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The *Rhizoma polypodii* Extract Prevents Lipid Peroxidation and Protects the Unsaturated Fatty Acids in the Environment with Radical Sourced Oxidations

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ABSTRACT

The present study investigated protective effects of the *Rhizoma polypodii (R.polypodii)* extract on unsaturated fatty acids and the prevention of lipid peroxidation (LPO) formation in the Fenton reagent environment.

Our findings confirm that *Rhizoma polypodii* extracts decreased the LPO level in the Fenton reagent environment and protected markedly the unsaturated fatty acids in the environment with radical sourced oxidations.

Key words: *Fenton Reagent, Flavonoids, Lipid Peroxidation, Radical Scavenging Effect, Rhizoma Polypodii.*

1. INTRODUCTION

Reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl, superoxide anions, nitric oxide radicals, play a key factor in oxidative stress related to the pathogenesis of various important diseases [18,17]. Antioxidants act as a major defense against radical mediated toxicity by preserving the damages. Some diseases, such as Alzheimer's disease, atherosclerosis, stroke, diabetes, and cancer have appeared to treat antioxidant-based drugs/formulations in the last three decades [12].

One of the main classes of secondary metabolites is phenolic compounds [28]. These phytochemicals are often described as antioxidants on account of their ability of protecting against damages caused by reactive oxygen species (ROS). By their ability to react and damage many structures in the body. Both synthetic and natural antioxidants have been shown to act against ROS and the use of antioxidants in food application has determined them to enhance product property, durability, and shelf life. Recent works have also mentioned the disadvantage of synthetic antioxidants, and their possible deleterious properties for human health compared to natural antioxidants [19].

The mechanism for the possible protective action of flavonoids is currently the subject of important research. The structural characteristics of antioxidants are reftlected their antioxidant properties. Flavonoids can act as antioxidants by several mechanisms, including donating hydrogen, quenching singlet oxygen, scavenging free radicals, and chelating redox active metals [31].

Besbase *(Rhizoma polypodii*) is the dried root and rhizome of *Polypodium vulgare* L. species. This species

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is a nonflower and a herbaceous plant which grows on rocks and walls. Their leaves are leather-like and have 20-40 lobes. It' s localized on the bases of the forests in the Western Anatolia region. It' s part under the soil contains saponine, volatile oils and tannin. It has laxative, vermifuge, bile and mucus expectorant effects [2,4,5].

In the present research, the effect of *Rhizoma polypodii* extracts on the formation of lipid peroxidation (LPO) of unsaturated fatty acids during autoxidation was determined. In addition, the scavenging features of extracts were studied on the DPPH radical.

2. EXPERIMENTAL

2.1. Chemicals

The following; oleic (18:1, n-9), linoleic (18:2, n-6), linolenic acid (18:3, n-3), Tween 20, tris-hydrochloride, quercetin, myricetin, resveratrol, cathechin, naringin, naringenin, kaempferol, HPLC grade methanol, acetonitrile, n-hexane, isopropyl alcohol, $FeCl_2$, H_2O_2, KH_2PO_4, butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), n-butanol, α,α-diphenyl-β-picryl-hydrazyl (DPPH•), dimethyl sulfoxide (DMSO), and ethyl alcohol were purchased from Sigma-Aldrich.

2.2. Preparation of the plant extracts

The *Rhizoma polypodii* sample is sold by the herb and spice seller in Elazıg as dehydrated and powdered in the bray. A g [1] herb material was homogenized in a 10 mL solution of 80% methanol. Homogenates were them centrifuged at 5000 rpm at +4ºC. After centrifugation, the flavonoid analysis of the supernatant was carried out using HPLC equipment. Then, the methanolic extract was concentrated and dried in a vacuum at 50ºC using a rotary evaporator. Each extract was then resuspended in DMSO as a stock solution.

2.3. Antioxidative activity testing of plant extracts

Antioxidative activities of the *Rhizoma polypodii* extract was determined by the method of Shimoi et al. with the following modifications. The Fe++ ($FeCl_2$ $2H_2O$) and hydrogen peroxide solutions were prepared fresh for every treatment using twice deionized water. Extract of the *Rhizoma polypodii* was also prepared fresh using DMSO. Oleic acid (3.35 mM), linoleic acid (9.01 mM), and linolenic acid (2.30 mM) were dissolved in the DMSO.

