TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TTDB

www.dergipark.gov.tr/turkjans

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

Antioxidant Capacity and Phenolic Compounds with HPLC of *Asphodelus ramosus* and Comparison of the Results with *Allium cepa* L. and *Allium porrum* L. Extracts[ð]

[1]Elif APAYDIN*, [2]Gülnur ARABACI

[1]Giresun University, Central Research Laboratory, 28100 Giresun, Turkey
[2]Department of Chemistry, Sakarya University, 54100 Sakarya, Turkey

*Sorumlu Yazar: eliferenapaydin@gmail.com

Received: 16.08.2017 Received in Revised: 21.09.2017 Accepted: 23.09.2017

Abstract

Asphodelus ramosus known as a yellow lily which has been a plant of onion for this reason, this plant was studied with about antioxidant activity of methanol, 50 % methanol, ethyl acetate and water extracts. The plant was dried at two different temperatures (35 ºC and 65 ºC) so as to understand how to work the difference in temperature effects. After extraction, all solvent extracts were studied for their total phenolic contents using Folin Ciocelteau reagent, total flavonoid content with aluminum nitrate method. In addition, DPPH free redical scavenging through the determination, the determination of the activity of removal of H_2O_2, copper (II) ion reducing antioxidant capacity determination (CUPRAC), removal activity of super oxide anion were studied and the results were supported with HPLC (High Performance Liquid Chromatography).

Keywords: *Asphodelus ramosus*, extraction, flavonoid, DPPH, H_2O_2, CUPRAC, HPLC

Asphodelus ramosus, Allium cepa L. ve *Allium porrum* L. Bitkilerinin Antioksidan Kapasitesi ve Fenolik Bileşenlerin HPLC ile Ayrımı

Özet

Sarızambak olarak bilinen ve latince adı *Asphodelus Ramosus* olan, çiriş bitkisinin antioksidan özellikleri incelenmiştir. Bu amaçla metanol, % 50 metanol, etil asetat ve su ekstraktları ile çalışılmıştır. Bitki iki farklı sıcaklıkta kurutulup (35 ºC ve 65 ºC), böylece sıcaklık farklılığının bir parametre oluşturması sağlanmıştır. Ekstraksiyon işleminden sonra tüm ekstraktların toplam fenolik bileşenlerine Folin Ciocalteu yöntemi ve toplam flavonoid bileşenlerine aluminum nitrat metodu uygulanmıştır. Buna ilaveten DPPH serbest radikal giderici etkisi, H_2O_2 süpürücü etkisi, bakır (II) iyonu indirgeme gücü (CUPRAC), süperoksit anyon radikali süpürme kapasitesi analiz edilmiş ve tüm bunlar HPLC (Yüksek Performanslı Sıvı Kromatografi) ile desteklenmiştir.

Anahtar Kelimeler: *Asphodelus ramosus*, ekstraksiyon, flavonoid, DPPH, H_2O_2, CUPRAC, HPLC

Introduction

Antioxidants significantly delay oxidation when have been low concentration in metabolizm and foods or the antioxidants prevent oxidation event (Ghosh and Mayers, 1998). Flavonoids are phenolic structure of human nutrition in the most abundant compounds in plants (Velika and Kron, 2012). Food source of flavonoids, flavonols and phenolic compounds like quercetin, kaempferol, gallic acid and myricetin has a wide range of biological as antibacterial, antiviral, antiallergic and antioxidant (Huang et al., 2005). With the increase of flavonoid consumption has an inverse relationship between the occurence of coronary heart disease (Depending on the antioxidant and antithrombotic effects) (Hertog et al., 1993). A study conducted in Japan, plasma total cholesterol and LDL-cholestrol concentrations decreased when increased flavonoid intaking. In other study in Finland quercetin-rich apple and onion consumption increases, coronary mortality found to be decreased (Coskun, 2005).

[ð]This research was produced Ms Thesis.

