Journal

J Biol Chem Environ Sci, 2007, Vol. 2(1): 233-262 www.acepsag.org

BIOCHEMICAL STUDIES OF SOME NATURAL ANTIOXIDANT COMPOUNDS AS FREE RADICAL SCAVENGERS *In-vitro*

Osman, M.E.A.*; Mustafa, A.A.*; Abedel Gawad, S.M. and Nermien, Z.A.****

** Biochemistry dept., Fac. of Agric., Cairo Univ., Egypt.*
*** Molecular Drug Evaluation Dept., National Organization for Drug Control & Research*

ABSTRACT

The antioxidant activity of different plant extracts (Parsley, Turmeric, Eucalyptus, Cumin, Basil, Olive, Lemon balm, Sage, Wild chicory, Rosemary, Lemon) were studied using *in vitro* and *in vivo* models. The ethanolic extracts of these plants were examined by DPPH "1,1- diphenyl-2-picryl hydrazyl method for evaluation the antioxidant activity of the different extracts using rutin and L-ascorbic acid as standard antioxidant agents. Also, the peroxide number and the acid value were determined for all plant ethanolic extracts to lookfore the antioxidant activity of each plant extract. *In vivo* experiment for assessment the antioxidant effect for each plant extract on Fe^{++}/Ascorbate model system by using (TBARS) thiobarbituric acid reactive substances method in rat liver mitochondria was performed.

The results revealed that the values of DPPH experiment were found to be less or comparable to those of ascorbic acid and rutin, the standard agents. The peroxide number increased daily up to 20 days compared by *butylated hydroxyl toluene (BHT)* as standard. *Petroselinum sativum* extracts by three concentrations "0.02, 0.04, 0.06 g/100g oil" exerted a near values as BHT "46.6, 45.97, 42.8" respectively. Also, *Saliva and Curcuma* showed a near values "43.27, 44.88" Meq./kg oil after 20 days of the experiment. The other extracts reached high values after 2 weeks. The acid values were increased in all different plant extracts according to the time intervals.

The experiment of TBARS of each extract with rat liver mitochondria exerts a significant antioxidant nature with different

activity, the highest one was *Petroselinum sativum* followed by *Saliva* extracts.

Key words: Antioxidants – Mitochondria - Rat liver - Free radical – Parsley – Turmeric – Eucalyptus – Cumin – Basil – Olive - Lemon balm – Sage - Wild chicory – Rosemary - Citrus Lemon – Hepatyoxicity - Free radical scavenging.

INTRODUCTION

Humans are subjected to various foreign chemicals such as: drugs, synthetic food additives, pollutants and synthetic drugs which have severe side effects especially with those tested for the dangerous diseases such as cancer. In fact, free radical production by such these compounds appears to be the most important mechanism causes pathological damage. Therefore, it is useful to return to the natural products and medicinal plants to overcome the side effects of these foreign materials (Bremness, 1994).

Curcumin is a naturally occurring phenolic compound isolated as a yellow pigment from turmeric (Buescher and Yang, 2000). This compound has been reported to possess a variety of biological and pharmacological activities, including antioxidative (Rukumani *et al.*, 2003), and anti-inflammatory (Chainani-Wu, 2003) activity.

Melissa officinalis L. (Lemon balm) is a member of the labiatae family. Phytochemical screening of this plant revealed the presence of flavonoids, terpenes and triterpene acids (Herodez *et al.*, 2003), essential oil (Sarer and Kokdil, 1991), and phenolic substances and tannin (Hohmann *et al.*, 1999). In phytotherapy, the plant is used as antioxidant (Ribeiro *et al.*, 2001).

Among plants that largely used are several species of saliva, particularly saliva libanotica belonging to the mint family Lamiaceae (Labiatae). It has been proven to have antioxidant activity (Dapkericius *et al.*, 1998) and anti-inflammatory properties (Schilcher, 1985), which could partly explain the reasons why this plant is so beneficial in the treatment of many human diseases. Interestingly, the quality and quantity of the essential oil extracted from S. libanotica, is vary greatly with the part of the plant used (Bellomaria *et al.*, 1992).

One of the principle causes of food quality deterioration is the oxidation of unsaturated lipids initiated by free radicals. When lipids are exposed to environmental factors such as: air, light and

temperature, oxidation reactions start to produce undesirable flavous, rancid odors, discoloration and other forms of spoilage. The primary autoxidation products are hydro peroxides, that have no taste and flavour, but their degradation products (aldehydes, ketones.....) are very potent taste and flavour modifiers (Gordon, 1991).

Administration of antioxidants greatly reduces or eliminates the biochemical and pathological changes brought about by lipid peroxidation (Horton and Fairhurst, 1987). A commonly used model procedure for studying non-enzymatic lipid peroxidation involves the use of mitochondria as lipid substrate (Tappel, 1973) and Fe^{++}/ Ascorbate as peroxidation inducers (Nagababu and Lakshmaiah, 1992).

Mitochondria are good models for peroxidation studies because the steady respiratory chain need mitochondria to exchange oxygen continuously, part of this oxygen give rise to reactive oxygen species (ROS) which can produce some damages including enzymes inactivation, DNA breakage and membrane destruction (Dalle-Donne *et al.*, 2003).

The search for safe and effective naturally occurring antioxidants is now focused on edible, especially spices and herbs (Nakatani, 1997). A large number of plants have been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for maintenance of health and protection from coronary heart diseases and cancer (Castenmiller *et al.*, 2002; Javanmardi *et al.*, 2003; Kaur and Kapoor, 2001). The evaluation of processing factors influencing the antioxidant activity is imperative to increase or preserve their efficacy and bio availability (Arabshahi *et al.*, 2007).

Prospective drugs for the treatment of chronic liver diseases should act through one or more of the following mechanisms: stabilization of the membrane of hepatocytes, protection against oxygen stress via the free radical scavenging effect and support of immunological defense of the organism (Feher *et al.*, 1987, and Xu *et al.*, 2003).

Recently, there is a considerable interest in the development of natural antioxidants of hepatoprotective agents from plant materials. Natural compounds with antioxidant properties can scavenge the free radical which damage lipid, protein, cell membrane and DNA (Starvic, 1994). Specific herbal medicine displayed a strong free

radical scavenging activity in the diphenylpicryl hydrazyl (DPPH) radical decolouration assay, also an investigation into their relative ability to scavenge the free radical DPPH (Feresin *et al.*, 2002).

Flavonoids demonstrated a wide range of biochemical and pharmacological effects including anti-oxidation, anti-inflammation, anti-platelet, anti-thrombatic, and anti allergic effects (Havsteen, 1983; Gryglewski *et al.*, 1987; Middleton and Kandaswami, 1992, and Cooks and Samman, 1996). Plant containing flavonoids and phenolics are known to possess strong antioxidant properties (Tripathi *et al.*, 1996).

The aim of this work is a trial to obtain a natural potentially active substances with cytotoxic and hepatoprotective activity from the Egyptian plant flora with more safe for mammals and human. As well as, to assessment the antioxidant power and activity of different herbal medicine to lookfore the highest activity of the antioxidant potent plant *in- vitro* and *in- vivo*.

MATERIALS & METHODS

2.1. Chemicals

All solvents used throughout the present work were obtained from different companies. L-ascorbic acid, Rutin and 2-Thiobarbituric acid were purchased from Sigma Chemical Co., St. Louis, USA. All other chemicals were obtained in the analytical and purified grade.

2.2. Oil:

Sunflower seed oil: Refined, bleached, and decolorized oil *(without antioxidant additives)* was obtained from Cairo oil and soap company, El-Badrashin, Giza, Egypt.

2.3. Plant Material

Aerial parts of Lemon balm and sweat basil; leaves of parsley, eucalyptus, rosemary, common sage, wild chicory and olives; seeds of cumin; fruits of lemon and rhizomes of curcuma (turmeric) were used and separated by ethanol 70% (Fig.3)

2.4. Natural antioxidant compounds

Table (1) illustrates some natural compounds in relation to their occurrence in particular families as given by Thomas (2000).