During the *in vitro* experiment, the first group was used as a control (n=5), the second group was the Fenton reagent group (n=5), and the third group was *Rhizoma polypodii* extract group (n=5). The control group was prepared with 0.5 mL of fatty acid and a buffer solution (0.2% Tween 20 / 0.05 M Tris•HCl / 0.15 M KCl, pH 7.4). The Fenton R group was prepared with 0.5 mL of fatty acid, a buffer solution, $FeCl_2$ (50 μM), and hydrogen peroxide (0.01 mM). The *Rhizoma polypodii* group was prepared with 0.5 mL of fatty acid, a buffer solution, FeCl2 (50 μM), hydrogen peroxide (0.01 mM). After incubation of the mixture at 37°C for 24 h, 100 μL of a 4% (w/v) BHT solution was added to prevent further oxidation. Then, 1 mL was taken each mixture and 1 mL of 0.6% TBA was added to the reaction mixture and incubated at 90°C for 45 min. Samples were allowed to cool to room temperature and the pink pigment produced was extracted with 3 mL of n-butanol. Samples were then centrifuged at 6000 rpm for 5 min and, the concentration of the upper butanol layer was measured by HPLC- fluorescence detector.

2.4. Quantification of LPO Level in the *in vitro* Environment

The products of the peroxidation of fatty acids in the reaction environment was determined by reading the fluorescence detector set at λ (excitation)= 515 nm and λ (emission)= 543 nm. Formation of the malonaldehyde *in vitro* environment was expressed as thiobarbituric acid reactive substances (TBARS) calculated from a calibration curve using 1, 1, 3, 3-tetraethoxypropane as the standard. The MDA-TBA (malondialdehyde-2-thiobarbituric acid) complex was analyzed using HPLC equipment. The equipment consisted of a pump (LC-10 AD*VP*), a Fluorescence detector (RF–10 XL), a column oven (CTO- 10AS*VP*), an autosampler (SIL-10AD*VP*), a degasser unit (DGU-14A), and a computer system with class *VP* software (Shimadzu, Kyoto Japan). An Inertsil ODS-3 column (15×4.6 mm, 5 μm) was used as the HPLC column. The column was eluted isocratically at 20ºC with a 5 mM sodium phosphate buffer (pH=7.0) and acetonitrile (85:15, v/v) at a rate of 1 mL/min [11].

2.5. Quantification of Remaining Fatty Acids in the *in vitro* Environment

Remaining mixtures of oleic (18:1 n-9), linoleic (18:2 n-6) and linolenic (18:3 n-3) acids in the test tube were converted to methyl esters by using 2 % sulfuric acid (v/v) in methanol [8]. The fatty acid methyl ester forms were extracted with n-hexane. Analysis was performed in a Shimadzu GC-17A instrument gas chromatograph equipped with a flame ionization detector (FID) and a 25 m 0.25 mm i.d. Permabond fused-silica capillary column (Machery- Nagel, Germany). The oven temperature was programmed for 160-215°C at 4°C / min. Injector and FID temperatures were 240 and 280°C, respectively. The nitrogen carrier gas flow was 1 mL/min. The methyl esters of oleate, linoleate, and linolenate were identified by comparison with authentic external standard mixtures analyzed under the same conditions. Class GC 10 software version 2.01 was used to process the data. The results were expressed as μmol/mL.

2.6. Antioxidant Assay by DPPH Radical Scavenging Activity

The free radical scavenging effect in the extracts was assessed by the decoloration of a methanolic solution of DPPH• according to the method of Brand-Williams et al. (1995). A solution of 25 mg/L DPPH in methanol was prepared and 3,9 mL of this solution was mixed with 50 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 [23]. Quercetin was

used as references. The absorbance at 517 nm was measured after reaction in the dark at 30°C for 30 min. Lower absorbance of the reaction mixture indicates high free radical scavenging activity. All tests were performed in triplicate, and the percentage inhibition of the radicals due to the antioxidant properties of the extracts were calculated as shown below:

DPPH radical scavenging activity (%)= [(Abscontrol – Abs sample)]/ (Abs control)] x 100
Where Abs control is the absorbance of the DPPH radical + methanol; the Abs sample is the absorbance of the DPPH radical + sample extract /standard.