Quercetin the most important compound of flavonoids and phenolic compound commonly found in plants and onions, cocumbers, broccoli, tomatoes, tea, red wine, fruits, olive oil and apple crust are rich in quercetin. Myricetin has been in cranberry, grape, red wine and kaempherol has been in leek, broccoli, lettuce, grapefruit and black tea (Arora and Kaur, 1999). Onion plants have antioxidant properties so as to *Asphodelus Ramosus* is an onion plants, it was investigated if it is source of flavonoids or not. In Turkey it grows widely in Marmara, Aegean, Mediterranean regions and high mountain areas (Ban et al., 2006). Flavonoids and volatile oil contained in plant is known in literature but is not registered (Zeybek and Zeybek, 1994). So that was investigated total phenolic and total flavonoid activities, free radical scavenging activity and for phenolic profile (gallic acid, chlorogenic acid, vanillic acid, caffeic ac id, naringin, quercetin, kaempferol) with HPLC of yellow lily.

Materials and Methods

Plant Material and Reagents

The plants which were oven-dried at 35 ºC and 65 ºC Asphodelus Ramosus, *Allium Cepa L*. and *Allium Porrum L*. and colleceted from Akyazi of Sakarya. The standarts for HPLC analysis were purchased from Sigma-Aldrich Chemie Gmbh (Germany) and Merck (Darmstadt, Germany). HPLC-grade methanol, acetic acid were obtained from Merc (Darmstadt, Germany). Folin Ciocalteu's phenol reagent and 1,1–diphenyl–2–picrylhydrazl (DPPH) were supplied from Merck (Darmstadt, Germany). All other chemicals and solvents were available in our laboratory.

Figure 1. Ground flowering part

Figure 2. After collected ground flowering part

Extraction

The plant samples were cutted little pieces with chopper after dried at different tempratures. The solvent was added as solvent ratio of plant (Plant (g): solvent (mL)) 1:20 and extracted in turbulent water bath for 8 hours at 250 rpm. Methanol, % 50 methanol, ethyl acetate and pure water were used as solvent. Solution was filtered using Whatman fitler paper type at the end of time (Linskens and Jackson, 1997a).

Solvents of filtrate were blown at 40 ºC in evaporator, stock solutions were prepared based on weighing the remaining solid material. These stock solutions were centrifugated at 5000 rpm for 15 minutes, stored in freezer (-8 ºC) as leaving sediment (Linskens and Jackson, 1997b).

Total phenolic compounds and total flavonoid compounds

Total phenols were obtained as gallic acid equavalents (GAE) and exspressed as mg gallic acid/gram extract (Gamez et al., 1999). 0.5 mL of sample, 2.5 ml Folin reagent (10%, v/v, in water) and 7.5 mL of sodium carbonate solution (20%, w/v, in water) were mixed in tube at room temperatur for 2 hours. The same event was used for standart gallic acid (used 0.05- 0.50 mg/mL concentrates). At the end of time the absorbance was measured at 750 nm and compared to gallic acid.

Total flavonoids were obtained as quercetin aquavalents (QE) and were expressed as mg Quercetin equavalent/g extract according to aluminum nitrate method which was modified (Moreno et al., 2000). 500 µl (10 mg/mL) extract was mixed 0.1 mL sodium acetate and after 1 minute 0.1 mL 10% (w/v) $Al(NO_3)_3$ was added and mixed, the volume was completed 5 mL with 96 % ethanol (v/v). The solutions were waited at room temperature for 40 minutes and were measured absorbance at 450 nm.

DPPH (1,1–diphenyl–2–picrylhydrazyl) scavenging activity

Trolox and BHT were used as standart. 1 mL DPPH solvent (50 µg - 1000 µg) was mixed with 4 mL DPPH solution. 1 mL methanol was used as control sample. After incubution at room tempreture the samples and control were measured absorbance at 517 nm (Blois, 1958). Samples absorbance were compared control samples and free radical scavenging activity calculated with this formule:

DPPH Scavenging activity (% Inhibition) = $(A_{control} - A_{Sample}) / A_{control} * 100$

Copper (II) ion reducing antioxidant capacity determination (CUPRAC)

System; the result which of copper (II) neokuproin complex from the addition of the antioxidant solution is based on the reduction of copper (I) neokuproin. Analysis have not been in antioxidants and against a reference at 450 nm obtained by measurement of absorbance value (Blois, 1958). Trolox, vitamin C and BHT were used as standart reference. The test results in terms of Troloks® equivalent antioxidant capacity was expressed as TEAC CUPRAC. Samples and standards were calculated with the curves of the correct equations for the results obtained from the absorbance results of the CUPRAC method against the antioxidant concentration. The slope of each line in the graph was proportioned to the curve of the correct equation for Trolox and the $TEAC_{CUPRAC}$ values were obtained.

