Bitlis Eren University
Journal of Science and Technology

ISSN 2146-7706
2, (2012), 52-56

Research Article

Some plant extracts that prevent lipid peroxidation and protect the unsaturated fatty acids in the fenton reagent environment

Oğuz Ayhan Kireçci [a], Zehra Gökçe [b], Ayşe Dilek Özşahin Kireçci [c,*], Gülcan Balcı [b], Ökkeş Yılmaz [b]

[a] *Hizan Vocational School, Bitlis Eren University, TR 13000 Bitlis, TURKEY*
[b] *Department of Biology, Faculty of Science and Arts, Firat University, TR 23119 Elazığ, TURKEY*
[c] *Department of Biology, Faculty of Science and Arts, Bitlis Eren University, TR 13000 Bitlis, TURKEY*
[*] *Corresponding author: molekuler@gmail.com*

Abstract

This study investigated protective effects of the *Laurus nobilis*, *Arum italicum* and *Malva sylvestris* extracts on the unsaturated fatty acids and the prevention of lipid peroxidation formation in the fenton reagent environment. Antioxidative activities of the plant extracts were determined with the following modifications. Lipid peroxidation level in the Fenton R group was significantly high when compared to the control group, but lipid peroxidation significantly decreased according to the Fenton R group ($p<0.0001$). It was determined that extracts had scavenging effect on the DPPH radical depending on the increase in the flavonoid concentration. Present results confirm that plants decrease lipid peroxidation level in the fenton reagent environment and protect markedly unsaturated fatty acids in the environment with radical sourced oxidations.

Keywords: Antioxidant capacity, *A. italicum*, *L. nobilis*, Lipid peroxidation, *M. sylvestris*

1. Introduction

Free radicals with the major species of reactive oxygen species (ROS) such as superoxide ($O^{-\cdot 2}$), hydroxyl radical (OH), oxide of nitrogen ($NO^{\cdot}$, $NO^{\cdot 2}$) and lipid radicals ($RO^{\cdot}$, $ROO^{\cdot}$, $R^{\cdot}$) are unstable and react readily with other groups or substances in the body, resulting in cell damage (Roberfroid & Colderon 1995). The ROS play an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts and inflammation (Kris-Etherton et al. 2004; Conforti et al. 2008).

Epidemiological and experimental studies reveal a negative correlation between the consumption of diets rich in fruit and vegetables and the risks for chronic diseases, such as cancer and cardiovascular diseases. These physiological functions of fruits and vegetables may be partly attributed to the abudance of phenolics in these (Saleem et al. 2002; Chen et al. 2005; Zhang et al. 2005).

Phenolic antioxidants are reported to quench oxygen-derived free radicals as well as substrate derived free radicals by donating a hydrogen atom or an electron to the free radical. Furthermore, phenolic extracts of plant materials have been shown to neutralize free radicals in various models (Conforti et al. 2006). The effect of plant extracts such as *L. nobilis*, *M. sylvestris* and *A. italicum* on pharmacological properties and potential health benefits have been studied in different parts of the world (Saglık et al. 2002; Politeo et al. 2007; Loizzo et al. 2008; Derwich et al. 2009; Barros et al. 2010; Marouane et al. 2011).

But until now the research properties the antioxidant activity of those plants were not fully investigated.

The aim of this work was to study the in vitro antioxidant activity of different extracts of *Laurus nobilis* L., *Malva sylvestris* L. and *Arum italicum* L., to assess their potential capacity as scavengers of free radicals and as inhibitors of lipid peroxidation.

2. Materials and Methods

2.1. Plant material

L. nobilis L., *M. sylvestris* L. and *A. italicum* L. were collected in spring and autumn of 2011 from the Hatay province of Turkey. The collected plant samples were dried in the dark.

2.2. Preparation of the extracts

5 g of dried plants were homogenized in 100 mL 80% methanol. Homogenates were centrifuged at 5000 rpm for 5 min at + 4°C. After centrifugation, the supernatant was concentrated to dryness under reduced 25 pressures at 50°C using a rotary evaporator. Each extract was resuspended in DMSO for stock solution, and stored at -20°C until analysis (Ozsahin et al. 2010).

2.3. Antioxidant assay by DPPH radical scavenging activity

The free radical scavenging effect in extracts was assessed by the decoloration of a methanolic solution of DPPH according to the method of Brand-Williams (1995). A solution of 25 mg/L DPPH in methanol was prepared and 4.0 ml portions of this solution were mixed with 50, 100 and 250 µL of extract in DMSO. The reaction mixture was left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. 1 µM quercetin was

Received 15 March 2012
Available online December 2012

used as a references. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control–Abs sample)] / (Abs control)] x 100.