2.7. Chromatographic conditions for flavonoid analysis

Chromatographic analysis was carried out by PREVAIL C18 reversed-phase column (15x4.6mm, 5µm), the mobile phase was methanol/water/acetonitile (46/46/8, v/v/v) containing 1.0% acetic acid [39]. This mobile phase was filtered through a 0.45 µm membrane fitler (Millipore), then deaerated ultrasonically prior to use. Catechin (CA), Naringin (NA), Rutin (RU), Resveratrol (RES), Myricetin (MYR), Morin (MOR), Naringenin (NAR), Quercetin (QU) and Kaempferol (KA) were quantified by DAD (Diode-Array Detection) following RP (Reversed phase) HPLC separation at 280 nm for CA and NA, 254 nm for RU, MYR, MOR and QU, 306 nm for RES, 265 nm for KA. Flow rate and injection volume were 1.0 ml/min and l0 µL, respectively. The chromatographic peaks of the analytes were confirmed by comparing their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak using external Standard method. All chromatographic operations were carried out at 25 ° C temperatures.

2.8. Statistical analysis

The experimental results were reported as mean ± SEM. Statistical analysis was performed using SPSS Software. Analysis of variance (ANOVA) and an LSD test were used to compare to the plant extract including groups compared with Fenton R group.

3. RESULTS

The present study findings showed that MDA-TBA level in the *Rhizoma polypodii* group was slightly lower than the control group ($p>0.05$), whereas, MDA-TBA level were about seven folds lower than that of the Fenton R. group ($p<0.001$) (Fig. 1). The difference between plant extracts and quercetin was statistically non-significant ($p>0.05$) when they were assessed in terms of DPPH capacity of free radical scavenging. Besides plant extracts were detected to have an effect of high radical scavenging just as quercetin (Table 1).

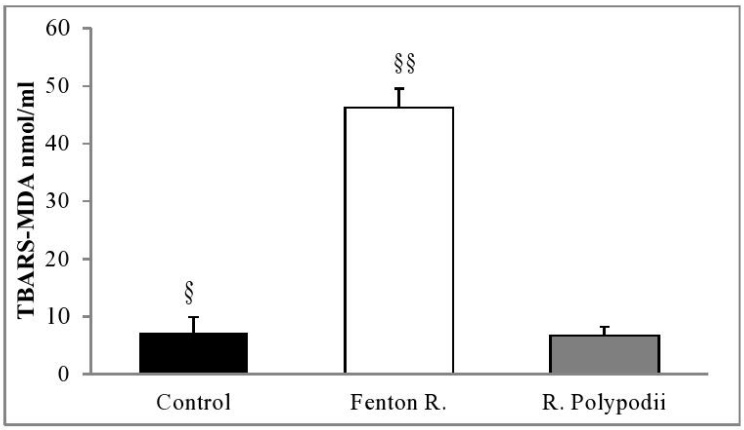

Fig 1. The level of TBARS-MDA (nmol/ml) *in vitro* environment of group § $p < 0.05$; §§ $p < 0.001$, compared with Rhizoma polypodii group (ANOVA, LSD-test). Values are given as mean ± S.E.M.

Table 1. The DPPH$^{\bullet}$ Scavenging effects of *R.poypodii* methanolic extracts (50 µL) The difference between plant extracts and quercetin was statistically non-significant (α $p>0.05$) when they were assessed in terms of DPPH capacity of free radical scavenging. (ANOVA, LSD-test). Values are given as mean ± S.E.M

Groups	Absorbance of 0 the minute	Absorbance of 30 the minutes	DPPH Scavencing effect Results (%)
DPPH Solution	0,56	0,56	0,56
DPPHSolution+*R.polypodii*	0,57	0,0293	94,76±0.37
DPPHSolution+*Quercetin*	0,55	0,0026	99,52±0,52

According to the fatty acids results, oleic, linoleic and linolenic acid amounts were significantly high in the plant extract group compared to the Fenton R. group ($p<0.001$) (Fig. 2). An important decrease in the Fenton R group compared to control group was detected ($p<0.001$) (Fig. 2) Also, the oleic acid level was higher than the control group ($p<0.001$) (Fig. 2). Depending on the flavonoid analysis results with HPLC-DAD, a significant amounts of catechin, naringenin, resveratrol and quercetin in the *R. polypodii* extract were present (Table 2).

