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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

A COMPARATIVE STUDY ON ANTIOXIDANT PROPERTIES AND METAL CONTENTS OF SOME EDIBLE MUSHROOM SAMPLES FROM KASTAMONU, TURKEY

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Abstract:

In this work *Pleurotus ostreatus*, *Agaricus bisporus* and *Lactarius delicious* were used to determine and compare their antioxidant capacities and metal contents. The edible mushroom samples were collected from Kastamonu in the West Black Sea region of Turkey. The antioxidant capacity studies were performed by DPPH (1, 1diphenyl-2-picryl hydrazyl) radical scavenging method and were expressed as Trolox equivalents with spectroscopic measurements. TEAC (Trolox Equivalent Antioxidant Capacity) values were found 0.302, 0.557 and 0.251 µM/g for *Pleurotus ostreatus*, *Lactarius delicious* and *Agaricus bisporus*, respectively. All samples were analyzed by X-ray fluorescence (XRF) spectrometry to obtain the concentration of Cr, Mn, Fe, Ni, Cu, Zn, Ca, Pb, Na, Mg and K. While maximum and minumum metal contents of mushrooms were found as mg/g for Na (96-14.9), Mg (8.83-2.60), K (4.05-3.16), Ca (0.089-0.019) and Fe (0.128-0.099), the maximum and minumum contents of mushrooms were found for Cr (8-5), Mn (12-11), Ni (15-6), Cu (30-20), Zn (7-3) and Pb (3-1) as mg/kg. Metal contents were determined together with antioxidant capacity of all analysed mushrooms. It was observed that although the Fe, Ni, Ca, Na, and Mg contents of *Agaricus bisporus* were lower, it had got higher inhibition than the other mushroom species.

Keywords: Antioxidant capacity, Metal contents, *Agaricus bisporus*, *Pleurotus ostreatus*, *Lactarius delicious*, TEAC values, Kastamonu

Introduction

There are many reactive oxygen species and free radicals that are formed as a result of the oxidation process which is an important process in terms of energy production in biological systems. These reactive species take part in degenerative processes and functional changes associated with diseases like cancer, rheumatoid arthritis, cirrhosis etc (Babu & Rao, 2013).

Antioxidants are substances that may help the body to protect against various types of oxidative damage and can prevent oxidation by various mechanisms such as scavenging free radicals, chelating pro-oxidant metal ions, quenching secondary oxidation products, and inhibiting prooxidative enzymes (Gülçin, 2012; Rajalingam *et al.*, 2013; Bakır *et al.*, 2013).

Recently, naturally occurring antioxidants for use in foods or medicinal materials have started to replace some synthetic antioxidants which are being restricted due to their negative health effect. Mushrooms have natural antioxidans and they have long been consumed as a part of the normal diet in many European countries because of their unique taste and subtle flavor. At the same time mushrooms which are a rich source of nutrients on account of carbohydrate, protein, ascorbic acid, tocopherols, iron, zinc, selenium, sodium, chitin, fibres and minerals have many biologically active components that offer health benefits and protection against degenerative diseases (Baysal *et al.*, 2007; Ouzouni *et al.*, 2009; Yılmaz *et al.*, 2016). These functional properties of mushrooms are mainly due to their chemical composition. Many wild edible mushroom species are known to accumulate high levels of heavy metals. Therefore, many studies have been made on their metal content (Isildak *et al.*, 2004; Cocchi *et al.*, 2006). Intensive research has been carried out to detect and explain the presence and distribution of several heavy metals in edible mushrooms, in particular arsenic, cadmium, cesium, copper, iron, lead, manganese, mercury, selenium, rubidium, and zinc (Cocchi *et al.*, 2006).

The chemical compositions of mushrooms caused some changes in their antioxidant properties and also their metal contents are effective in the change of these properties. But investigations which display the changes of the antioxidant properties with their metal content are very few. The minerals can be accumulated in mushrooms, and this accumulation is generally species metabolism-dependent and also strongly affected by the chemical composition of the substrate from which mushrooms get their nutrients (Radulescu *et al.*, 2010).

Atomic absorption spectrometry (AAS) has been one of the most used techniques for elemental analysis; however, the suitability of X-ray fluorescence (XRF) technique has been established to be suitable to determine the elemental content in biological samples with the big advantage of being non–destructive (Carvalho *et al.*, 2005).

Agaricus bisporus and *Pleurotus ostreatus* preferred by the people because of easily and rapidly cultured, *Lactarius delicious* preferred for its flavor and these mushrooms are always have a comprehensive and regular trade in our country. Previously antioxidant activity wasn't been assessed with metal content together in these mushroom species which were collected from Kastamonu region. In this work we determined the antioxidant capacity in order to identify how changes their antioxidant capacity with concentration of heavy metals in these mushroom species. For this purpose, we calculated antioxidant capacity of mushrooms using DPPH (1, 1diphenyl-2-picryl hydrazyl) radical scavenging method to compare trolox equivalent capacity. We used XRF Spectrometry to find heavy metal concentrations in these edible mushrooms.

Materials and Methods

Study Field and Labratory Works

Pleurotus ostreatus colected from Bozkurt at May 2016 and *Lactarius delicious* collected at November 2016 from Devrekani in Kastamonu. *Agaricus bisporus* samples taken from Kastamonu University Mushroom Research and Application Center. Mushroom species was confirmed by Prof. Dr. Sabri Unal at Mushroom Research and Application Center, Kastamonu University.