Table (1): Illustrates some natural compounds in relation to their occurrence in particular families as given by Thomas (2000)

No	Latin name	Family	English name	Arabic name	Part used	Source
1	Petroselinum sativum	Apiaceae	Parsley	البقدونس	leaves	Market
2	Cuminum cyminum	Apiaceae	Cumin	الكمون	Seeds	Attar
3	Curcuma longa	Zingibraceae	turmeric	الكركم	Rhizomes	Attar
4	Cichorium endivia	Asteraceae	Wild chicory	الشيكوريا	leaves	ES of NODCAR
5	Eucalyptus globules	Myrtaceae	Eucalyptus	كافور	Leaves	ES of NODCAR
6	Melissa officinalis	Labiatae	Lemon balm	الميلسيا	Aerial parts	ES of NODCAR
7	Rosmarinus officinalis	Labiatae	Rosemary	حصالبان	Leaves	ES OF NODCAR
8	Saliva officinalis	Labiatae	Common sage	المرمرية	Aerial parts	ESMP
9	Ocimum basilicum	Labiatae	Sweat basil	الريحان	Aerial parts	ES OF NODCAR
10	Olea europea	Oleaceae	Olives	الزيتون	Leaves, fruits	ESFA
11	Citrus aurantifolia	Rutaceae	Lemon	الليمون	fruits	Market

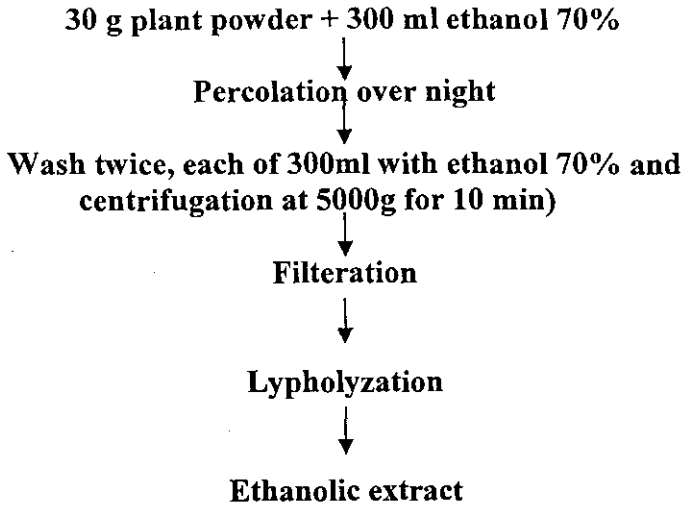
ESMP: Experimental station of Medicinal plants, Fac. Of Pharmacy, Cairo University.
ESFA : Experimental station of Fac. Of Agriculture, Cairo University.
ES of NADCAR: Experimental station of National Organization for Drug control & Research, Kafer El-Gable.

2.5. Preparation of samples

All collected plant samples were cleaned, air dried in shade, and then grinded to fine powder before extraction. Such powdered samples were kept in dark glass bottles ready for further investigations.

2.5.1. Ethanol extracts (Et1):

The air dried materials (10g) were extracted three times successively using 70% ethanol at the rate of 3 x 10 ml per gram of plant materials. The supernatant liquid was decanted, centrifuged at 5000 g for 10 min, and then filtered. The collected supernatants were lyophilized to give a final weight of 2.5% of the initial amounts for dried powder of ethanol extract (Lee *et al.*, 2002).



2.6. Determination of some Chemical properties:

a- Acid value:

Acid value was determined as (Oleic acid %) according to the method described in A.O.C.S (2000).

b- Peroxide value:

The peroxide value was determined as (Meq. O_2/Kg oil), according to the method described in A.O.C.S (2000).

Each extract was added to sunflower oil in the following quantities: 0.02 g/100 g oil of "BHT" as reference standard; petroselinum 0.02, 0.04, 0.06 g/100 g oil; 0.02 g/100 g oil of saliva; 0.02 g/10g oil of curcumin; 0.02 g/100 g oil of eucalyptus; 0.02 g/100 g

oil of Olea and 0.02 g/100 g oil of citrus. For the control, the sample without addition of antioxidant was used.

After careful mixing, the samples were exposed to sun light for 20 days. Three replicate samples were stored, oxidative stability was determined by measuring peroxide and acid values every 24 hrs.

2.7. Determination of the peroxide number and acid values at the presence of each plant extract

* Five kg of sunflower seeds was purchased, the oil was extracted, refined, bleached, and decolorized (without any addition of antioxidant) to study the effect of the natural plant extracts on it according to the plant with higher antioxidant scavenge activity.
* The acidity as oleic acid and the peroxide value as (Meq. O_2/kg oil) was determined.
* The ethanol extracts of the plant powder after percolation was passed through different processes: washing, centrifugation, filtration and Lypholization
* The plants which used according to their antioxidant activity are:
  - Petroselinum sativum
  - Saliva officinalis
  - Curcuma longa
  - Eucalyptus globulus
  - Citrus aeurantifolia
  - Olea europea
* At the beginning of the experiment the Acid value and the peroxide value were determined in the sunflower oil without the plant extracts (Zero time) at 200 ppm/kg oil (A.O.C.S, 2000).
* Add 0.02 g of plant extract to 100 g oil for each plant then exposed these plant extracts to heat and light daily for one month.
* Determination the acid and the peroxide values daily for each mixture "oil with extract".

The induction period was considered as the number of days needed for the peroxide value of the sample to become 20 Meq O_2/kg of fat (Economou *et al.,* 1991). This is in agreement with a general consideration that oils become rancid at peroxide values higher than 20.

2.8. Free radical scavenging assay:

The free radical scavenging effect of the eleven plant extracts were assessed by the decolouration of a methanolic solution of DPPH (Diphenylpicryl hydrazyl) according to Viturro *et al.* (1999), Astudillo

et al. (2000), Feresin *et al.* (2002) and (Lee *et al.*, 2002). The quenching of free radicals by extracts was evaluated spectrophotomertically at 517 nm against the absorbance of the DPPH radical.

A freshly prepared DPPH solution (20 mg/l) was used for the assay. Samples were dissolved in methanol and the methanolic DPPH served as a control. The degree of decolouration indicates the free radical scavenging efficiency of the substances. Rutin and vitamin C was used as a reference free radical scavenger. The percentage of DPPH decolouration was calculated as follows:

$$\textbf{DPPH decolouration} = 100 \times (A_1 - A_2 / A_2)$$

A1: was the absorbance of the control

A2: was the absorbance in the presence of the tested extract

Extracts were assessed at (100, 50, 25, and 15 µg/ml). All reactions were carried out in triplicate treatments.

2.9. Bioassay In-vitro hepatoprotective assay

2.9.1. Preparation of mitochondria from rat liver for lipid peroxidation assay:

Mitochondria preparation was performed by using the methods of (Ham and Liebler, 1995; Hanna *et al.*, 1994 and Yen and Hsieh, 1998) (Fig.1):

Experimental animals

Twenty male albino rats weighing about 150-180g were used for *in-vitro* study. The rats were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt.

Reagents

The following reagents were used in the preparation of mitochondria from rat liver:

1) 0.25 M sucrose buffer (pH 7.4). It was prepared by dissolving 85.6 g of sucrose, 0.474 g of Tris-Hcl and 0.038 g of EDTA in distilled water, the pH was adjusted to 7.4. The final volume was completed to 500 ml using distilled water.
2) Krebs-Ringer phosphate buffer (pH 7.4). This buffer was prepared by dissolving 7.131g of NaCl, 0.303 g of $MgSO_4.7H_2O$, 0.363 g of KCl and 14.93 g of $Na_2HPO_4.12H_2O$ in 500 ml of distilled water and the pH was adjusted to 7.4. The final volume was completed to one liter.

Procedure

Rats were killed by decapitation after fasting for 24h and their liver tissues were quickly removed. The liver tissues were cut into small pieces in 0.25 M sucrose buffer (pH 7.4) at 4°C and then homogenized with nine volume of sucrose buffer using Teflon homogenizer (CAT R18).

The protein in the isolated mitochondria was measured by the method of Lowry *et al.* (1951).