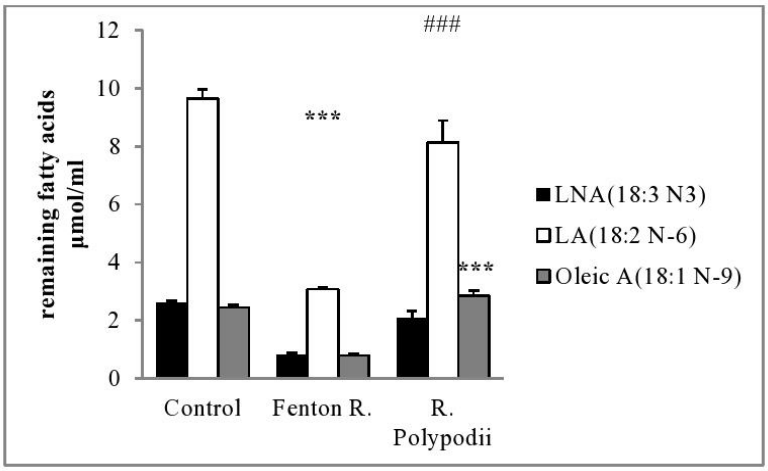

Fig 2. The levels of remaining fatty acids *in vitro* environment (µmol/ml) *** $p < 0.001$ compared with control group; ### $p < 0.001$, compared with Fenton R. group. (ANOVA, LSD-test). Values are given as mean ± S.E.M.

Table 2. The contents of flavonoids in the extracts of *R.polypodii* (µg/1 g)

Flavonoids	*R.polypodii*
Quercetin	0,25
Catachin	1296,5
Narıngenin	696,5
Resveratrol	597,75
Total	2591

4. DISCUSSIONS

In aerobic cells the energy required to fuel biological functions is produced in the electron transport system of mitochondria. In addition to energy, ROS generated in the cells as a normal product of cellular metabolism and are formed which have the potential to cause progressive oxidative and cellular damage. Reactive oxygen species can damage to cellular components such as DNA, RNA, lipid and proteins which theoretically contributes to the physiology of aging. Hydrogen peroxide (H_2O_2) is a major Reactive Oxygen Species. Catalase and superoxide dismutase help to minimize the damaging effects of hydrogen peroxide and breakdown it into oxygen and water, however this conversion is not efficient, and remnant peroxides persist in the cellular environment. While ROS are produced as a product of normal cellular metabolism, excessive ROS can induce oxidative damage in cell constituents and promote some degenerative diseases and aging [30].

In our findings it was determined that in vitro environments where unsaturated fatty acids, H_2O_2 and Fe^{+} ions exist that the the level of MDA- TBA, which is one of the most important criteria of LPO [38], is not statistically differences in the plant extract group compared with control group ($p>0.05$). At the same time, when *R. polypodii* and control group were compared with Fenton R. group, our data was demonstrated that LPO level in the Fenton R. group was high seven folds ($p<0.001$) (Fig. 2).

In this study the decrease in high level of LPO in the plant extracts that determined only in the Fenton including group, is believed to be related to its flavonoid ingredients. Catechin, naringenin, resveratrol and quercetin are the flavonoid contents of *R.polypodii* (Fig. 1, Table 2).

Flavonoids are found in plants, foods, and the most common phenolic compounds belong to the flavonoids [20]. Stratil et al. 2006 found relationship between the ingredient of phenolic substances and the total antioxidant activity of samples.

Flavonoids have been called to as "nature's biological response modifiers" through their beneficial effects to help the body resist to viruses, allergens, and carcinogens. Azmi et al. have studied that plant polyphenols activate endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage. Besides, flavonoids have anti-allergic, anti-inflammatory [36], anti-microbial, anti-viral and anti-

cancer activities [9]. The hypothesis that flavonoids may reduced heart disease rate in 1993 when Hertog et al. In men ın the Netherlands reported an statistically inverse correlation between the intake of five major flavonoids (quercetin, kaempferol, myricetin, luteolin, and apigenin) and mortality or coronary heart disease incidence [21].