Table 1. CUPRAC TEAC values for referance standarts

	Trolox	BHT	Vitamin C
Curve	16.603	20.164	22.82
$TEAC_{CUPRAC}$	**1**	**1.2145**	**1.3744**

Table 2. CUPRAC TEAC values for samples

SAMPLES	T ˚C	water	MeOH	water:MeOH (1:1)	Ethyl acetate
Asphodelus ramosus	35 ˚C	**0.1565**	**0.1708**	**0.1605**	**0.1501**
	65 ˚C	**0.2153**	**0.1868**	**0.1867**	**0.1594**
Allium cepa L.	35 ˚C	**0.2717**	**0.1288**	**0.3001**	**0.1747**
	65 ˚C	**0.2673**	**0.2382**	**0.3291**	**0.2126**
Allium porrum L.	35 ˚C	**0.1185**	**0.1338**	**0.1169**	**0.1735**
	65 ˚C	**0.1279**	**0.1300**	**0.1253**	**0.1723**

Removal activity of super oxide anion radical

This method is based on clearing of assay buffer adjusted to pH = 8 nicotinamide adenine dinucleotide (NADH) with phenazine methasulfate (PMS) between the reaction results released superoxide anion radical ($O_2^{-\bullet}$), Nitro Blue Tetrazolium (NBT) dye color. Superoxide when reacted with NBT, occurs monoformazo firstly and then diformazo. Diformazo gives maximum absorbance at 560 nm wavelength but NBT color is not.

HPLC analysis

Fragmantation profiles was obtained in HPLC system for identified the phenolic compounds and these conmpounds were concluded by comparing with standart peak retention times run under the same HPLC conditions. The HPLC was Shimadzu Prominance and the fragmantation was done with a reverse phase colomn Inertsil ODS-2 GL Sciences Inc. 5 µm (4.6x250 mm) C18 and used Shimadzu SPD-M10 A*vp* PDA detector (270 nm) (Liu, 1997). System flow was 1 mL/min and system temperature was 25 Cº (Miura et al., 2002).

Mobile phase was used as;

A: methanol: water: acetic acid (10:88:2, v/v/v)

B: methanol: water: acetic acid (90:8:2, v/v/v)

Statistical analysis

Results were evaluated by integration of the account. Peak fields were calculated for each standart reagent using the calibration curves as concentration. Standard deviation was used for all the values. The correlations were statistical significant ($p<0.05$). The result is in accordance with literature searchs that shows that a high values in phenolics is often associated with tandem capacity of scavenging radicals (Oomah et al., 2011).

Result and Discussion

Determination of total phenolic compounds was analyzed using Folin Ciocalteu reagent, the most frequently gallic acid was used as a standard (Rodriguez-Delgado et al., 2001) and it was analyzed 0.05-0.5 mg/mL concantration range. Total flavonoid compounds were determined to be equivalent to quercetin with aluminum nitrate method.

Results of yellow lily the dried plant samples at 65 ºC temperature were showed to have higher than the samples which was dried at 35 ºC. The

extracts which dried plant of 35 ˚C are sorted according to differences insolvent:

