ISBN 978-81-934174-2-3
6th International Conference on Healthcare, Environment, Food and Biological Sciences (HEFBS-17)
Istanbul (Turkey) Sept. 8-10, 2017

Study on Total Phenolic, Flavonoid Contents and Antioxidant Activity of Water Extract of Endemic Plant Satureja Hortensis Leaves

Abolfazl Kamkar[1]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Iran

***Abstract*:** *Synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxy toluene are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens. Keeping in this view, there has been a considerable interest by the industry and a growing trend in consumer preferences for natural antioxidants, in this study, total phenolics, flavonoids contents , antioxidant activity and the protective effects of water extract of Satureja hortensis L., in stabilizing soybean oil against oxidation at different concentrations (200 and 400 ppm) were tested.The aerial parts of S. hortensis L. were extracted with 100 ml water. Soybean oil was selected due to its unsaturated fatty acids variety. Antioxidant activities of the water extract of the Satureja hortensis was evaluated by peroxide values and 2-thiobarbituric acid tests in soybean oil for 49 days every 7 days. To assess the antioxidant efficacy of extract, the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and β- carotene/linoleic acid systems were used. Total phenolic and flavonoid contents of samples were investigated. Oxidation experiments were carried out in triplicates; results were averaged and statistically analyzed with one-way ANOVA by using SPSS 19. water extract could significantly lower the peroxide value and thiobarbituric acid value of oil ($p \leq 0.05$). The IC50 value for extract was 21.5 ± 0.7 µg/ml. In the β- carotene/linoleic acid system, aqueous extract exhibited 89.5 ± 1.20 % inhibitions against linoleic acid oxidation. The total phenolic and flavonoid contents of extract was (161.12 ± 0. 28 m g/ g) and (76.39 ± 0. 22 mg/ g), expressed in Gallic acid and Quercetin equivalents, respectively.the leaves of satureja hortensis could be a good source of antioxidant and water extract of satureja may have potential application as natural antioxidant in the edible oil and food industry.*

Keywords: *Total Phenolic, Flavonoid Contents, Antioxidant Activity, Water Extract, Satureja hortensi*

1. Introduction

Vegetable oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acids (PUFAs) are more susceptible to oxidation .Oxidation of oils modifies their organoleptic properties, nutritional value, and affecting the shelf life of the product. Thus, both natural and synthetic antioxidants are widely used (Kamkar et al., 2014).

Synthetic antioxidants which are commonly used such as butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens (Hossain et al., 2011).

Thus, there has been a considerable interest by the industry and a growing trend in consumer preferences for natural antioxidants. Natural antioxidative compounds are found in numerous plant materials such as oilseeds, vegetables, leaves, spices and herbs (Kamkar et al., 2014).

Phenolic compounds from plant sources may act as antioxidants .A large number of vegetables such as kale, spinach, broccoli, onion, rosemary, and satureja are known to be rich sources in antioxidant .Plants belonging to

the Labiates family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties (Kamkar et al., 2014).

Satureja hortensis known medicinal plant which is widely distributed in different parts of Iran. Besides of its usual use in food industry as an aromatic and flavoring agent, it has been received major consideration regard to having anti-inflammatory, antioxidant, antibacterial and antifungal activities (Kamkar et al., 2014).

Several principles have to be considered before making a decision to choose appropriate solvent for plant extraction, the nature of the assayed components, the physicochemical properties of the matrix, the availability of reagents and equipment, the cost and safety concerns.

Recovery of antioxidant compounds from plant materials is typically accomplished through

different extraction techniques taking into account their chemistry and uneven distribution in the plant matrix. However, the extract yields and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent.

Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix.

This may be due to the fact that phenolic compounds are often extracted in higher amounts in more polar solvents.

In other words, extracts prepared with the addition of water exhibited the highest polyphenol and flavonoid content and antioxidant activity, while organic polar solvents were the least efficient extract ants. Due to its advantages, accelerated solvent extraction is an appropriate method for industrial application; for domestic purposes, traditional maceration also affording a very active extract can be recommended (Sultana et al., 2009; Pietrzak et al., 2014).

