technol. 106: 424-431.

9- Shantha, N. C. and Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J. AOAC Int. 77: 421-424.

10- Silverstein, R.; Bassler, G. and Moril, T. (1981). Spectrometric identification of organic compounds. John wiley and sons, U.S.A., 4th ed.

11- Tanaka, M.; Sugita, S.; Nagashima, Y. and Taguchi, T. (1990). Influence of water activity on the development of antioxidative effect during the Maillard reaction between histidine and glucose. Nippon suisan Gakkaishi 56: 525-530.

12- Wang, L.; Yen, J.; Liang, H. and Wu, M. (2003). Antioxidant effect of methanol extracts from lotus plumule and Blossom. J. Food and Drug analysis. 11: 60-66.
13- Yakeda, Y.; Fukumoto, K.; Tchibana, M.; Shingu, Y.; Fugita, T. and Ichihara, T. (1990). Monoterpene glycosides having a cross conjugated dienone system from spiraea cantoniensis. Phytochem. 29: 1591.
14- Yen, G. C. and Chen, H. Y. (1995). Antioxidative activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food chem. 46: 844-854.

مجلة البصرة للعلوم الزراعية المجلد 20 العدد 1, 2007

التأثير المضاد للأكسدة للتربينات الأحادية المعزولة من بذور نبات الدبق *Loranthus europaeus L. seeds*

عدنان جاسم محمد الفرطوسي — ***قسم الكيمياء – كلية العلوم***

علي خضير جابر الركابي — ***قسم علوم الأغذية والتقانات الاحيائية – كلية الزراعة***

جامعـــــة البصــرة – البصــرة – العراق

الخلاصة

أستخلص زيت بذور نبات حب الدبق *Loranthus europaeus L. seeds* باستخدام مذيب الهكسان وشخصت هويته الكيميائية باستخدام تحاليل كيميائية نوعية بعدة تقنيات منها : كروموتوكرافيا الطبقة الرقيقة ، مطيافية الأشعة تحت الحمراء ومطيافية الأشعة فوق البنفسجية . أظهرت النتائج احتواء الزيت على خمسة مركبات كيميائية عائدة إلى عائلة التربينات الأحادية وان جميعها تمتلك مركبات سكرية بشكل كلوكوسيدات كما تم دراسة الفعالية المضادة للأكسدة لمستخلص التربينات الأحادية المعزولة من بذور نبات حب الدبق باستخدام اختبارات متعددة ، إذ أظهر المستخلص فعالية مضادة لأكسدة حامض اللينوليك في النظام النموذجي. و امتلك قابلية اختزال قوية وقدرة لاقتناص أيون الحديدوز وكذلك اظهر المستخلص فعالية جيدة لأعاقة أكسدة زيت الذرة.