Waterextract> water: methanol (1:1) extract> met hanoleextract

Table 3. Amounts of total phenolic compounds of plant extracts

Samples	T (ºC)	Water	MeOH	Water: MeOH (1:1)	Ethyl acetate
Asphodolus ramosus	35 ˚C	33.51 ±0.33	28.15 ±0.03	29.73 ±0.07	26.01 ±0.07
	65 ˚C	44.46 ±0.07	41.86 ±0.03	42.09 ±0.10	27.55 ±0.03
Allium cepa L.	35 ˚C	25.96 ±0.07	22.09 ±0.26	22.07 ±0,03	16.23 ±0.03
	65 ˚C	35.85 ±0.65	32.29 ±0.36	41.86 ±0.03	38.09 ±0.03
Allium porrum L.	35 ˚C	27.98 ±0.98	18.48 ±0.16	22.83 ±0.13	26.95 ±1.40
	65 ˚C	33.65 ±0.13	23.24 ±0.26	27.50 ±0.10	27.32 ±2.51

*The amount of phenolic substances: phenolic compounds gallic acid is equivalent to the unit was expressed as mg/g extract. Results are average of 3 parallel test, the standard deviation values were considered

Because of water: methanol (1:1) extract and methanol extract values are very close of 65 ºC extracts, we can use the same sort like plants which dried at 35 ºC temperature. Hydroxyl groups of phenols due to the power to destroy free radicals that describes a very important plant components (Singleton et al., 1999). The studies indicates a paralel relationship between total phenol and antioxidant activity (Hatano et al., 1989).

Table 4. Amounts of total flavonoid compounds of plant extracts

Samples	T (ºC)	Water	MeOH	Water: MeOH (1:1)	Samples
Yellow lily	35 ˚C	**754.33** ±0.83	714.03 ±1.75	542.65 ±4.87	733.46 ±1.84
	65 ˚C	713.31 ±2.76	742.24 ±4.14	**881.04** ±2.76	736.71 4.60

*The amount of phenolic substances: phenolic compounds quercetin is equivalent to the unit was expressed as µg flavonoid content / g extract. Results are average of 2 parallel test, the standard deviation values were considered

Results of total flavonoid contend suggest that there is no difference between the temperatures to form a parameter. It was concluded that while the water plant extract which dried at 35 ºC has the highest flavonoid value, the highest flavoniod value of dried plant at 65 ºC is methanol-water (1:1) extract.

DPPH (1,1–diphenyl–2–picrylhydrazyl) scavenging activity;

Although the decrease in absorbance value of the expectedincrease in concentration. Because the absorbance which was decreasing shows that the rest of the DPPH solution amount and that means removal of free radicals.

DPPH free radical scavenging activity comparisons were made on the basis of standard reagents which been trolox and BHT. According to results, trolox with the highest value at 87.69 % (±0.19) and the second was BHT with 74.44 % (±0.15). It was asked to be seen how effect created by different temperature and reported that there is no difference by temperature and recorded that solvents were exchange factor for activity with not to be different effects.

HPLC Analysis; To determine the amount of phenolic acids and recognition of the UV spectra and retantion times were compared with standards (Ozturk et al., 2007). Retantion times of standart reagents could be seen with chromatogrom. Most of phenolic acids make maximum UV absorbance at a wavelength of 270 nm (Vinson et al., 1998).