Many researcher have studied the effects of flavonoids on LPO [1, 7, 13, 15]. Resveratrol is able to inhibit lipid peroxidation induced ethanol as reported Kasdallah et al, Duthie et al. have assessed that quercetin sweeped the reactive oxygen species. Resveratrol reduced the mitochondrial LPO[37]. Kostiuk et al. have assessed that antioxidative activity of rutin and quercetin in different systems of LPO.

Dorozhko et al. have researched the antiradical and chelating effect of one of the phenolic compounds of rutin during peroxidation of lipids from microsomes and liposomes. Vafeiadou et al. have reported that a type of flavonoid naringenin impedes inflammatory signalling in glial cells and protects against neuroinflammatory damage.
We assess that a relationship between the reductions in the level of lipid peroxide of the *Rhizoma polypodii* extract and scavenging activity of the DPPH radical. Also, it was determined that a statistically non difference between *Rhizoma polypodii* vs quercetin and that the extract has high level of DPPH radical scavenging ability as quercetin (Table 2).

Hosseinimehr et al. have researched that total flavonoids, phenol contents and radical scavenging activity of five plants extracts using DPPH method. The other study Srinivasan et al. have investigated that protective effect of Caesalpinia digyna root (CDM) of using DPPH method. Johnson and Loo (2000) effects of epigallocatechin gallate (EGCG) and quercetin on oxidative injury to cellular DNA of using DPPH method. They suggested that low concentrations of EGCG and quercetin scavenged free radicals, however inhibiting oxidative DNA brekage. But, high concentrations of these extracts caused cellular DNA damage. Fagali and Catala have reported that antioxidant activity of linoleic acid by DPPH method.

Here in the present, when fatty acid amount was studied the unsaturated fatty acids in *Rhizoma polypodii* extract are high compared with Fenton R group (Fig. 3). So we assessed that the flavonoid of extract may protect the three fatty acids from radical sourced oxidations.

Huang et al. have studied the oxidative damage of genistein in endothelial cells. Naderi et al. have researched some flavonoids like morin, genistein, apigenin and biochanin A, naringin and quercetin at different concentration. This research showed that flavonoids prevented in vitro LDL oxidation and probably would be important to prevent atherosclerosis.

Davalos et al. have evaluated commercial dietary antioxidant supplements for their in vitro antioxidant capacity (by different methodologies: antiradical activity against DPPH, inhibition of methyl linoleate autoxidation, and resistance to ion-dependent oxidation of human low-density lipoprotein).

Depending on the results, we approve that organisms will prevent oxidation in the unsaturated fatty acids of membrane structure as a result of consume of these plants as dietary, and that they will reduce the number of lipid peroxidation level. Consequently, we think that there is relationship among *Rhizoma polypodii*'s phenolic compound, avoiding lipid peroxidation and significantly inhibiting the lipid peroxidation. Also, it was determined that the plant extract has a high level capacity to scavenge the radical of DPPH. Antioxidants are frequently used in food and pharmacological industry. However, this compound could be evaluated in these points.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

[1] Akkaya, H., Yilmaz, O., 'Antioxidant Capacity and Radical Scavenging Activity of the *Silybum marianum* and *Ceratonia siliqua L*' ***Ekoloji*** 21, 82: 9-16 (2012).

[2] 'Atropa -poisining with alkaloide-', ***Sisli Child Hospital Medical Bulletin*** 8, 183 (1974).

[3] Azmi, A.S., Bhat, S.H., Hanif, S., Hadi S.M., 'Plant polyphenols mobilize endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: a putative mechanism for anticancer properties' ***Federation of European Biochemical Societies Letters*** 580(2): 533-8 (2006).

[4] Baytop, A., 'Wild Solanaceas of Anatolia' ***Pharmacy Bulletin*** 3: 136 (1961).

[5] Baytop, T., 'Poisonous plants, plant poisoning, and treatment methods in Turkey' ***Istanbul University Pharmacy Faculty*** 51 (1989).