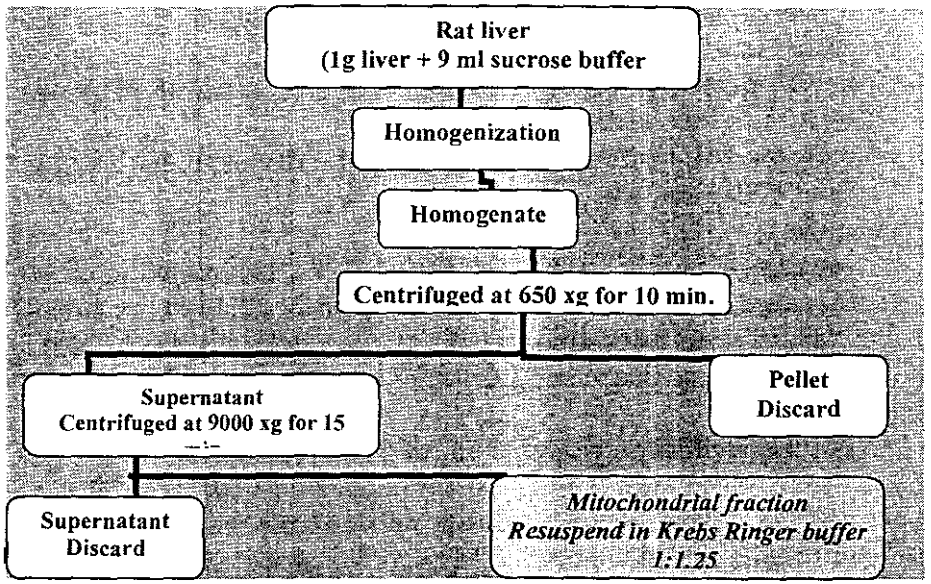


Fig. (1): Schematic outline of the preparation of mitochondrial fractions.

2.9.2. Measurement of lipid peroxidation in mitochondria:

Lipid peroxide formation was determined as thiobarbituric acid reactive substances (TBARS). It was determined according to the method Fe^{++}/Ascorbate induce lipid peroxidation of Nagababu and Lakshmaiah (1992) for mitochondria.

Principle

The principle of the assay depends on a colourimetric determination of pink pigment products, resulting from the reaction of TBARS as malondialdehyde with thiobarbituric acid in an acidic medium, at high temperature. The chromogen was extracted with n-

butanol, and its absorbance was measured at 535 nm using Unicom spectrometer.

Reagents

1- HEPES buffer (pH 7.4) It was prepared by dissolving 14.17g of HEPES in 500 ml of distilled water and the pH was adjusted to 7.4. The final volume was completed to one liter using distilled water.
2- 10 μM $FeSO_4$. 0.00287g of $FeSO_4$ was dissolved in one liter of distilled water.
3- 0.2 mM ascorbate. It was prepared by dissolving 0.0352g of L-ascorbic acid in one liter of distilled water.
4- Thiobarbituric acid (TBA) solution (1%). One gram of TBA was dissolved in 100 ml of distilled water.
5- Trichloroacetic acid (TCA) 2.8%. 2.8g of TCA were dissolved in 100 ml of distilled water.

Procedure

A mixture of mitochondrial suspension (0.5 ml), containing 3.0 mg of protein, HEPES buffer (0.1 ml), $FeSO_4$ (0.1ml), ascorbate (0.1ml) and the indicated amounts of various plant extracts (0.25, 0.5 and 1.0 mg) was incubated at 37°C for 30 min in a final volume of 1.0 ml.

After the incubation period, 1 ml of TBA (1%) and 1ml of TCA (2.8%) were added, and then tubes were heated at 100°C for 30 min. After cooling, 2.5 ml of n-butanol was added to the reaction mixtures and centrifuged at 3500 r.p.m. for 15 min. The absorbance of the pink colour was measured at 535 nm using Unicam spectrophotometer. Rutin was used as a positive control.

Calculations

Mitochondrial lipid peroxidation was calculated using the following equation:

Inhibition % (I) = [(At-Ac)/Ac] x 100

Where: A_t: is the absorbance of the test extracts and A_c: is the absorbance of the control containing all reagents except plant extract under study.

RESULTS AND DISCUSSION

The extracts of different herbal medicine were assessed for their antioxidant activities by the following determinations:

I- Determination the free radical scavenging activity of the ethanolic extracts of these plants.

II- Assessment the antioxidant activity of some of these plants by using sunflower oil without additives "special antioxidants" by determining the peroxide and acid values.

III- In vitro study on the antioxidant activity of the plant extracts in the presence of rat liver mitochondrial fractions by Fe^{++}/Ascorbate induced lipid peroxidation.

I- Determination the free radical scavenging activity of the ethanolic plant extracts by DPPH method

The compiled data in Table (2), and Fig. (2) Revealed the DPPH decolouration reaction of different plant ethanolic extracts in different concentrations comparative against antioxidant standards "Rutin (Casa *et al.,* 2000) and L-Ascorbic acid". These ethanolic extracts exhibited a strongest antioxidant activity as evidence by their concentrations at "100 µg/ml". The values were found to be less or comparable to that standard rutin and less than L-Ascorbic acid standard (Badami *et al.,* 2003).

As indicated from the results that the high concentration of the plant extracts exerted a highly potent of antioxidant activity. As well as, the low concentrations of each plant extracts revealed a lowest response of antioxidant activity. *Eucalyptus globules and Rosmarinus officinalis* extracts showed a strong free radical scavenging effect in the DPPH decolouration assay; this activity may be due to the active ingredients of each extract such as flavonoids and phenolic compounds.

Miean and Suhaila (2001) found a different flavonoids such as: "Myriccetin, quercetin, kaempferol, luteolin, and apigenin" contents in edible tropical plants. The major flavonoid in these plant extracts was quercetin, followed by myricetin and kaempferol. In vegetables, quercetin glycosides predominate, but glycosides of kaempferol, luteolin and apigenin are also present. Fruits contain almost exclusively quercetin glycosides, whereas kaempferol and myricetin glycosides are found only in trace quantities.

Melissa officinalis L. extract given to the hyperlipidemic rats lowered the serum AST activity in a significant manner when compared to control rats ($p<0.0001$). Serum ALT and AST activities were significantly lowered when compared to untreated hyperlipidemic rats ($p<0.0001$). The effect of Melissa officinalis L. extract on rat liver glutathione and lipid peroxidation was investigated by (Bolkent *et al.*, 2005).

Table (2): Free radical scavenging activity of ethanol extracts of plants under investigation

Samples	[a] DPPH decolouration %			
	15 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)
1) Rutin hydrate (st.)	44.0	66.1	92.1	*93.0*
2) L-Ascorbic acid	70.0	98.5	96.2	*98.0*
3) Eucalyptus globules	17.9	36.7	62.5	*95.4*
4) Rosmarinus officinalis	12.0	28.6	61.2	*93.0*
5) Saliva officinalis	14.3	21.9	33.9	*63.3*
6) Melissa officinalis	4.3	12.0	30.6	*44.1*
7) Olea europea	5.2	12.0	16.3	*42.4*
8) Ocimum basilicum	8.9	19.6	24.5	*39.5*
9) Citrus aurantifolia	7.14	12.9	15.2	*28.3*
10) Petroselinum sativum	7.6	11.3	18.2	*27.1*
11) Curcuma longa	5.4	9.7	10.2	*25.0*
12) Cuminum cyminum	5.9	11.5	17.0	*21.7*
13) Cichorium intybus	3.4	7.6	10.5	*19.9*

[a] (%) was expressed as the absorbance of the sample with absorbance of the control.
DPPH: Diphenyl picryl hydrazyl radical

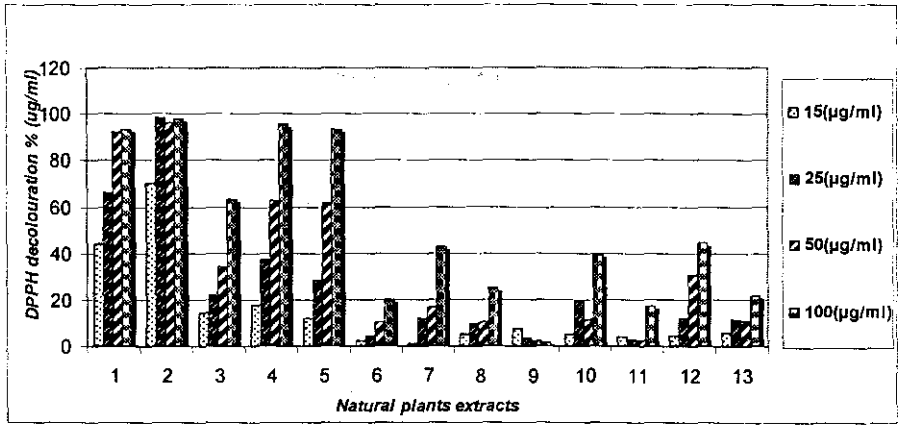


Fig (2): Free radical scavenging activity of the natural plant extracts

II- Assessment the effect of plant extracts in the mixture with sunflower oil by peroxide and acid values

Table (3) and Fig (3) illustrated the effect of each plant extracts on the peroxide value of sunflower oil "without antioxidant additives" during 20 days of incubation periods. The results showed that the peroxide value was enhanced by time intervals "time-dependent" as compared to *"BHT" butylated hydroxyl toluene* as standard. The highest values were observed up to 20 days for *Petroselinum Sativum* under the effect of the three concentrations "0.02, 0.04, 0.06 g/ 100g oil" by 46.6, 45.97, 42.82 Meq./kg oil, respectively. As well as, the extracts of *Saliva officinalis and Curcuma longa* exerted approximately a related values of peroxide numbers by 43.27 and 44.88 Meq./kg oil after 20 days (Selvam *et al.,* 2005).