2.4. Antioxidative activity testing of the plant extracts

Antioxidative activities of the plant extracts were determined by the method of Shimoi et al. (1994) with the following modifications. 1 mM $FeCl_2$ ($FeCl_2.H_2O_2$) and 3 µM hydrogen peroxide (H_2O_2) solutions were prepared fresh for every treatment using doubly deionized water. Extracts of plants were also prepared fresh using DMSO. Oleic acid (3.19 mM) and linoleic acid (8.6 mM) were dissolved DMSO. The buffer solutions were prepared with 0.2% Twin 20, 0.05 M Tris-HCl-Base and 0.15 M KCl (pH=7.4).

During *in vitro* experiment, the first group was used for control, the second group was the Fenton reagent group, ($FeCl_2$ H_2O_2 +, Fenton R), and the third group was the Fenton R plus *L. nobilis* extract and the fourth group was the Fenton R plus *A. italicum* extract, and the fifth group was the Fenton R plus *M. sylvestris* extract. The 1st group was used as a control group and 0.4 ml fatty acid mixture (102.67 µM 18:2 and 42.53 18:1) µM was suspended in a 5 ml buffer solution. The 2nd group was the Fenton reagent group, and 0.4 ml fatty acid mixture was suspended in a 5 ml buffer solution and was added FeCl2 (50 µM) and hydrogen peroxide (0.01 mM). The 3-5th groups were the Fenton reagent plus plant extracts, and 0.4 ml fatty acid mixture were suspended in a 5 ml buffer solution and were added $FeCl_2$ (50 µM), hydrogen peroxide (0.01 mM) and 1 ml plant extracts. All of the groups were incubated at 37°C for 24 h. After the incubation, 100 µL of the 4% (w/v) BHT solution was added to prevent further oxidation. Then, from each, 1 ml mixtures were taken and 1 ml % 0.6 of 2-thiobarbituric acid (TBA) was added to the reaction mixture and incubated at 90°C for 40 min. Samples were allowed to cool to room temperature and the pigment produced was extracted with 3 ml of n-butanol. Samples were then centrifuged at 6000 rpm for 5 min and the concentration of upper butanol layer was measured at a HPLC-fluorescence detector.

2.5. Quantitation of LPO level *in vitro* environment

The products of the peroxidation of the fatty acids in vitro environment were determined by reading the fluorescence detector set at λ (excitation) 515 nm, and λ (emission) 543 nm. Formation of the malonaldehyde in vitro enviroment expressed as thiobarbituric acid-reactive substances (TBARS) were calculated from a calibration curve using 1, 1, 3, 3-tetraethoxypropane as the standard. The MDA-TBA complex was analyzed using the HPLC equipment (Shimadzu, Kyoto Japan). Inertsil ODS 3 column (15×4.6 mm, 5 µm) was used as the HPLC column. The column was eluted isocratically at 20°C with a 5 mM sodium phosphate buffer (pH=7.0) and acetonitrile (85:15, v/v) at a rate of 1 ml/min (De Las Heras et al. 2003).

2.6. Quantitation of the remaining fatty acids *in vitro* enviroment

The remaining mixtures of oleate and linoleate in the test tube were converted to methyl esters by using 2% sulfuric acid (v/v) in methanol (Christie 1992). Fatty acid methyl ester analysis was performed in a Shimadzu GC-17A intstrument gas chromatograph equipped with a flame ionization detector (FID) and a 25 m, 0.25 mm i.d. Permabond fused-silica capillary column (Machhery-Nagel, Germay). The oven temperature was programmed between 160-215°C, 4°C/ min. The injector and FID temperatures were 240 and 280°C, respectively. The nitrogen carrier gas flow was 1 ml/min. The results were expressed as µmol/ml.

3. Results and Discussion

According to the results of DPPH free radical cleaning, it was detected that *L. nobilis* and *A. italicum* extracts were significantly effective between concentrations of 50 mL (99.27%, 86.2%) and 100 mL (95.75%, 89.63%), respectively ($p<0.0001$), however *L. nobilis* and *A. italicum* groups showed an antagonistic effect at concentrations higher than 250 mL see Figure 1. *M. sylvestris* extracts were reported to display antioxidant activity starting from 50 µL (50.77%) concentration and this activity was observed to increase as the concentration increased (Figure 1). L. nobilis was found to have more significant radical cleaning characteristics than the other groups in all concentrations. When the groups were compared depending on the increasing concentration the group having the highest antioxidant capacity was found to be the *L. nobilis* group as seen in Figure 1.