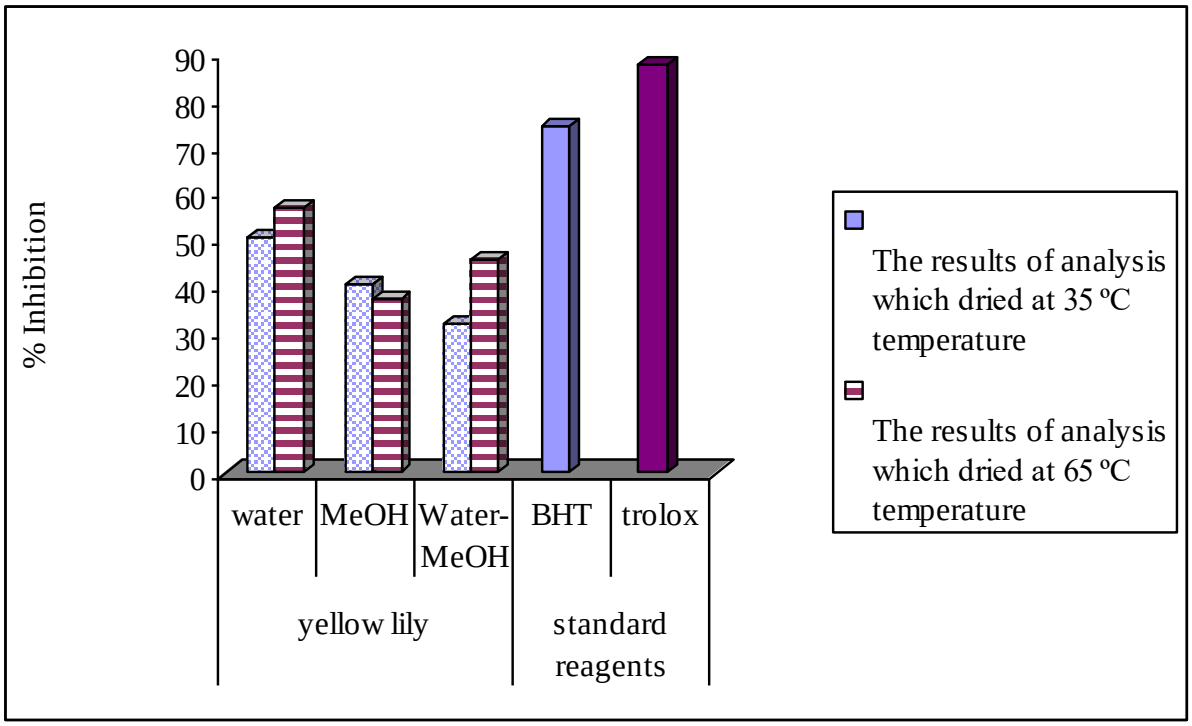

Figure 4. % Inhibition values of DPPH free radical scavenging activity of plant extract (The results of the antioxidant activity are average of two parallel tests)