The peroxide value of sunflower oil with single extract is presented in Fig. (5). *Petroselinum* by the three concentrations retarded the hydroperoxide formation significantly. Also, *Saliva and Curcuma* retarded the hydroperoxide formation significantly (Lugasi and Hovari, 2000).

The addition of these plant extracts lowered the final peroxide value after 20 days as by control sample. After 8 days, *eucalyptus, citrus, and olea* extracts showed a slight anti-oxidative effect toward the oxidative stability of sunflower oil. The determination of hydroperoxides is, in that phase, slowed by their decomposition into

Table (3): Determination the effect of the plant extracts in mixture with sunflower oil by peroxide value

Plant extracts / Duration periods	Oil only Meq./1.0g sample	Butylated Hydroxyl Toluene 0.02g/100g oil	Petroselinum Sativum 0.02g/100g oil	Petroselinum Sativum 0.04g/100g oil	Petroselinum Sativum 0.06g/ 100g oil	Saliva officinalis 0.02g / 100g oil	Curcuma longa 0.02g/ 100g oil	Eucalyptus Globuuules 0.02g/100g oil	Citrus Aurantifolia 0.02g/100g oil	Olea europea 0.02g/100g oil
1	*2.622 meq. / 1.0g sample without any addition of plant extracts*	2.74	2.00	2.39	4.10	4.75	2.49	6.99	3.69	2.79
2		2.81	2.20	2.46	4.21	4.81	2.52	7.07	3.74	2.83
3		3.25	3.78	4.6	6.40	5.90	5.30	10.20	4.99	5.20
4		4.01	4.90	5.99	8.50	7.89	8.40	12.60	6.50	9.30
5		4.46	5.95	6.84	9.33	8.39	9.97	14.21	7.46	11.06
6		6.70	7.60	10.50	12.5	11.40	12.90	18.39	14.40	15.30
7		10.7	14.0	14.3	15.6	14.0	16.3	20.3	22.0	18.90
8		11.8	16.01	17.61	17.73	16.43	18.17	27.09	22.25	24.28
9		13.2	18.3	19.1	17.90	17.90	18.90	29.0	24.0	25.0
10		15.4	20.0	21.3	19.20	19.40	19.40	31.3	28.3	26.9
11		17.0	22.7	23.6	21.3	21.0	21.3	40.6	30.5	29.0
12		19.1	24.6	23.6	24.2	23.7	22.0	45.7	37.2	30.6
13		21.9	26.3	25.2	26.9	25.5	24.8	50.8	39.9	35.9
14		23.28	28.63	26.35	28.07	26.25	25.84	57.50	44.75	40.29
15		26.20	30.6	28.0	30.02	28.0	26.4			
16		28.30	34.7	30.81	34.18	30.31	29.7			
17		30.90	37.0	33.17	36.51	36.41	32.7			
18		34.99	39.90	40.60	40.09	39.99	36.90			
19		38.99	46.59	45.97	42.82	43.27	44.88			

All values are mean of three replicates

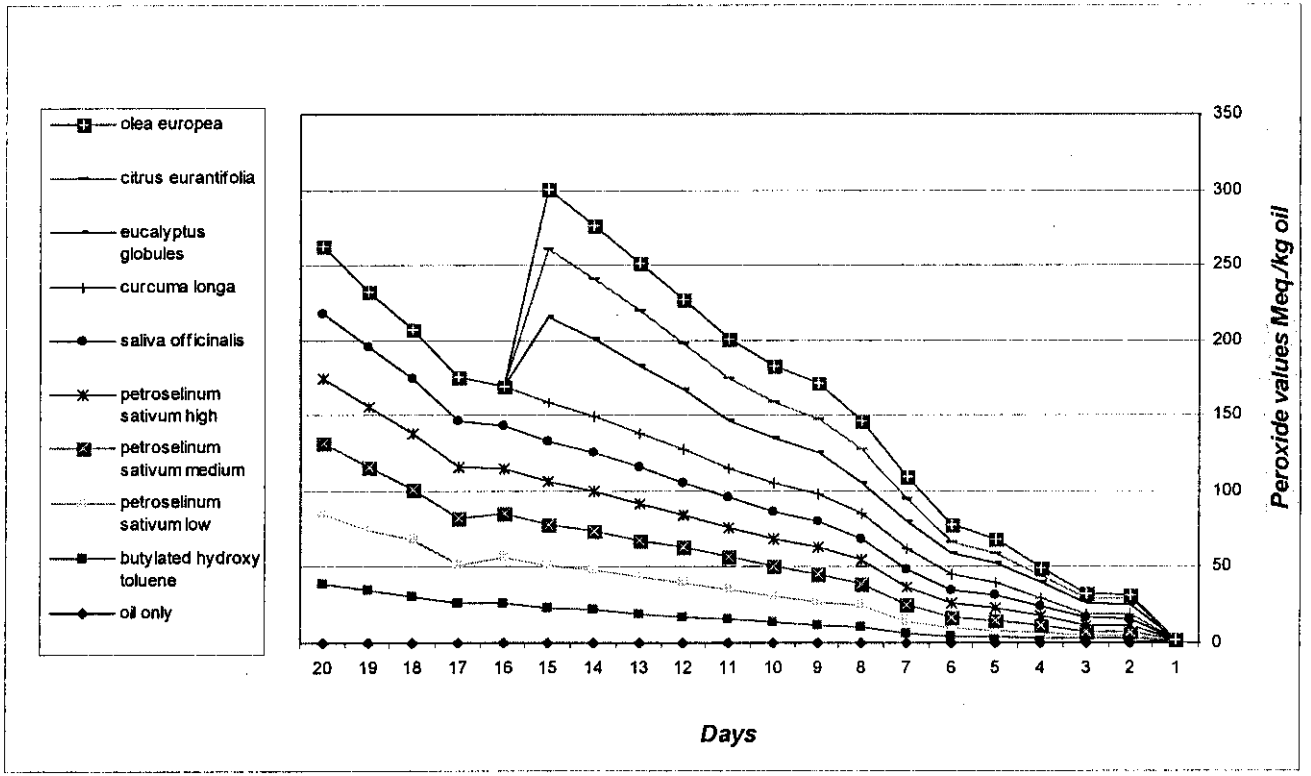


Fig (3): The peroxide values of the sunflower oil at the presence of each ethanolic extracts

secondary products. This is in agreement with the findings of (Hras *et al.,* 2000).

The plant extracts of *Eucalyptus, Citrus, and Olea* revealed a high peroxide values reached to 57.5, 44.75, and 40.29 Meq./kg oil after 15 days only. These results indicated that the first group of plant extracts which complete 20 days at the limit of peroxide values in the range 42.82 – 46.59 Meq./kg exerted a highly potent of antioxidant activity . It is appeared to little more values above the standard "BHT" compounds (38.99 Meq./kg). While the other group of plant extracts which stopped at the high values after 2 weeks only but not extend to 20 days exerted a less potent activity of the antioxidant power.

These results in agreement with (Wagner *et al.,* 1984, and Feresin *et al.,* 2002), they found that the occurrence of steroids triterpenoids and flavonoids are responsible for the antioxidant activity of the plant extract which are led to the identification of flavonoids and phenolics as active components of the ethanolic plant extracts.

Table (4) and Fig. (4) illustrated the acid value of sunflower oil with added the plant extracts of each material alone. As indicated from the data that the acid values were increased from the 1st day between 0.091–0.095 up to 15 days between 0.167–0.187 for the standard "BHT" and other extracts. On the day 14, the maximum acid value was observed for all the extracts, while after that, the values seems to be decreased in BHT to 0.175; *petroselinum* to 0.166 at a concentration of 00.04 g/100g oil and saliva at 0.02 g/100 g oil to 0.142. The other extracts exerted a higher value by 0.343. 0.350 at the end of experiment "20 days". Curcuma exerted an increase values, then followed by decreased values "0.167" at 20 days. *Eucalyptus, citrus, and olea* exerted the maximum acid values "0.185, 0.184, 0.184" after two weeks as compared from the standard "BHT" which have 0.183 Meq./1.0 g.