[6] Brand-Williams, W., Cuvelier, M.E., Berset, C., 'Use of a free radical method to evaluate antioxidant activity' ***Lebensmittel-Wissenschaft & Technologie*** 28 (1): 25-30 (1995).

[7] Brown, J.E., Khodr, H., Hider, R.C., Rice-Evans, C.A., 'Structural dependence of flavonoid interaction with Cu^2 ions: implications for their antioxidant properties' ***Biochemical Journal*** 330: 1173-1178 (1998).

[8] Christie, W.W., 'Gas Chromotograhy and Lipid' ***The Oil Pres Glaskow*** 302 (1992).

[9] Cushnie, T.P.T., Lamb, A.J., 'Antimicrobial activity of flavonoids' ***International Journal of Antimicrobial Agents*** 26 (5): 343-356 (2005).

[10] Dávalos, A., Gómez-Cordovés., C., Bartolomé, B., 'Commercial dietary antioxidant supplements assayed for their antioxidant activity by different

methodologies' ***Journal of Agricultural and Food Chemistry*** 23;51(9): 2512-9 (2003).

[11] De Las Heras, A., Schoch, A., Gibis, M., Fischer, A., 'Comparison of methods for determining malondialdehyde in dry sausage by HPLC and the classic TBA test' ***The journal European Food Research and Technology*** 217 (2): 80-184 (2003).

[12] Devasagayam, T.P.A., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S., Lele, R.D., Review: Free radicals and antioxidants in human health: Current status and future prospects, ***Journal* of the *Association* of *Physicians* of India** 52: 794–804 (2004).

[13] Devi, R.P., Ganasoundari, A., Vrinda, B., Srinivasan, K.K, Unnikrishnan, M.K., 'Radiation protection by the ocimum flavonoids orientin and vicenin: mechanisms of action.' ***Radiation Research*** 154: 455-460 (2000).

[14] Dorozhko, A.I., Brodskii, A.V., Afanas'ev, I.B., 'Chelating and antiradical effect of rutin during peroxidation of lipids from microsomes and liposomes' ***Biokhimiia*** 53(10): 1660-6 (1988).

[15] Duthie, S.J., Collins, A.R., Duthie, G.G., Dobson, V.L., 'Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidized pyrimidines) in human lymphocytes' ***Mutation Research*** 393: 223-231 (1997).

[16] Fagali, N., Catalá, A., 'Antioxidant activity of conjugated linoleic acid isomers, linoleic acid and its methyl ester determined by photoemission and DPPH techniques.' ***Biophysical Chemistry*** 137(1): 56-62 (2008).

[17] Finkel, T., Holbrook, N.J., 'Oxidants, oxidative stress and the biology of aging' ***Nature*** 408: 239–247 (2000).

[18] Halliwell, B., J.M.C., 'Gutteridge, *Free Radicals in Biology and Medicine*, 3rd ed.', ***Oxford University Press, Oxford*** (1999).

[19] Harborne, J.B., Williams, C.A., 'Advances in flavonoid research since 1992' ***Phytochemistry***, *55*: 481- 504 (2000).

[20] Hendricks, J.J.A., de Vries, H.E., van der Pol, S.M.A., van der Berg, T.K., van Tol, E.A.F., Dijkstra, C.D., Flavonoids inhibit myelin phagocytosis by macrophages; a structure-activity relationship study. ***Biochemical Pharmacology*** 65: 877–885 (2003).

[21] Hertog, M.G., Feskens, E.J., Hollman, P.C., Katan, M.B., Kromhout, D., 'Dietary antioxidant flavonoids and risk of coronary heart disease' ***the Zutphen Elderly Study Lancet*** 23;342(8878): 1007-11 (1993).

[22] Hosseinimehr, S.J., Pourmorad, F., Shahabimajd, N., Shahrbandy, K., Hosseinzadeh, R., 'In vitro antioxidant activity of Polygonium hyrcanicum, Centaurea depressa, Sambucus ebulus, Mentha spicata and Phytolacca americana' ***Pakistan Journal of Biological Sciences*** 15;10(4): 637-40 (2007).

[23] Hsu, B., Coupar, I. M., 'Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica' ***Food Chemistry*** 98: 317–328 (2006).