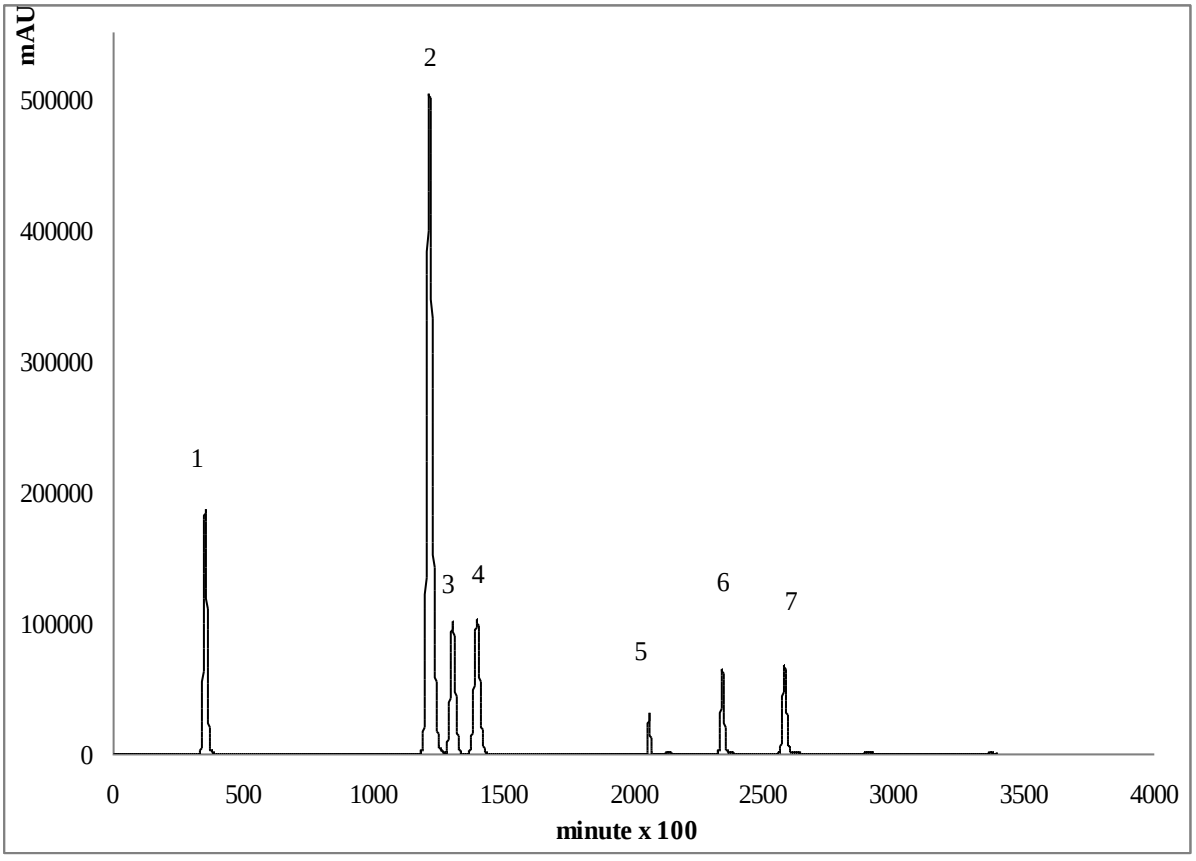

Figure 5. HPLC determination of the optimum conditions of some phenolic acids. 1: gallic acid, 2: chlorogenic acid, 3: vanillic acid, 4: caffeic acid, 5: naringin, 6: quercetin, 7: kaempferol

Table 5. The phenolic compounds of yellow lily dried at 35 ºC of all extracts

		Peak no	Phenolic and flavonoid compounds	Retantion time (minute)	Area	Quantity (mg/10mg/ mL extract)
Yellow lily 35 ˚C	Water extract	1	Gallic acid	3.03	13586	0.016
		2	Chlorogenic acid	11.04	5599	0.002
		5	Naringin	22.79	3864	0.001
		6	Quercetin	24.32	1980	0.020
		7	Kaemferol	27.76	1221	0.004
	Methanol extract	1	Gallic acid	3.25	222522	0.039
		2	Chlorogenic acid	11.01	73642	0.009
		5	Naringin	22.03	95022	0.047
		7	Kaemferol	27.58	85432	0.013
	Metanol:water (1:1) extract	1	Gallic acid	2.93	252355	0.042
		2	Chlorogenic acid	11.06	59407	0.008
		5	Naringin	22.35	120093	0.059
		6	Quercetin	24.35	2678	0.020
		7	Kaemferol	27.72	56789	0.010

Table 6. The phenolic compounds of yellow lily dried at 65 ºC of all extracts

		Peak no	Phenolic and flavonoid compounds	Retantion time (minute)	Area	Quantity (mg/10mg/mL extract)
Yellow lily 65˚C	Water extract	1	Gallic acid	3.53	67124	0.022
		2	Chlorogenic acid	11.43	10579	0.002
		4	Cafeic acid	16.86	3813	0.049
		5	Naringin	22.44	179299	0.089
		6	Quercetin	25.65	2163	0.020
		7	Kaemferol	27.80	1174	0.004
	Methanol extract	1	Gallic acid	3.12	172045	0.033
		2	Chlorogenic acid	10.94	86789	0.011
		3	Vanilic acid	14.05	92768	0.129
		4	Cafeic acid	14.90	76890	0.053
		5	Naringin	22.74	394728	**0.197**
		7	Kaemferol	27.89	98763	0.014
	Metanol: water (1:1) extract	1	Gallic acid	2.93	184874	0.035
		2	Chlorogenic asit	10.89	56276	0.008
		5	Naringin	22.74	310154	0.154
		6	Quercetin	24.40	3456	0.020
		7	Kaemferol	27.79	59890	0.010

Conclusion

According to identification with HPLC of yellow lily which dried at two different temperature was concluded that a plant rich in phenolic compounds. Seven phenolic compounds are used as the standard reagent were found in plant. Different extract have been contained different phenolics. Water extraction which made with the plant was dried at 65 ºC have been caffeic acid as different from 35 ºC's one (0.049 mg caffeic acid / 10mg/mL plant extract). Values of water extracts of phenolic compounds, as shown in the tables were very close to each other. Drying temperature difference of the plants has not established values very close results. Dried 65 ºC in the methanol extraction had caffeic acid and vanilic acid as different from dried 35 ºC temperature. This result concludes that 65 ºC temperature lets to pass plant substance from plant to extraction solution better than 35 ºC.