These results indicated that, the antioxidative extract may have a potency to decrease the acid hydrolysis such as saliva and petroselinum (Hras *et al.,* 2000, and Che Man and Tan, 1999).

Table (4): Determination the effect of the plant extracts in mixture with sunflower oil by Acid value

Plant extracts / Duration periods	Oil only Meq./1.0g sample	Butylated Hydroxyl Toluene 0.02g/100g oil	Petroselinum Sativum 0.02g/100g oil	Petroselinum Sativum 0.04g/100g oil	Petroselinum Sativum 0.06g/ 100g oil	Saliva officinalis 0.02g / 100g oil	Curcuma longa 0.02g/ 100g oil	Eucalyptus Globuuules 0.02g/100g oil	Citrus Aurantifolia 0.02g/100g oil	Olea europea 0.02g/100g oil
1	*0.306 mg KOH / 1.0g sample without any addition of plant extracts*	0.093	0.091	0.091	0.095	0.091	0.093	0.091	0.094	0.095
2		0.093	0.091	0.091	0.095	0.091	0.093	0.091	0.094	0.095
3		0.093	0.091	0.091	0.095	0.086	0.093	0.091	0.099	0.092
4		0.091	0.090	0.090	0.093	0.086	0.180	0.091	0.181	0.092
5		0.091	0.089	0.089	0.093	0.086	0.186	0.091	0.182	0.092
6		0.092	0.090	0.089	0.097	0.089	0.186	0.095	0.095	0.092
7		0.095	0.095	0.093	0.179	0.093	0.096	0.096	0.095	0.094
8		0.095	0.096	0.095	0.187	0.095	0.096	0.097	0.095	0.096
9		0.095	0.096	0.095	0.187	0.099	0.096	0.097	0.097	0.096
10		0.095	0.096	0.099	0.187	0.140	0.100	0.097	0.097	0.096
11		0.100	0.134	0.129	0.187	0.167	0.140	0.139	0.139	0.167
12		0.143	0.156	0.146	0.186	0.184	0.167	0.169	0.0169	0.179
13		0.167	0.179	0.187	0.186	0.184	0.187	0.184	0.184	0.182
14		0.183	0.182	0.187	0.186	0.184	0.264	0.185	0.184	0.184
15		0.181	0.223	0.182	0.227	0.173	0.239			
16		0.179	0.263	0.176	0.268	0.163	0.215			
17		0.177	0.303	0.171	0.309	0.153	0.191			
18		0.175	0.335	0.169	0.330	0.142	0.170			
19		0.175	0.343	0.166	0.350	0.142	0.167			

All values are mean of three replicates

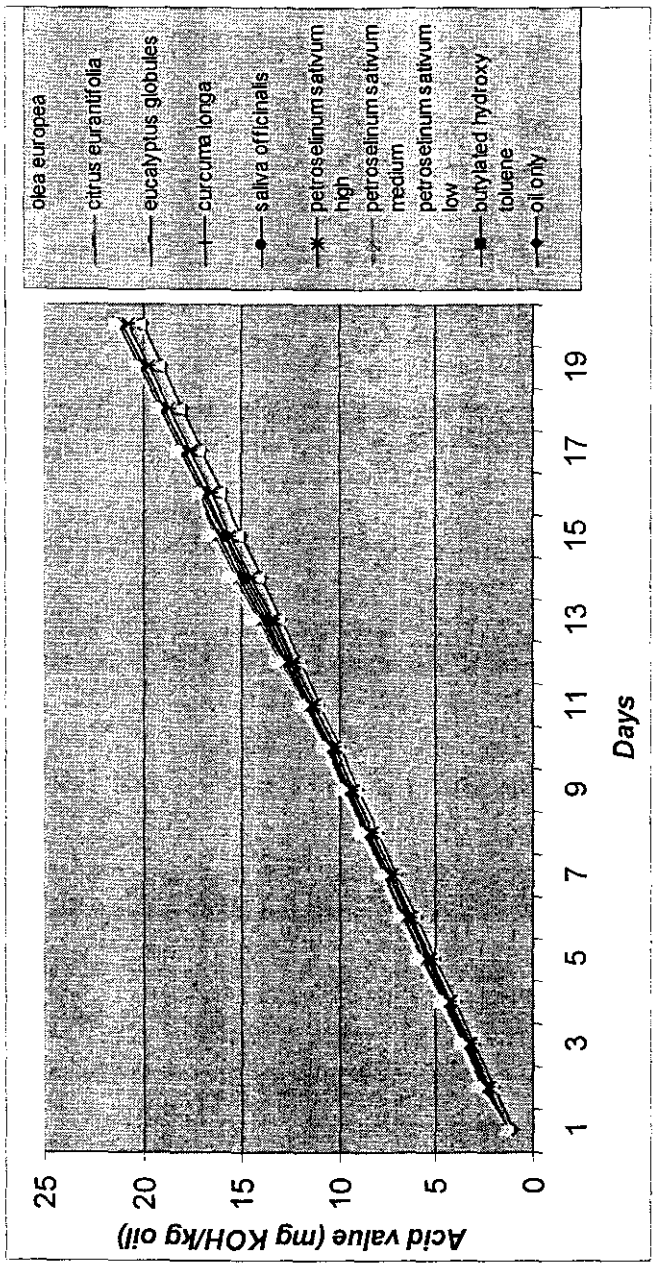


Fig (4): The acid values of the sunflower oil at the presence of each ethanolic extracts

III- In vitro study of Fe^{++}/Ascorbate to induce lipid peroxidation and measurement the formation of TBARS of rat liver mitochondria at the presence of plant extracts

Combination of Fe^{++} and a reducing reagent is an extensively used system for generating hydroxyl radicals to induce lipid peroxidation (Kamat and Delvasagayam, 1995). Table (5) shows the Fe^{++}/Ascorbate induced lipid peroxidation of rat liver mitochondria under an aerobic atmosphere. The concentrations of TBARS increased quickly by addition of Fe^{++} and ascorbate in dose dependant manner. It is evident from results that petroselinum, saliva, and curcuma inhibited Fe^{++}/Ascorbate - induced TBARS formation and the inhibition degree correlated dose dependently with the concentration of extracts "0.25 mg and 1.0 mg" (Wei *et al.*, 2006).

As revealed from the data, the high percentage of inhibition was to the high concentration of plant extract as compared with rutin as antioxidant standard. Administration of rutin decreased TBARS and lipid hydro peroxides and increased the non-enzymatic antioxidants significantly. The data shows that rutin exhibits antioxidant activity (Kamalakkannan and Prince, 2006).

Petroselinum, saliva and curcuma extracts exerted a highly inhibitory effect at the high concentration by 72.6% and 72.3%, and 72.1%, while the lower inhibitory effect was observed at ocimum, cichorium and cuminum by 46.1%, 46.1% and 44.7% respectively. It was investigated by Reddy and Lokesh (1994) that curcumin is a potent antioxidant, as it is able to scavenge ROS decrease lipid peroxidation and increase GSH content (Venkatesan *et al.*, 2000). These antioxidative effects of curcumin might be useful for the prevention or treatment of inflammatory processes (Strasser *et al.*, 2005).

Curcumin exhibits marked antioxidant and anti-inflammatory properties, thus curcumin scavenges active oxygen species including superoxide, hydroxyl radical and NO (Sreejayan and Rao, 1997). It's elevates the activities of detoxification enzymes of xenobiotic metabolism, such as glutathione transferase and NAD(P)H: quinine reductase (Arbiser *et al.*, 1998 , and Ukill *et al.*, 2003).