For this reason, polar solvent was used for the extraction in this work and total phenolic, total flavonoid contents, and antioxidant properties of the obtained extract was assayed by different tests.

In the present study, the antioxidant activity of the water extract (high polarity) of satureja hortensis was assayed through various in vitro models. So far we know, this is the first report on total phenolic, total flavonoids and antioxidant activity of water extract of satureja hortensis leaves.

2. Materials and Methods

2.1 Plant Material and Extraction

The aerial parts of Satureja hortensis L. were collected from Isfahan province (Iran) and dried in the shade, ground in a grinder and a portion (15g) of dried plant material was extracted with 100 ml of distilled water by using an electrical shaker for 6 hours. The extract was filtered using Whatman filter paper (No.1) and concentrated in vacuum at 40°C using a rotary evaporator extractor. Extract was kept in the dark at 4°C until used (Kamkar et al., 2014).

2.2 Samples and Reagents

Chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), and Merck (Germany).

Refined, bleached and deodorized soybean oil without any antioxidants was taken from the manufacturing company (Bahshahr, Oil Factory, and Tehran, Iran).

2.3 Antioxidant Activity

2.3.1 Scavenging Effect on DPPH Radicals

Bleaching of purple colored methanol solution of DPPH was applied to determine the capability of the related extracts and some pure compounds regarding the hydrogen atom or electron donation. This spectrophotometer assay treats constant radical diphenyl picrylhydrazyl as a reagent (Burits and Bucar, 2000).

Fifty microliter of various concentrations of the extract in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percentage terms (I %) was calculated in the following way:

I %=(Ablank-Asample/A blank) ×100

Where A blank is the absorbance of the control reaction (containing all reagents except the test compounds) and A sample is the absorbance of the test compounds. Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract. All tests were done in triplicate. Values (mean ± SD) of the extract were compared with those values of BHT one – way ANOVA.

2.4 β-Carotene-Linoleic Acid Assay

The β -carotene bleaching method is based on the loss of the yellow color of β -carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β -carotene bleaching can be impeded in the presence of antioxidants (Dapkevicius et al., 1998).A stock of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1ml of chloroform (HPLC grade); 25 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water, saturated with oxygen (30 min 100 ml/min), was added with vigorous shaking. Two thousand five hundred micro-liters of this reaction mixture were dispensed to test tubes and 350 µl portions of the extract prepared at 2 g / l concentration was added and emulsion system was incubated for 48h at room temperature. The same procedure was repeated with synthetic antioxidant, BHT, as positive control, and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidant capacities of the extracts were compared with those of BHT and blank.

2.5 Determination of Total Phenolic Content

The total phenolic contents of extract were determined using the Folin–Denis method (Swain and Hills, 1959) with a little modification. This method is based on the reducing power of the phenolic hydroxyl groups; 0.1 ml of extract (10 mg/ml in ethanol) and 1.0 ml Folin–Ciocalteu reagent (1:1 with water) were mixed. After 5 min, 1 ml of 10% Na2CO3 was added and the volume was accurately adjusted to 25 ml. Then the mixture was left at room temperature for 60 min. The absorbance was measured with a spectrophotometer (Shimadzu UV-2550; Shimadzu, Kyoto, Japan) at 765 nm against a blank. The determination was performed three times. Total phenolic contents were calculated from standard curve of Gallic acid, and then expressed as mg / g sample.

2.6 Total Flavonoid Content

Total flavonoid content was determined by the method of Kumaran and Joel Karunakaran (2007).To 2.0 mL of extract solution, 2.0 mL of 2% AlCl3 ethanol and 3.0 mL (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was recorded after 2.5 h at 20 ◦C. Extract samples were evaluated at a final concentration of 0.1 mg/ mL. Total flavonoid contents were calculated from standard curve of quercetin, and then expressed as mg / g sample.

2.7 Oxidative Stability Experiment

2.7.1 Selection of oils

The selection of test oil was based on the presence of varying PUFAs composition. Soybean oil which rich in linolenic acid (18:3n-3) in addition to linoleic acid. Schaal oven test as described by Economou et al. (1991) was conducted to evaluate the effect of antioxidants against oxidation during the accelerated oxidative storage of oils.