In MeOH: water (1:1) mix, temperature has no effect which can be difference for analysis results and as to results naringin is main compound in the both of different temperature extract.

References

Arora, D., Kaur, J. 1999. Antimicrobial activity of spices. International Journal of Antimicrobial Agents, 12: 257-262.

Ban, J.Y., Cho S.O., Koh S.B., Song K.S., Bae K., Seong, Y.H. 2006. Protection of amyloid β protein (25-35) induced neurotoxicity by methanol extract of Smilacis chinae rhizome in cultured rat cortical neurons. Journal of Ethnopharmacology, 106: 230-237.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.

Coskun, T. 2005. Health benefits of functional foods. Journal of Child Health and Diseases, 48: 69-84.

Gamez-Meza, N., Noriega-Rodriguez, J.A., Medina-Juarez, L.A., Ortega-Garcia, J., Cazarez-Casanova, R., Angulo-Guerrero, O. 1999. Antioxidant activity in soybean oil of extracts from Thompson grape bagasse. Journal of the American Oil Chemists' Society, 76: 1445-1447.

Ghosh, J., Myers, C.E. 1998. Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. Proceeding of National Academy of Sciences, 95: 13182-7.

Hatano, T., Edamatsu, R., Mori, A., Fujita, Y., Yasuhara, E. 1989. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chemical and pharmaceutical bulletin, 37: 2016-2021.

Hertog, M.G., Feskens, E.J., Hollman, P.C., Katan, M.B., Kromhout, D. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen Elderly Study, Lancet, 342: 1007-1011.

Huang, D., Ou, B., Prior, R.L. 2005. The Chemistry behind antioxidant capacity assays. Journal of Agriculture and Food Chemistry, 53: 1841-1856.

Linskens, H.F., Jackson, J.F. 1997a. Plant volatile analysis. Modern Methods of Plant Analysis, Vol. 19 Springer, Germany.

Linskens, H.F., Jackson, J.F. 1997b. Essential Oils and waxes, Modern Methods of Plant Analysis, Vol. 12 Springer, Germany.

Liu, F., OOI V.E.C. and Chang, S.T. 1997. Free radical scavenging activity of mushroom polysaccharide extracts, Life Science. 60: 763-771.

Miura, K., Kikuzaki, H., Nakatani, N. 2002. Antioxidant activity of chemical components from Sage (*Salvia officinalis L*.) and Thyme (*Thymus vulgaris L*.) measured by the oil stability index method. 2002. Journal of Agriculturel and Food Chemistry, 50: 1845-1851.

Moreno, M.I.N., Isla, M.I., Sampietro, A.R., Vattuone, M.A. 2000. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. Journal of Ethnopharmacol, 71: 109-114.

Oomah, B.D., Caspar, F., Malcolmson, L.J., Bellido, A.S. 2011. Phenolics and antioxidant activity of lentil and pea hulls. Food Research International, 44(1): 436-441.

Ozturk, N., Tuncel, M., Tuncel, N.B. 2007. Determination of phenolic acids by a modified HPLC: Its application to various plant materials. Journal of Liquid Chromatography and Related Technologies, 30: 587-596.

Rodriguez-Delgado, M.A., Malovana, S., Perez, J.P., Borges, T., Garcia Montelonga, F.J. 2001. Journal of Chromatography, 912, 249.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. 1999. Anaysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. Methods in enzymology, 299: 152-178.

Velika, B., Kron, I. 2012. Antioxidant proporties of benzoic acid derivates. *Free* Radical Antioxidant, *2*: 62-67.

Vinson, J.A., Yong, H., Xuchui, S., Zubik, L. 1998. Phenol antioxidant quantity and quality in foods: Vegetables. Journal of Agriculturel and Food Chemistry, 46: 3630-3634.

Zeybek, N., Zeybek, U. 1994. Pharmaceutical botany hardwoods, systematic and significant items. Ege University, Faculty of Pharmacy, Publication number: 2 Ege University Press.