Oxidative stress plays a major role in the pathogenesis of various diseases including hypoxia and cancer. Curcumin exhibits strong antioxidant activity, comparable to vitamin C and E (Toda *et al.*, 1985). Curcumin with its proven anti-inflammatory and antioxidant

Table (5): Relative inhibitory effect of ethanol extract (E1) of plants under investigation on Fe^{++}/ascorbate model system induced lipid *peroxidation in vitro in rat liver mitochondria.*

Samples	*Relative change*		
	0.25 mg	1.0 mg	Activity number
Rutin hydrate (+ve)	63.6%	71.2%	St.
Saliva officinalis	40.2%	72.3%	2
Eucalyptus globules	52.0%	65.0%	4
Rosmarinus officinalis	44.9%	48.5%	8
Olea europea	42.6%	63.4%	6
Citrus auratifolia	15.1%	65.0%	5
Ocimum basilicum	46.8%	46.1%	9
Petroselinum sativum	59.1%	72.6%	1
Cuminum cyminum	77.3%	44.7%	11
Melissa officinalis	45.0%	59.1%	7
Curcuma longa	62.4%	72.1%	3
Cichorium intybus	52.0%	46.1%	10

Table (6): Comparison between Inhibition % of plant extract only and the inhibition in rat liver Mitochondria

Plant extract	Inhibition % of plant extract by DPPH	Plant extract	Inhibition % of plant extract in rat liver mitochondria
	High dose		High dose
Euclyptus globules	95.4%	Petroselinum sativum	72.6%
Rosmarinus officinalis	93.0%	Saliva officinalis	72.3%
Saliva officinalis	63.3%	Curcuma longa	72.1%
Melissa officinalis	44.1%	Euclyptus globules	65.0%
Olea europea	42.4%	Citrus auratifolia	65.0%
Ocimum basilicum	39.5%	Olea europea	63.4%
Citrus auratifolia	28.3%	Melissa officinalis	59.1%
Petroselinum sativum	27.1%	Rosmarinus officinalis	48.5%
Curcuma longa	25.0%	Ocimum basilicum	46.1%
Cuminum cyminum	21.7%	Cichorium intybus	46.1%
Cichorium intybus	19.9%	Cuminum cyminum	44.7%

properties has been shown to have several therapeutic advantages. It was shown to be a potent scavenger of a variety of reactive oxygen species including super oxide anion radicals and hydroxyl radicals (Reddy and Lokesh, 1994). It was also shown to inhibit lipid peroxidation in different animal models (Sreejayan and Rao, 1997). Curcumin protected oxidative cell injury of kidney cells by inhibiting lipid degradation, lipid peroxidation and cytolysis (Cohly et al., 1998).

The protective effect of curcumin was attributed to its antioxidant properties by inhibiting free radical generation (Manikandan *et al.*, 2004). It caused a decrease in the degree of degradation of the existing collagen matrix and collagen synthesis, these effects were attributed to free radical scavenging properties and inhibition of lysosomal enzyme release by curcumin (Nirmala *et al.*, 1999). Treatment with curcumin showed beneficial effects on renal injury by its ability to inhibit the expression of the apoptosis-related genes Fas and Fas-L (Jones *et al.*, 2000, and Thiyagarajan and Sharama, 2004).

These results indicated that the highly antioxidant contents have more potent of inhibitory effect. Cao *et al.* (1998) reported that consumption of controlled diets high in fruits and vegetables increased significantly the antioxidant capacity and this increase could not be explained by the increase in the α-tocopherol or carotenoid concentration in plasma. Thus, flavonoids represent an important source of antioxidant activity in the human diet (Hertog *et al.*, 1993).

It was found a variation in the level of the antioxidant compound, the concentrations of flavones and flavonols, like those of all secondary plant metabolites, vary within certain limits and are dependent on a number of factors such as, growing condition degree of ripeness, size of the fruit and variety (Crozier *et al.*, 1997). The antioxidant substances capable of settling on the membrane and counteracting lipid peroxide formation (De Groot *et al.*, 1996 and Halliwell, 1996)

Recently results show that among flavonoids there are strong scavengers of lipid radicals (Bombardelli and Morazzoni, 1993; Wolf *et al.*, 1994, and Catapeno, 1997). Rutin has been found to be an important antioxidant agent (Metodiewa *et al.*, 1997) and also has well established properties against lipid peroxidation (Bombardelli and Morazzoni, 1993).

The presented data in Table (6) elicited the comparative studies between the percentage inhibition of plant extracts by DPPH and the

inhibition in rat liver mitochondria. As revealed from the results that the extracts of *eucalyptus, rosmarinus, and saliva* exerted a highly significant inhibitory effect by DPPH reaction "95.4%, 93.0%, 63.3%", respectively. These plant extracts exerted an inhibitory effect in rat liver mitochondria by 65% for eucalyptus, 48.5% for *rosmarinus* and 72.3% for saliva. The lowest inhibitory effect by DPPH was observed to *cuminum* (21.7%), and *cichorium* (19.9%), while the inhibitory effect of these extracts was changed in rat liver mitochondria by 44.7% for *cuminum,* and 46.1% for *cichorium.* So, the inhibitory effect was altered in either DPPH or in rat liver mitochondria.

Lipid peroxidation also yields a wide range of cytocolic products most of which are aldehydes, as exemplified by MDA, which can be measured following the TBA method (Ohkawa *et al.,* 1979). In the present study, the inhibitory effect of the plant extracts caused a significant decrease in the level of TBARS when compared to control of rat liver mitochondria.

The alteration in the inhibitory effect between all the ethanolic plant extracts was dose-dependent and dependent on the active ingredients of antioxidant compounds in each extracts. Several flavonoids and phenolic oxygen heterocyclic is found to be the major constituent in some herbal medicine and found to possess antioxidant activity. The observed antioxidant activity of these plant extracts may be due to the presence of flavonoids and phenolic compounds present in them *(Tripathi et al.,* 1996 and Badami *et al.,* 2003).

CONCLUSION & RECOMMENDATION

The *in-vitro* antioxidant screening using DPPH method showed strong antioxidant activity of the extracts of Eucalyptus globules and *Rosmarinus officinalis* at the concentration of 100 μg/ml.

Fe^{++}/Ascorbate model system induced lipid peroxidation in rat liver mitochondria *in-vitro* at the presence of each plant extract revealed that the highly inhibitory effect was observed for the ethanolic extracts of *petroselinum sativum* by 72.6% followed by *saliva officinalis* (72.3%), *Cuminum cyminum* (44.7%), *cichorium intybus* (46.1%), and *ocimum basilicum* (46.1%). The antioxidant activity and the inhibitory effect of each plant extract were dose and time dependent.

In comparative studies between the inhibitory effect measured by DPPH and Fe^{++}/Ascorbate in mitochondria, the plant extract which have a more potent effect in each method did not exerted the same level of inhibitory effect in other technique. Eucalyptus extract exerted a high percentage (95.4%) of inhibitory effect by DPPH, it was not the high percentage by Fe^{++}/Ascorbate models "65% and so on.....

The peroxide value was increased depending on the antioxidant activity of each plant extract and time. The acid values were also enhanced by time increase up to 20 days.

Hence, the plant merits farther investigation for identifying antioxidant nature of its constituents.

REFERENCES

AOCS (2000): In: official methods and recommended practices of the American oil chemist's society method cd 8-53 and method cd 1890. (4^{th} ed.) Champaign: American Oil Chem. Soc.

Arabshahi, D.S.; Devi, D.V. and Urooj, A. (2007): Evaluation of antioxidant activity of some plant extracts and their heat, pH, and storage stability. Food Chem. 100:1100-1105.

Arbiser, J.L.; Klauber, N.; Rohan, R.; Van Leeuwen, R.; Huang, M.T.; Fischer, C.; Flyynn, E. and Byers, H.R. (1998): Curcumin is an in vivo inhibitor of angiogenesis. Mol. Med. 4: 376-383.

Astudillo, L.; Schmeda, H.G.; Herrea, J.P. and Cortes, M. (2000): Proimate composition and biological activity of Chilean Prosopis species. J. Sci. Food Agri., 80: 567-573.

Badami, S.; Moorkoth, S.; Rai, S.R.; Kannan, E. and Bhojraj, B. (2003): Antioxidant activity of Caesallpinia sappan heartwood.. Biol. Pharm. Bull. 26(11): 1534-1537.

Bellomaria, B.; Arnold, N. and Valentini, G. (1992): Contribution to the study of the essential oils from three species of saliva growing wild in the Eastern Mediterranean region. J. Ess. Oil Res. 4: 607-614.

Bolkent, S.; Yanardag, R.; Karabulut, B.O. and Yesilyaprak, B. (2005): Protective role of Melissa officinalis L. extract on liver of hyperlipidemic rats: A morphological and biochemical study. J. Ethno. 99: 391-398.

Bombardelli, E. and Morazzoni, P. (1993): The flavonoids: new perspectives in biological activities and therapeutics. Chem. Org. 11: 25-28.

Bremness, L. (1994): The complete book of Herbs. Dorling Kindersley Ltd. London. P: 304.