2.7.2 Sample preparation

Antioxidative effects of the extract on lipid peroxidation were evaluated in the soybean oil. Each sample (50 ml) was transferred to a series of dark capped glass test bottles. Then Satureja hortensis extract (200, 400

ppm) and BHT (200 ppm) were added to the dark capped glass test bottles and put in an oven at 60 °C. The stability of oil to oxidation was evaluated each week over a 7-week period by analyzing the peroxide values (PVs) and TBARS levels.

At least three samples of each category were analyzed to fulfill the requirements for statistical analyses.

2.7.3 Peroxide value

Peroxide values were measured by AOCS cd 8-53 Official Method (1990).For this purpose, a known weight of oil sample (3 g) was dissolved in glacial acetic acid (30 ml) and chloroform (20 ml). Then saturated KI solution (1 ml) was added. The mixture was kept in the dark for 15 min. After adding of distilled water (50 ml), mixture was titrated against sodium thiosulphate (0.02 N) using starch as an indicator. A blank titration was done parallel to treatment and PVs (meq of oxygen/kg) was calculated using the following formula:

Peroxide value = 1000 S × N/W.

In this formula, S is the volume of sodium thiosulphate solution (blank corrected) in ml; N is the normality of sodium thiosulphate solution (0.02 N) and W is the weight of oil sample (gram).

2.7.4 Thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances were determined weekly, using the method of AOCS (1998).This procedure allows the direct determination of TBARS in oils and fats without preliminary isolation of secondary oxidation products. Oil sample (50-200 mg) was solubilized in10 ml of 1-butanol, mixed with 10 ml of 0.2% TBA in 1-butanol, incubated 2 h in a 95°C water bath and cooled for 10 min under tap water. The absorbance was measured at 532 nm against a corresponding blank (reaction with all the reagents and treatments except the oil).

The standard curve was determined by the TBARS reaction of a series of aliquots (0.1-1ml) of 0.2 mM TMP (1, 1, 3, 3-tetra-ethoxypropane) (Merck, S4258497) prepared in 1-butanol .The results were expressed as μmol malonaldehyde (MDA)/g of oil (n=3).

2.8 Statistical Analysis

Oxidation experiments were carried out in triplicates, and during analysis each measurement was repeated three times. Results were averaged and statistically analyzed with one-way ANOVA by using SPSS 19. Differences between oxidative indicators for the various treatments were calculated by post hoc comparisons of means according to Duncan’ s multiple range tests. Alpha in all cases was 0.05($p \leqslant 0.05$).

3. Results

3.1 Scavenging effect on DPPH radicals

In this study, Satureja hortensis water extract was evaluated considering the capability to control free radicals and to prevent lipid oxidation. The DPPH method with the stable organic radical 1, 1-diphenyl-2-picrylhydrazyl is used for the determination of free radical scavenging activity, usually expressed as IC50, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. According to the results, radicals were controlled more effectively by increasing the extracts concentration. Lower IC50 value indicates a higher antioxidant activity. Fig.1 shows IC50 values for extract and BHT in DPPH assay. The water extract of Satureja hortensis has IC50 value of 21.5 ± 0.7 μg/ml, which is, inversely related to its antioxidant ability. These studies showed that water extract of Satureja hortensis extract possess significant antioxidant activity. The IC50 of BHT was 24.00± 00 μg/ml. Overall antioxidant activity of the water extract was higher than BHT ($p \leq 0.05$).

3.2 β-carotene-linoleic acid assay

This method was based on the loss of the yellow color of β -carotene due to its reaction with radicals formed after linoleic acid oxidation in emulsion. The rate of β -carotene bleaching can be impeded in the presence of antioxidants. Fig. 2 shows inhibition on lipid peroxidation in response to extract. Water extract and BHT

inhibited the linoleic acid oxidation as much as 89.5 % and 88.86%, respectively. In addition, the water extract showed higher inhibition compared to the control (6.55%).