[24] Huang, G., Liu, Y., Chang. H., Zhang. X., Ren, D., Wilson, J.X., Cao, X., 'Effects of genistein on oxidative injury in endothelial cells.' ***Journal of Nutritional Science and Vitaminology*** (Tokyo) 54(5): 402-8 (2008).

[25] Johnson, M.K., Loo, G., 'Effects of epigallocatechin gallate and quercetin on oxidative damage to cellular DNA.' ***Mutation Research*** 28;459(3): 211-8 (2000).

[26] Kasdallah, Grissa., A., Mornagui, B., Aouani, E., Hammami, M., Gharbi, N., Kamoun, A., El-Fazaa, S., Protective effect of resveratrol on ethanol-induced lipid peroxidation in rats. ***Alcohol*** 41(3): 236-9 (2006).

[27] Kostiuk, V.A., Potapovich, A.I., Tereshchenko, S.M., Afanas'ev, I.B., 'Antioxidative activity of flavonoids in various systems of lipid peroxidation' ***Biochemistry*** 53(8): 1365-70 (1988).

[28] Macheix, J. J., Fleuriet, A., Billot, J., '*Fruit phenolics'* ***CRC Press***: Boca Raton, Florida, USA 105-148 (1990).

[29] Naderi, G.A., Asgary, S., Sarraf-Zadegan, N., Shirvany, H., 'Anti-oxidant effect of flavonoids on the susceptibility of LDL oxidation' ***Molecular Cellular Biochemistry*** 246(1-2): 193-6 (2003).

[30] Patel, R.P., Cornwell, T., Darley-USMAR, V.M., 'The biochemistry of nitric oxide and peroxynitrite: implications for mitochondrial function. In: Understanding the process of ageing: The roles of mitochondria, free radicals, and antioxidants. Eds: E Cadenas and L Packer, Marcel Dekker, Inc Lounge in New York, ***Academic Press,*** pp 39-40. 29 Basel 39-40 (1999).

[31] Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., 'Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of Vaccinium species' ***Journal of Agricultural and Food Chemistry*** 46: 2686-93 (1998).

[32] Shimoi, K., Masuda, S., Furugoru. M., Esaki, S., Kinae, N., 'Radioprotective effect of antioxidative flavonoids in X-ray irradiated mice' ***Carcinogenesis*** 15: 2669-2672 (1994).

[33] Srinivasan, R., Chandrasekar, M.J., Nanjan, M.J., Suresh, B., 'Antioxidant activity of Caesalpinia digyna root' ***Journal of Ethnopharmacology*** 5;113(2): 284-91 (2007).

[34] Stratil, P., Klejdus, B., Kublan, V., 'Determination of total content of phenolic compounds and their

antioxidant activity in vegetables-evaluation of spectrophometric methods' ***Journal of Agricultural and Food Chemistry*** 54: 607-616 (2006).

[35] Vafeiadou, K., Vauzour, D., Lee, H.Y., Rodriguez-Mateos, A., Williams, R.J., Spencer, J.P., 'The citrus flavanone naringenin inhibits inflammatory signalling in glial cells and protects against neuroinflammatory injury' ***Archives of Biochemistry and Biophysics***1; 484(1): 100-9 (2009).

[36] Yamamoto, Y., Gaynor, R.B., 'Therapeutic potential of inhibition of the *NF-KB* pathway in the treatment of inflammation and cancer' ***Journal of Clinical Investigation.*** 107 (2): 135 (2005).

[37] Yousuf, S., Atif, F., Ahmad, M., Hoda, N., Ishrat, T., Khan, B., Islam, F., 'Resveratrol exerts its neuroprotective effect by modulating mitochondrial dysfunctions and associated cell death during cerebral ischemia' ***Brain Research*** 23, 1250: 242-53 (2009).

[38] Yilmaz, O., Akkaya, H., 'Radical scavenging Activity of *Thymbra spicata* and *Quercus ithaburensis* in Fenton Reagent environment and Their Protective Effects on the Unsaturated Fatty Acids' ***Ekoloji*** 21, 85: 34-40 (2012).

[39] Zu, Y., Li, C., Fu, Y., Zhao, C., 'Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD' ***Journal Pharmaceutical and Biomedical Analysis.*** 41: 714–719 (2006).