Buescher, R. and Yang, L. (2000): Turmeric, in: G. J. Lauro, F. J. Francis (eds.), Natural Food Colorants, Marcel Dekker, New York. 205-226.

Cao, G; Booth, S.L.; Sadowski, J.A. and Prior, R.L. (1998): Increases in human plasma Antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am. J. Clin. Nutr. 68: 1081-1087.

Casa, C.L.; Villegas, I.; Alarcon de la Lastra; Motilva, C. and Martyin Calero, M.J. (2000): Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. J. Ethnopharmacol. 71: 45-53.

Castenmiller, J.J.M.; Linssen, J.P.H.; Heinonen, I.M.; Hopia, A.I.; Schwartz, K. and Hollmann, P.C.H. (2002): Antioxidant properties of differently processed spinach products. Nahrung/ Food 46: 290-293.

Catapeno, A.L. (1997): Antioxidant effects of flavonoids. Angiology. 48:39-44.

Chainani-Wu, N. (2003): Safety and anti-inflammatory activity of curcumin: a component of turmeric (curcuma longa). J. Altern. Complement. Med. 9: 161-168.

Che Man, Y.B., and Tan, C.P. (1999): Effects of natural and synthetic antioxidants on changes in refined, bleached, and deodorized palm olein during deep-fat frying of potato chips. J. of Am. Oil Chem. Soc. 76:331-339.

Cohly, H.H.; Taylor, A.; Angel, M.F., and Salahudeen, A.K. (1998): Effect of turmeric, turmerin and curcumin on H_2O_2-induced renal epithelial (LLC-PK1) cell injury. Free radical Biolo. And Med. 24(1): 49-54.

Cooks, N.C. and Samman, S. (1996): Flavonoids. chemistry, metabolism, cardio protective effects and dietary sources. J. Nutr. Biochem. 7: 66.

Crozier, A.; Michael, E.J.L.; Mc Donald, M.S., and Black, C. (1997): Quantitative analysis of the flavonoid content of commercial

tomatoes, anions, lettuce, and celery. J. Agric. Food Chem., 45: 590-595.

Dapkericius, A.; Venskutonis, R.; Beek, T. and Linssen, J. (1998): Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agric. 77: 140-146.

Dalle-Donne, I.; Rossi, D.; Giustarini, D.; Milzani, A. and Colombo, R. (2003): Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chem. Acta 329: 23-38.

De Groot, H.; Dehmmlow, C. and Raven, U. (1996): Tissue injury by free radicals and the protective effects of flavonoids. Methods find. Exp. Clin. Pharmacol. (supp. B). 18: 23-25.

Economou, K.D.; Oreopoulou, V. and Thomopoulos, C.D. (1991): Antioxidant activity of some plant extracts of the family Labiatae. J. Am. Oil Chem. Soc. 68: 109-113.

Feher, J.; Cornides, A. and Cosmos, G. (1987): Antioxidant and immunomodulant effect of hepatoprotective drugs. In. Assessment and management of hepatobiliary disease. (Sanyi, O.L.; Csomus, G. and Crepaldi, G. Eds.). Springer-Verlag, Berlin Heidelberg, P. 261.

Feresin, G.E.; Tapia, A.; Angel, G.R.; Delporte, C..; Erazo, N.B. and Schmeda, H.G. (2002): Free radical scavengers, anti-inflammatory and analgesic activity of Acaena magellanica. J. Pharm. Pharmacol., 54: 835-844.

Gryglewski, R.J.; Korbut, R.; Robak, J. and Sueis, J. (1987): On the mechanism of antithrombotic action of flavonoid. Biochem. Pharmacol. 37: 317.

Gordon, M.H. (1991): Oils and Fats? Taint or flavour. Chem. in Britain, Nov. 1020-1022.

Halliwell, B. (1996): Antioxidant in human health and disease. Ann. Rev. Nutr. 16: 33-50.

Ham, A.L. and Liebler, D.L. (1995): Vitamin E oxidation in rat liver mitochondria. Biochemistry, 34: 5754-5761.

Hanna, A.N.; Sharma, H.M.; Kauffman, E.M. and Newwan, H.A.I. (1994): *In vitro* and *in vivo* inhibition of microsomal lipid peroxidation by MA-631. *Pharmacol. Biochem. Behav.*, 39: 505-510.

Havsteen, B. (1983): Flavonoids, A class of natural products of high pharmacological potency. Biochem. Pharmacol. 32 (7): 1141-1148.

Herodez, S.S.; Hadolin, M.; Skerget, M. and Knez, Z. (2003): Solvent extraction study of antioxidants from balm (Melissa officinalis L.) leaves. Food Chem. 80: 275-282.

Hertog, M.G.L.; Hollman, P.C.H.; Katan, M.B. and Kromhout, D. (1993): Intake of potentially anticarcinogenic flavonoid and their determinants in adults in the Netherlands. Nutr. Cancer, 20:(1): 21-29.

Hohmann, J.; Zupko, I.; Redei, D.; Sanyi, M.C.; Faikay, G.; Mathe, I. and Janicsak, G. (1999): Protective effects of the aerial parts of saliva officinalis, Melissa officinalis and Lavandula angustifolia and their constituents against enzyme dependent and enzyme independent lipid peroxudation. Planta Medica 65: 576-578.

Horton, A.A., and Fairhurst, S. (1987): Lipid peroxidation and mechanisms of toxicity. CRC Crit Rev. Toxicol. 18: 27-79.

Hras, A.R.; Hadolin, M.; Knez, Z. and Bauman, D. (2000): Comparision of antioxidative and synergestic effects of rosemary extract with α-Tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem. 71: 229-233.

Javanmardi, J.; Stushnoff, C.; Locke, E., and Vivoanco, J.M. (2003): Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem. 83: 547-550.

Jones, E.A.; Shahed, A. and Shoskes, D.A. (2000): Modulation of apoptotic and inflammatory genes by bioflavonoid and angiotensin II inhibition in Urethral obstruction. . Urology 56(2): 346-351.

Kamalakkannan, N. and Prince, P.S. (2006): Antihypercaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin – induced diabetic Wister rats. Basic Clin. Pharmacol. Toxicol. Jan; 98(1): 97-103.

Kamat, J.P. and Delasagayam, T.P.A. (1995): Tocotrienols from palmoil as potent inhibitors of lipid peroxidation and protein oxidation in rat brain mitochondria. Neurosci. Lett. 195: 179-182.

Kaur, C. and Kapoor, H.C. (2001): Antioxidants in fruits and vegetables. The millennium's health. Inter. J. Food Sci. and Techno. 36: 703-725.

Lee, J.C.; Kim, H.R.; Kim, J. and Jang, Y.S. (2002): Antioxidant property of an Ethanol extract of the stem of *Opuntia ficus-indica var. Saboten. J. Agric. Food Chem.*, 50: 6490-6496.

Lowry, O.H.; Rosebrough, N.I.; Farr, A.L. and Randall, R.J. (1951): Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.

Lugasi, A. and Hovari, J. (2000): Flavonoid aglycons in foods of plant origin I. vegetables. Acta Alimentarii. 29:345-352.

Manikandan, P.; Sumitra, M.; Aishwarya, S.; Manohar, B.M.; Lokandam, B. and Puvanakrishnan, R. (2004): Curcumin modulates free radical quenching in myocardial ischaemia in rats. Inter. J. Biochem. and cell Biolo. 36(10): 1967-1980.

Metodiewa, D.; Kochman, A. and Karolizak, S. (1997): Evidence for antiradical and antioxidant properties of four biological active N,N- diethylamino ethyl ethers of flavonone oximes: a comparison with natural polyphenolic flavonoid (rutin) action. Biochem. Mol. Biol. Int. 41: 1067-1075.

Middleton, E.J.R. and Kandaswami, C. (1992): Effect of flavonoids on immune and inflammatory cell function. Biochem. Pharmacol. 43(6): 1167-1179.

Miean, K.H. and Suhaila, M. (2001): Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) content of edible tropical plants. J. Agric. Food Chem. 49: 3106-3112.

Nagababu, E. and Lakshmaiah, M. (1992): Inhibitory effect of eugenol on non enzymatic lipid peroxidation in rat liver mitochondria. Biochem. Pharmacol., 43: 2393-2400.

Nakatani, N. (1997): Antioxidants from spices and herbs. In: Shhidi, F. (ed.), Natural antioxidants. Chem. Health effects and app. P: 64-73. Champaign. IL. AOCS Press.