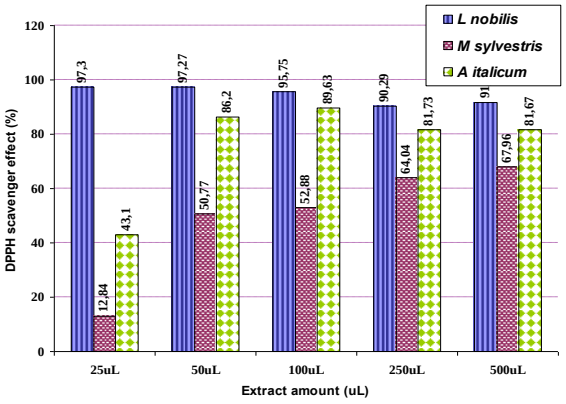

Figure 1. DPPH• scavenger effect of plant extracts (%)

The obtained results could point to strong quenching activities of flavonoids present in the leaves of *L. nobilis* against DPPH radicals (Fejes et al. 2000). It can be supposed that such antiradical activity is also caused, besides flavonoids, by terpenoids, since nonpolar solvents also exhibited high antiradical potential. Antioxidant activity of *L. nobilis* leave great demonstrated activity for methanolic, ethanolic and water extracts (Kang et al. 2002; Conforti et al. 2006; Papageorgiou et al. 2008; Dall'Acqua et al. 2009). EC_{50} values for DPPH free radical scavenging were 24% for *M. sylvestris* (Della Greca et al. 2009), 0.97 mg/mL for *M. sylvestris* (Fejes et al. 2000; Kumarasamy et al. 2007). These data are changeable with the data presented in the present study (Fejes et al. 2000; Kang et al. 2002; Conforti et al. 2006; Kumarasamy et al. 2007; Papageorgiou et al. 2008; Dall'Acqua et al. 2009; Della Greca et al. 2009).

LPO level was found to be significantly high in the group containing the Fenton reagent (44.77 nmol/ml) ($p<0.0001$), when compared to the control group (8.51

nmol/ml) shown Figure 2. When the groups containing plant extracts (16.08-47.72 nmol/ml) and the control group (8.51 nmol/ml) were compared, a partial increase was observed in *L. nobilis* (16.08 8.51 nmol/ml) and significant increases were observed in *A. italicum* and *M. sylvestris* ($p<0.05$, $p<0.001$). On the other hand, the MDA-TBARS level in the groups containing Fenton reagent was ($p<0.0001$). When the groups containing plant exracts were compared among each other, the LPO level in *L. nobilis* was observed to be lower than the *A. italicum* and *M. sylvestris* levels ($p<0.0001$). No statistical difference was observed between the Fenton reagent and *M. sylvestris* (Figure 2).

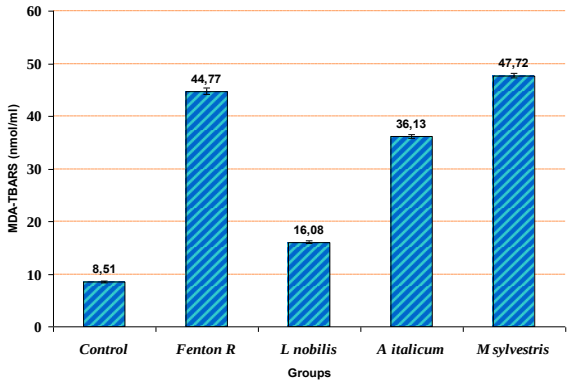

Figure 2. The levels of MDA-TBA in the environments of Fenton reagent and Fenton reagent with plant extracts (Fenton R: Fenton reagent)