3.3 Total phenolic and flavonoid contents

Table 1 shows the total phenolic and flavonoid contents in water extract of satureja hortensis. According to the results the total phenolic and flavonoid contents of water extract was 161.12 ± 0. 28 m g/ g) and (76.39 ± 0. 22 mg/ g), expressed in Gallic acid and Quercetin equivalents, respectively.

3.4 Oxidative stability of oil

PVs levels during the storage of soybean oil over a 49 days period containing different concentrations of water extract (200, 400 ppm) are shown in comparison to BHT (200 ppm) and the negative control in figures 3.The initial PVs levels in the oil were 2.4 meq/kg. We have shown that water extract reduced PVs at different concentrations (200, 400 ppm) and incubation time points compared to the control (0 ppm). However, in all samples PVs showed a trend to increase from beginning of the storage period to the end of experiment. For the control oil sample the gradual increase in PVs from seventh day to end of the storage period was significantly higher than the treated samples and BHT. ($p \leqslant 0.05$). Overall, the amounts of PVs reduced with increase of the extract concentration ($p \leqslant 0.05$).

Fig. 4 shows the values of TBARS in BHT (200 ppm) and water extract (200, 400, ppm) compared to the control (0 ppm). TBARS values of treatment oil samples were lower than the control during days 7 until the end of storage period ($p \leqslant 0.05$). TBARS values of oil samples containing water extract (400 ppm) at the end of experiment (days 42 and 49) were significantly lower than the containing BHT oil sample ($p \leqslant 0.05$).

We have found that PVs and TBARS values of oil treated with all concentrations of the water extract and BHT were lower than the control over the 49 days of storage ($p \leqslant 0.05$).

4. Discussion

The DPPH scavenging capability has been widely used to evaluate the antioxidant capacity of extracts from different plant materials and is a stable organic nitrogen free radical. DPPH is scavenged by reductants contained in the tested materials through the donation of hydrogen, forming its reduced form. The color changes from purple to yellow after reduced, which can be quantified by its decrease of absorbance at the wavelength of 517 nm (Yang et al., 2014).

In this study, Iranian satureja hortensis water extract was evaluated considering the capability to control free radicals and to prevent lipid oxidation. Antioxidant activity of water extract and their abilities in inhibiting lipid oxidations was different, and radicals were controlled more effectively by increasing the extracts concentration.

In this research he DPPH free radical-scavenging capabilities of water extract of S. hortensis was 21.5 μg/ml , that was higher than those reported by Kamkar et al.,(2014) for methanol (31.5±0.7 μg/ml) and ethanol (37±0 μg/ml) extracts of this plant. The ability to control free radicals by water extract was higher compared to BHT. The differences between S. hortensis extracts and BHT, especially methanol ones are very weak. Moreover, the DPPH radical scavenging activity of water extract in this study was lower than results obtained for water extracts of P. gnaphalodes (Kamkar et al., 2012) , Mangifera indica leaf (Kawpoomhae et al., 2010) , and hydroalcoholic extract of Mediterranean dietary plants (Conforti et al., 2008).

Some studies have indicated that inhibitory potency of free radicals may be related mainly to phenolic compounds of plants such as flavonoids and phenolic acid in polar extract of plants. In fact, it has been found that antioxidant molecules such as polyphenols, flavonoids, and tannins reduce and discolor DPPH due to their hydrogen donating ability (Msaada et al., 2014). These kinds of extracts have superior control effect on free radicals in comparison with non-polar ones. (Gulcin et al., 2004; Bektas, et al., 2005; Sharififar et al., 2007; Sarikurkcu et al., 2008).

As a result, β-carotene is oxidized and broken down in part; subsequently the system loses its chromospheres and characteristic orange color, which is monitored spectrophotometrically (Kamkar et al.,

2014) .As can be seen from the Fig. 2, the % inhibition capacity of the water extract (89.5 %) was found higher to the inhibition capacity of the positive control BHT (88.86%). On the other hand, BHT had lower antioxidant activities than water extract. Other studies showed that BHT was more potent than the plant extracts (Tosun et al., 2009; Wu et al., 2009).Previous study indicated that the inhibition ability of methanolic and ethanolic extracts of Satureja hortensis was lower than aqueous extract (Kamkar et al., 2014). Another study showed that the inhibition capacity of methanol extract of M. globosum was highest and almost equal to the BHT. Weakest activity was related to essential oil (Sarikurkcu et al., 2008).