Nirmala, C.; Anand, S. and Puvanakrishnan, R. (1999): Curcumin treatment modulates collagen metabolism in isoproterenol induced myocardial necrosis in rats. Molecular and Cellular Biochem. 197(1-2): 31-37.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): assay for lipid peroxidation animals tissues by thiobarbituric reaction analytical Biochem. 95: 351-358.

Reddy, A.C., and Lokesh, B.R. (1994): Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive

oxygen species and the oxidation of ferrous iron. Moll. Cell Biochem. 137 (1): 1-8.

Ribeiro, M.A.; Bernardo- Gil, M.G. and Esquivel, M.M. (2001): Melissa officinalis L. : study of antioxidant activity in supercritical residues. J. Supercritical fluids 21: 51-60.

Rukumani, R.; Balasubbashini, M.S., and Menon, V.P. (2003): Protective effects of curcumin and photo-irradiated curcumin on circulatory lipids and lipid peroxidation products in alcohol and polyunsaturated fatty acids–induced toxicity. Phyto. Res. 17: 925-929.

Sarer, E., and Kokdil, G. (1991): Constituents of the essential oil from Melissa officinalis. Planta Medica. 57: 89-90.

Schilcher, H. (1985): Effects and side effects of essential oils. In: Svendson, B. and Scheffer, J.J.C. (eds.). Essential oils and Aromatic plants. Junk, Dordrecht, The Netherlands. P:217-231.

Selvam, C.; Jachak, S.M.; Thilagavathi, R. and Chakraborti, A.K. (2005): Design, synthesis, biological evaluation and molecular docking of curcumin analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents. Bioorg. Med. Chem. Lett. Apr.1; 15(7): 1793-7.

Sreejayan, A. and Rao, M.N.A. (1997): Nitric oxide scavenging by curcuminiods. J. Pharm. Pharmacol. 49: 105-107.

Starvic, B. (1994): Antimutagens and anticarcinogens in food. Food Chem. Toxicol., 32: 79-90.

Strasser, E.M.; Wessner, B.; Manhart, N. and Roth, E. (2005): The relationship between the anti-inflammatory effects of curcumin and molecular glutathione content in myelomonocytic cells. Biochem. Pharmacol. 70: 552-559.

Tappel, A.L. (1973): Lipid peroxidation damage to cell components. Fed. Proc. Fed. Amsoc. Exp. Biol. 32:1870-1874.

Thiyagarajan, M. and Sharama, S.S. (2004): Neuroprotective effect of curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Life Sci. 74(8): 969-985.

Thomas, S.C. (2000): In: Medicinal plants culture, utilization, and phytopharmacology. Technomic Publishing Company, Inc. 851 New Holland Avenues, Lancaster, Pennsylvania. 17694 U.S.A

Toda, S.; Miyase, T.; Arichi, H.; Tanizawa, H. and Takino, Y. (1985): Natural antioxidants. III Antioxidative components isolated from

rhizome of curcuma longa L. Chem. and Pharma. Bull. 33(4): 1725-1728.

Tripathi, Y.B.; Chaurasia, S.; Tripathi, E.; Upadhyay, A. and Dubey, G.P. (1996): Indian J. Exp. Biol. 34:523-526.

Ukill, A.; Maity, S.; Karmakar, S.; Datta, N.; Vedasiroononi. J.R. and Das, P. K. (2003): Curcumin, the major components of food flavour turmeric, reduces mucosal injury in triinitrobenzene sulphonic acid-induced colitis.. Br. J. Pharmcol. 139: 209-218.

Venkatesan, N.; Punithavathi, D. and Arrumugam, V. (2000): Curcumin prevents adriamycin nephrotoxicity in rats. Br. J. Pharmacol. 129:231-4.

Viturro, C.; Molina, A. and Schmeda, H.G. (1999): Free radical scavengers from *Mutisia friesiana* (Asteraceae) and *Sanicula gravelens* (Apiaceae). Phytother. Res. 13: 422-424.

Wagner, H.; Bladt, S. and Zgainski, E. (1984): Plant Drug Analysis. Springer Verlag, Berlin.

Wei, Q.Y.; Chen, W.F.; Zhou, B.; Yang, L. and Liu, Z.L. (2006): Inhibition of lipid peroxidation and protein oxidation in rat liver mitochondria by curcumin and its analogues. Biochem. and Biophys. Acta 1760: 70-77.

Wolf, B.; Christa, M. and Manfred, S. (1994): Flavonoids antioxidants: rate constants for reactions with oxygen radicals. Methods Enzymol. 234: 420-429.

Xu, J.; Ma, M. and Purcell, W.M. (2003): Characterization of some cytotoxic endpoints using rat liver and Hep G2 spheroid *in vitro* models and their application in hepatotoxicity studies. 1. Glucose metabolism and enzyme release as cytotoxic markers. Toxicol. Appl. Pharmacol., 189:100-111.

Yen, G.C., and Hsieh, C.L. (1998): Antioxidant activity of extracts from Du-zhong *(Eucommia ulmoides)*toward various lipid peroxidation models *in vitro. J. Agric. Food Chem.*, 46: 3952-3957.

دراسات بيوكيميائية لبعض المركبات الطبيعية المضادة للأكسدة ككاسحات للشقوق الحرة

محيى الدين على عثمان* عمرو أحمد مصطفى*
سامى محمود عبد الجواد** نيرمين زكريا أحمـــــد**
* جامعة القاهرة – كلية الزراعة – قسم الكيمياء الحيوية
** الهيئة القومية للرقابة والبحوث الدوائية – التقييم الدوائى الجزيئى

تم دراسة التأثير المضاد للأكسدة لمجموعة من النباتات المختلفة باستخدام أنظمة خارج الجسم وداخلة وتم استخدام طريقة (DPPH)لنزع اللون لأختبار المستخلصات الكحولية لمجموعة من النباتات وهى البقدونس و المريمية و الكركم و الكافور و الليمون و الكمون و الميلسيا و الزيتون و أكليل الجبل (حصا اللبان) و الشيكوريا و الريحان على أجزاء مختلفة فى النبات منها: الأوراق و الثمار و الجزء الهوائى كلة والجذور وذلك لتقييم النشاط المضاد للأكسدة بالمقارنة بمواد قياسية مثل الروتين وحمض الأسكوربيك وكذلك تم تقدير رقم البيروكسيد ورقم الحموضة لمجموعة محددة منهم والتى تم تحديد أعلاها في النشاط من خلال تجربة (DPPH)وذلك لمعرفة نشاطهم المضاد للأكسدة لكل مستخلص نباتى.

كذلك أجريت تجربة لتقييم النشاط المضاد للأكسدة لكل مستخلص ايثانولى باستخدام نظام Fe^{++}/ Ascorbate واستخدام تفاعل TBARS فى جسيمات الميتوكوندريا لكبد الفئران فى وجود كل مستخلص على حدة.

وقد أظهرت النتائج باستخدام تجربة نزع اللون بالمركب DPPH أن بعض المستخلصات النباتية لها نفس التأثير أو أقل من المواد القياسية المستخدمة وهى الروتين وحمض الأسكوربيك .

أوضح رقم البيروكسيد زيادة يوميه حتى اليوم العشرون باستخدام زيت عباد الشمس منزوع منة المواد النشطة بالمقارنة بمادة قياسية وهى (BHT) هيدروكسى بيوتيل تولوين فى بعض المستخلصات النباتية ذات النشاط العالى والبعض الأخر ذات النشاط المنخفض وأظهر رقم البيروكسيد زيادة حتى أسبوعين فقط.

أظهر نبات البقدونس بالتركيزات الثلاثة المستخدمة (0.02 , 0.04 , 0.06 g/100 g Oil) مع زيت عباد الشمس قيم مرتفعة قريبة من المادة القياسية ونشاطها بتركيزات 46.6, 45.97, 42.8 Meq./ Kg Oil على التوالى.

وقد أظهرت باقى المستخلصات الكحولية للنباتات المتبقية أعلى مستوى للنشاط لها بعد اليوم الرابع عشر. وقد أظهر رقم الحموضة سرعة التحليل الحمضى للزيت فى وجود المستخلصات النباتية وقد لوحظ زيادة رقم الحموضة فى بعض الأحيان بزيادة الوقت اللازم للتحليل خلال أيام التجربة.

وقد أظهرت تجربة Fe^{++}/Ascorbate على خلايا الميتوكوندريا لكبد الفئران باستخدام المستخلصات النباتية أن أفضل هذه المستخلصات ذات النشاط المضاد للأكسدة هو البقدونس ثم المريمية والكركم على التوالى.