In previous phytochemical investigations on *L. nobilis* leaves and fruits, different groups of chemical were observed to be isolated: flavones, flavonols, sesquiterpene lactones, alkaloids, glycosylated flavonoids and monoterpene (Pech & Bruneton 1982; Vidal-Olliver et al. 1989; Kaurinovic et al. 2010). Matsuda et al. (2002) have also established that the methanolic extract from the leaves of *L. nobilis* inhibit nitric oxide production in lipopolysaccharide activated mouse peritoneal macrophages. Since lipid peroxidation causes oxidative damage to cell membranes and all other systems that contain lipids (Chatterjee 1988), in the investigation of the total antioxidative activity of extracts it is necessary to investigate their effects on lipid peroxidation. High inhibitory effect of this extract and its components towards Fe^{2+} dependent LP of liposomes can be related to the presence of flavonoids in the extracts. It was established that flavonoids act as mighty scavangers of free radicals (Robak & Gryglewski 1988). It is also known that antiradical potential of flavonoids are most pronounced towards OH, peroxy- and alkoxy radicals, which are formed in the process of lipid peroxidation (Sichel et al. 1991). These data are consistent with the data presented in the present study (Pech & Bruneton 1982; Chatterjee 1988; Robak & Gryglewski 1988; Vidal-Olliver et al. 1989; Sichel et al. 1991; Matsuda et al. 2002; Kaurinovic et al. 2010).

Oleic acid, linoleic acid and linolenic acid amounts were observed to decrease in all groups (3.60-3.89, 7.73-8.86 and 1.24-1.52 umol/ml) when compared to the control group (4.11, 9.87 and 2.2 umol/ml), respectively as seen in Figure 3. When the fatty acid amounts in the reaction media was compared with the control group, significant decreases were observed in Fenton R and plant extract groups ($p<0.0001$). When the groups containing plant extract were compared according to the Fenton R group (3.83, 7.23 and 1.11 umol/ml), it was found that oleic, linoleic and linolenic acid amounts were significantly higher in *L. nobilis*, *A. italicum* and *M. sylvestris* see in Figure 3. When the plant extracts were compared to each other, it was observed that the fatty acid levels in the *L. nobilis* group were much higher than those of the *A. italicum* and *M. sylvestris* groups ($p<0.001$) see Figure 3.

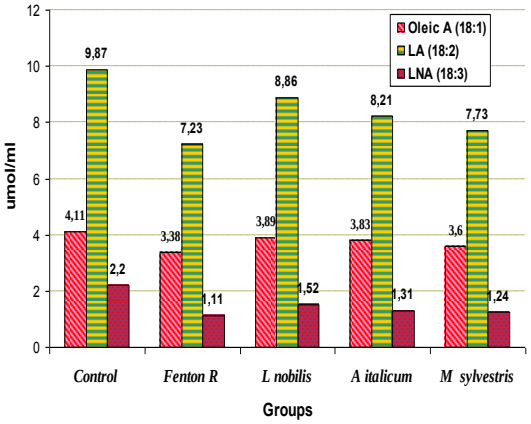

Figure 3. The levels of LA (18:2, n6) and oleic acid (18:1, n9) in the reaction environment (μmol/1 ml)

However, no statistically significant difference was found between the *A. italicum* and *M. sylvestris* groups ($p>0.05$). Looking at the MDA-TBA formation results, it was detected that the oleic, linoleic and linolenic acids which were added into the reaction environment were higher in each group into which three plants were added in comparison with the Fenton R group. Especially, it was detected that oleic, linoleic and linolenic acid levels were higher in the *L. nobilis* group in comparison to *M. sylvestris* and *A. italicum*. We think that the increase in these groups arise particularly from the quantity of phytochemicals. So it is presumed that the flavonoids of extracts may protect unsaturated fatty acids from radical sourced oxidations. All favonoids acted as antioxidants on oxidation of methyl linoleate although the antioxidant response of kaemferol and rutin is weak. Quercetin and myricetin inhibits the hydro peroxide formation in methyl linoleate environment (Ozsahin & Yilmaz 2010). In our findings, both the characteristic of scavenging DPPH radical, and in the environment in which oleic, linoleic and linolenic acid exist, the decrease of MDA-TBA formation was arisen from the flavonoids inside it. Moreover, as mentioned in the literature works, it can be said that flavonoids are also effective in this case (Shahidi & Marian 2003; Skerget et al. 2005; Ozsahin & Yilmaz 2010; Ozsahin et al. 2010).

4. Conclusion

As a result, as it is suggested in literary works, we confirm that *L. nobilis*, *M. sylvestris* and *A.italicum* have a strong antioxidant activity. Apart from that, it can be emphasized that *L. nobilis*, *M. sylvestris* and *A.italicum* have a protective effect on polyunsaturated fatty acids in the studies carried out with the molecules which existed in the cell membranes in in vitro environment.

We assume that these results confirm the idea of using natural antioxidant compounds in a safer way instead of using artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) that are used to preserve food products.

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