These compounds in extract inhibit formation of conjugated compounds more effectively than scavenging of free radicals, but polar section has high effects against both tests. In this study, polar section of extract had a higher inhibitory effect on linoleic oxidation. Antioxidants reduce oxidation rate of lipid compounds in cell walls or prevent formation of volatile organic combinations and dien conjugated hydroproxides .Here again, polar extracts have more effective activities in comparison with non-polar . Difference in antioxidation power of various extracts may be as a result of quantity and type of phenolic compounds. Moreover, presences of non-phenolic antioxidants such as vitamin C and A and β-Carotene will also be helpful in performance of the antioxidant (Dapkevicius et al., 1998; Gulcin et al., 2004; Sharififar et al., 2007; Sarikurkcu et al., 2008;Monfarred etal.,2012). It should be noted that phenolic compounds operate more effectively as hydrogen supplier and therefore they can provide the impressive antioxidants (Gulluce et al., 2007; Senji and Yuuya, 2008).

Several methods have been used to measure of variety of oxidation products. Lipid hydroperoxides is one commonly measured parameter. Iodometric titration has been the preferred method for measurement of hydroperoxides as peroxide value (PV). Common measures of secondary lipid oxidation products are, for example, the anisidine value and malonaldehye (MDA) (Halvorsen et al.,2011).

PVs and TBARS levels in the soybean oil containing different concentrations of water extracts (200, 400 ppm) are shown in comparison to BHT (200 ppm) and the negative control (0 ppm) in figures 3, 4. Amounts of these parameters were 2.4 meq kg-1 and 43 nmol kg-1, respectively in initial time.

This study results demonstrated that the water extract of S.hortensis are able to decrease both primary and secondary oxidation of soybean oil during storage. While, PVs and TBARS levels of soybean oil in the control group showed a rapid increase after 7 days of incubation, but a slight increase were shown in the treated oil samples with water extract. The amounts of PVs and TBARS reduced with increase of the extract concentration.Stability effects of methanolic and ethanolic extracts of satureja hortens in soybean oil in previous study were proven (Kamkar et al., 2014). The stability effect of water extract was higher than the methanolic and ethanolic extracts. On the other hand, the stability effects of water extract (WE) ,methanolic extract(ME), and ethanolic extract(EE) were inorder:WE>ME>EE.

Already antioxidative effects of various plant extracts in vegetable oil have been reported. For example the inhibitory effect of sorghum crude phenolic extract in sunflower oil on primary oxidation was lower than synthetic antioxidant of TBHQ; regardless their abilities for inhibiting the secondary oxidation were similar. (Anwar et al. 2007; Sikwese and Duodu 2007).Water extract of Harng Jyur (Chrysanthemum morifolium) varieties in soy bean oil emulsion showed a higher inhibition on both PV and TBARS values (Duh ,1999).

Ethanol extract of Satureja hortensis L. had strong antioxidative action during oxidation of sunflower oil at 100 °C (Marinova and Yanishlieva ,1997).Ether and water extracts of the Urtica dioica L. (Monfared et al. (2011), water and methanol extracts of Mentha pulegium (Kamkar et al., (2010) showed a higher inhibition capacity in creation of both peroxide and MDA. Researchers conclude that the potent antioxidant compounds like flavonoids, terpenoids and poly phosphates which exist in leaves of vegetables possess higher water solubility than the other solvents (Rahmat et al. 2003, Arumugam et al. 2006) and it can be the reason of superiority of water extract in some cases compared to other extracts.

Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of Gallic acid equivalents as described above. As can be seen from the table 1, data obtained from the total phenolic and flavonoid assays support the key role of phenolic compounds

in free radical scavenging and/or antioxidant systems. As expected, amount of the total phenolic and total flavonoid were high in water fraction (table 1).Previous study indicated that total phenolic and flavonoid contents of metanolic and ethanolic extracts of Satureja hortensis were lower than water extract (Kamkar et al., 2014). According to Sarikurkcu et al., (2008) amount of the total phenolic was highest in polar sub-fraction, but total flavonoid content has been found equal for polar sub fraction and non-polar sub fraction.

The total phenol and flavonoid concentrations of extracts were strongly affected by extractants.Besides; a positive correlation is between antioxidant activity potential and amount of phenolic compounds of the extracts (Maizura et al. (2011).

This feature can be due to the presence of water soluble active ingredients such as flavonoids in polar extract of plants (Arumugam et al., 2006; Rahmat et al., 2003).Such results have attributed to the presence of several antioxidants with a range of solubility. Generally, higher extract yields, phenolic contents and plant materials antioxidant activity were obtained using aqueous organic solvents, as compared to the respective absolute organic solvents (Sultana et al., 2009).

On the other hand, extract prepared with water – polar solvent mixture displayed the highest TPC, TFC,and antioxidant activity , while organic polar solvents were the least efficient extractants (Pietrzak et al.,2014).

5. Conclusion

We have shown that water extract of S. hortensis can be considered as an antioxidant and antiradical. Aqueous extract showed antioxidative potency when added to soybean oil. The protective effect of the S. hortensis extract was comparable with widely used synthetic antioxidant BHT.

Thus, water extract of S.hortensis could be prepared and added to the commercial vegetable oils as natural antioxidant and suitable alternative for synthetic antioxidants such as BHT.

6. Conflict of Interest

The author declares that there are no conflicts of interest.

7. References

[1] American Oil Chemists' Society (AOCS). (1990). Official Method Cd 8–53 and Method Cd 1890. 4th ed.

[2] American Oil Chemists' Society (AOCS). (1998). Official Method Cd 19-90. 2-Thiobarbituric Acid Value. 5th ed.

[3] Anwar F., Siddiq A., Iqbal S., Asi M.R. (2007). Stabilization Of Sun-Flower Oil With Moringa oleifera Leaves under ambient storage. Journal of Food Lipids. 14: 35-49.
https://doi.org/10.1111/j.1745-4522.2006.00069.x

[4] Arumugam P., Ramamurthy P., Santhiya S.T., Ramesh A. (2006). Antioxidant Activity Measured In Different Solvent Fractions Obtained from Mentha Spicata Linn.: An Analysis By ABTS+ Decolorization Assay. Asia Pacific Journal of Clinical Nutrition. 15: 119-124.

[5] Bektas T., Dimitra D., Atalay S., Munevver S., Moschos P.(2005). Antimicrobial And Antioxidant Activities of the Essential Oil and Various Extract of Saliva tomentosa miller (Lamiaceae). Food Chemistry, 90: 333-340.
https://doi.org/10.1016/j.foodchem.2003.09.013

[6] Burits M., Bucar F. (2000). Antioxidant Activity of Nigella Sativa Essential Oil. Phytotherapy Research. 14: 323-328.
https://doi.org/10.1002/1099-1573(200008)14:5%3C323::AID-PTR621%3E3.0.CO;2-Q

[7] Conforti F., Sosa S., Marrelli M., Menichini F., Statti G.A ., Uzunov D. (2008). In Vivo Anti-Inflammatory and In Vitro Antioxidant Activities of Mediterranean Dietary Plants. Journal of Ethnopharmacology; 116: 144–151.
https://doi.org/10.1016/j.jep.2007.11.015

[8] Dapkevicius A., Venskutonis R., Van Beek T.A., Linssen J.P. (1998). Antioxidant Activity of Extracts Obtained By Different Isolation Procedures From Some Aromatic Herbs Grown In Lithuania. Journal of the Science of Food and Agriculture. 77: 140-146.

https://doi.org/10.1002/(SICI)1097-0010(199805)77:1%3C140::AID-JSFA18%3E3.0.CO;2-K

[9] Duh P.D. (1999). Antioxidant Activity of Water Extract of Four Harng Jyur (Chrysanthemum Morifolium Ramat) Varieties In Soybean Oil Emulsion. Food Chemistry. 66: 471-476.

[10] Economou K.D., Oreopoulou V., Thomopoulos C.D. (1991). Antioxidant Activity of Some Plant Extracts of The Family of Labiate. Journal of the American Oil Chemist's Society, 68 (2): 109-113.

https://doi.org/10.1007/BF02662329

[11] Gulcin I., Kufrevioglu I.R., Oktag M., Buyukokuroglu E.M. (2004). Antioxidant, Antimicrobial, Antiulcer and Analgesic Activities of Nettle (Urtica Dioica L.). Journal of Enthnopharmacology, 90: 205-215.

https://doi.org/10.1016/j.jep.2003.09.028

[12] Gulluce M., Sahin F., Sokmen M., Ozer H., Daferera D., Sokmen A., Polissiou M., Adiguzel A., Ozkan H., Hajhashemi V., Ghannadi A., Pezeshkian S.K. (2007). Antinociceptive and Anti-Inflammatory Effects of Satureja Hortensis L. Extracts and Essential Oil. Journal of Ethnopharmacology. 82: 83-87.

[13] Halvorsen BL., Blomhoff R.(2011). Determination of Lipid Oxidation Products in Vegetable Oils and Marine Omega-3 Supplements. Food Nutrition Researcn; 55:10

https://doi.org/10.3402/fnr.v55i0.5792

[14] Hossain AM., Dawood Shah M., Gnanaraj C., Iqbal M. (2011). In Vitro Total Phenolics, Flavonoids Contents and Antioxidant Activity of Essential Oil, Various Organic Extracts From The Leaves Of Tropical Medicinal Plant Tetrastigma From Sabah. Asian Pacific Journal of Tropical Medicine, 11,717-721.

https://doi.org/10.1016/S1995-7645(11)60180-6

[15] Kamkar A., Javan A.J., Asadi F., Kamalinejad M. (2010). The Antioxidative Effect of Iranian Mentha Pulegium Extracts and Essential Oil in Sunflower Oil. Food and Chemical Toxicology. 48: 1796-1800.

https://doi.org/10.1016/j.fct.2010.04.003

[16] Kamkar A., Shariatifar N., Jamshidi A.H., Jebelli Javan A., Sadeghi T., Zeagham Monfared M.M. (2012). In vitro Evaluation of Antioxidant Activity of Iranian Mentha Longifolia Essential Oil and Extracts. Journal of Medicinal Plants, 11: 185–194.

[17] Kamkar A., Tooriyan F., Jafari M., Bagherzade M., Saadatjou S., Molaee Aghaee E. (2014).

[18] Antioxidant Activity of Methanol and Ethanol Extracts of Satureja hortensis L. in Soybean Oil,

[19] Journal of Food Quality and Hazards Control, 1: 113-119

[20] Kawpoomhae K., Sukma M., Ngawhirunpat T., Opanasopit P., Sripattanaporn A. (2010). Antioxidant and Neuroprotective Effects of Standardized Extracts of Mangifera Indica Leaf. The Thai Journal of Pharmaceutical Sciences, 34: 32–43.

[21] Kumaran A., Karunakaran R.J. (2007). Antioxidant Activity of Cassiauriculata Flowers. Fitoterapia. 78: 46-47.

https://doi.org/10.1016/j.fitote.2006.09.031

[22] Maizura M., Aminah A., Wan Aida W.M. (2011). Total Phenolic Content and Antioxidant Activity of Kesum (Polygonum Minus), Ginger (Zingiber Officinale) and Turmeric (Curcuma Longa) Extract. International Food Research Journal. 18: 529–534.

[23] Marinova E., Yanishlieva N. (1997). Antioxidative Activity of Extracts from Selected Species of the Family Lamiaceae in Sunflower Oil. Food Chemistry, 58: 245-248.

https://doi.org/10.1016/S0308-8146(96)00223-3

[24] Msaada K., Ben Jemia M., Salem N., Bachrouch O., Sriti J., Tammar S., Bettaieb I., Jabri I., Kefi S., Limam M., Brahim M. Marzouk.(2014). Antioxidant Activity of Methanolic Extracts From Three Coriander (Coriandrum Sativum L.) Fruit Varietie. Arabian Journal of Chemistry.inpress.

[25] Monfared M., Kamkar A., Khaligh S.G., Javan A.J., Asadi F., Basti A.A. (2011). Antioxidative Effects of Iranian Urtica dioica L. Extracts on the Oxidation of Sunflower Oil. Journal of Medicinal Plants Research. 5: 4438-4445.

[26] Pietrzak W., Nowak R., Olech M. (2014). Effect Of Extraction Method On Phenolic Content and Antioxidant Activity Of Mistletoe Extracts From viscum album subsp.abietis. Chemical Papers ,68 (7) 976–982

https://doi.org/10.2478/s11696-013-0524-4

[27] Rahmat A., Kumar V., Fong L.M., Endrini S., Sani H.A. (2003). Determination of Total Antioxidant Activity in Three Types of Local Vegetables Shoots and the Cytotoxic Effect of Their Ethanolic Extracts Against Different Cancer Cell Lines. Asia Pacific Journal of Clinical Nutrition. 12: 308-311.

[28] Sharififar F., Moshafi M.H., Mansouri M., Khodashenas M., Khoshnoodi N. (2007). In vitro Evaluation of Antibacterial and Antioxidant Activities of The Essential Oil and Methanolic Extract of Endemic Zataria Multiflura Boiss. Food Control, 18(7): 805-800.

https://doi.org/10.1016/j.foodcont.2006.04.002

[29] Sarikurkcu C., Tepe B., Daferera D., Polissiou M., Harmandar M. (2008). Studies on The Antioxidant Activity of The Essential Oil and Methanol Extract of Marrubium Globosum Subsp. Globosum (Lamiaceae) By Three Different Chemical Assays. Bioresource Technology, 99: 4239-4246.

https://doi.org/10.1016/j.biortech.2007.08.058

[30] Senji S., Yuuya I. (2008).Comparison of Antioxidant Properties of Persimmon Vinegar and Some Other Commercial Vinegar In Radical-Scavenging Assays and On Lipid Oxidation in Tuna Homogenates. Food Chemistry, 107(2): 744-739.

[31] Sikwese F., Duodu K.G. (2007). Antioxidant Effect Of A Crude Phenolic Extract From Sorghum Bran In Sunflower Oil In The Presence of Ferric Ions. Food Chemistry. 104: 324-331.

https://doi.org/10.1016/j.foodchem.2006.11.042

[32] Sultana B., Anwar F., Ashraf M. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules, 14, 2167-2180.

https://doi.org/10.3390/molecules14062167

[33] Swain T., Hillis W. (1959). The Phenolic Constituents of Prunus Domestica. The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture, 10: 63-68.

https://doi.org/10.1002/jsfa.2740100110

[34] Tosun M., Ercisli S., Sengul M., Ozer H., Polat T., Ozturk E. (2009). Antioxidant Properties and Total Phenolic Content of Eight Salvia Species from Turkey. Biological Research, 42: 175-181.

https://doi.org/10.4067/S0716-97602009000200005

[35] Wu N., Fu K., Fu Y.J., Zu Y.G., Chang F.R., Chen Y.H., Liu X.L., Kong Y., Liu W., Gu C.B. (2009). Antioxidant Activities of Ex-Tracts and Main Components of Pigeonpea [Cajanus Cajan (L.) Millsp.] Leaves. Molecules, 14: 1032-1043.

https://doi.org/10.3390/molecules14031032

[36] Yang J., Chen1 C., Zhao S., Ge F., Liu D. (2014). Effect of Solvents on the Antioxidant Activity of Walnut (Juglans regia L.) Shell Extracts . Journal of Food and Nutrition Research, 2, (9) 621-626.

https://doi.org/10.12691/jfnr-2-9-15