All chemicals which were analytical grade provided from Sigma-Aldrich Co. LLC. In each stage deionized purity water was used. Absorbents was measured using a SHIMADZU the UVM-1240 UV-Visible spectrophotometer (Shimazu Corp., Kyoto, Japan manufactures) with a pair of identical quartz cuvette of 1 cm thickness at 517 nm. XRF measurements were made with X-Ray Fluorescence Spectrometer (Spectro Xepos II).

Determination of DPPH Activity

Free radical scavenging effects of trolox and mushroom extracts was performed by using DPPH method. DPPH radical available as commercially and one of the stable radicals that used in the antioxidant capacity and activity assay. Its ethanol solution was purple and gives the maximum absorbance at 515-517 nm. When reduced by antioxidants, its color turns lighter and progress of the reaction can be monitored with a spectrophotometer. The amount of antioxidant which required to reduce DPPH concentration by 50% is a commonly used parameter to measure the antioxidant activity and it is called IC_{50} (mg/mL) (Frankel & Meyer, 2000).

Preparation of mushroom extracts

Pieces of 1 gram were taken from each mushroom species and was pulverized in a porcelain crucible. Then they were dissolved in 10 ml, 75% ethanol solution. The mixture was filtered through a filtration cloth after waited 30 minutes at room temperature. The resulting homogenate centrifuged at 5000 rpm for 10 minutes (at 18ºC). The supernatant that received from this process centrifuged at 7500 rpm for 10 min (at 4ºC) again. The final supernatant was taken and used (100 mg / ml) for DPPH and XRF measurements (Pedraza-Chaverri, *et al.* 2004; Lee *et al.*, 2004).

Preparation of Trolox solution

100 mg Trolox ($C_{14}H_{18}O_4$) was taken and dissolved in 100 ml 75% ethanol ($4x10^{-3}$ M). Then $8x10^{-5}$ M concentration was obtained by diluting this solution.

Preparation of DPPH calibration solutions

123 mg DPPH ($C_{18}H_{12}N_5O_6$) was dissolved in 50 ml of absolute alcohol ($6.25x10^{-3}$ M). Then diluted from this solution and concentration of $1.25x10^{-3}$ M as well, $2.5x10^{-4}$ M and $5x10^{-5}$ M DPPH calibration solutions were prepared. Absorbance of the DPPH solutions were read and the calibration graph is obtained. And the calibration equation were shown for DPPH solutions at the concentration range $5\text{-}25x10^{-5}$ M.

For this study, first of different concentrated DPPH calibration solutions prepared with ethanol incubated for 15 minutes at room temperature and in the dark and then absorbance at 517 nm were recorded corresponding to the blank. In the same way ethanol -DPPH solution which prepared for control was used as a standard for sample studies.

Determination of Total Antioxidant Status

Preparation of Sample [TR + Ethanol + DPPH] system solution

The solution was prepared as follows: 3 ml (stock $2.5x\ 10^{-4}$ M) DPPH + X ml TR (Trolox) + (3-X) ml of absolute ethanol; total volume of 6 ml of the reaction mixture.

Preparation of Sample [Mushroom Extract + Ethanol + DPPH] system solution

The solution was prepared as follows: 3 ml (stock $2.5x\ 10^{-4}$ M) DPPH + X mL Mushroom Extract + (3-X) ml of absolute ethanol; total volume of 6 ml of the reaction mixture.

Percentage of radical scavenging activity is calculated by the following formula:

$$\% \text{ Inhibition} = [(C_0 - C_1) / C_0] \times 100$$

C_0: Concentration of control solution (no antioxidant added) and C_1: Concentrations of sample solutions (when antioxidant was present) (Huang *et al.*, 2005).

The IC_{50} value was determined from the graph slope "y = mx + c" formula that obtained from the graph for standard trolox and mushroom extracts (Mukherjee *et al.*, 2011).

Study of Metal Content

The collected mushroom samples dried in drying oven (NUVE KD 400) at 105ºC for 24 hours and then pulverized and stored in polyethylene bottles prior to analysis. These samples (1 g) were digested with 12 ml of HNO_3 (65%) and 4 ml of H_2O_2 (30%) in a microwave digestion system for 45 min and diluted to 20 ml with deionized water. Prepeared samples were analysed by X-Ray Fluorescence Spectrometer for three times, repeatedly (Mendil *et al.*, 2004).

Statistical Analysis

The relationship between Trolox and antioxidant concent of mushrooms were calculated using descriptive statistical analysis with Microcal Origin Pro 8.5.1 (Origin Lab. Corp., Northampton, MA, USA). Statistically significant effects were investigated using SPSS software (SPSS Inc., Chicago, IL, USA) for Windows version 13.

Results and Discussion

Antioxidant activity

In this study, we used DPPH radical quenching method and trolox which is a water-soluble antioxidant as a standart. Free radical scavenging effects of trolox and mushroom extracts was performed by using DPPH method. Therefore, we calculated calibration equation $y=7.62x10^3c-0.018$ ($R^2=0.999$) with the help of different DPPH concentrations. We use this calibration equation for calculating percent of inhibition of Trolox and mushroom species.

Decreasing absorbance values of samples that prepared with Trolox and mushrooms extracts gave remaining DPPH solution values so free radical scavenging activity. And we showed inhibition values and concentration equals of Trolox solution at various concentrations. To determine the (unitless) TEAC coefficient of each compound, the ratio of the slope **(*m*)** of the linear regression curve of the tested compound to that of Trolox was used:

$$TEAC = m_{compound} / m_{Trolox}$$

Then the calculated trolox equivalents can be used for comparative analysis of the antioxidant capacity of the various mushroom samples. As can be seen from Table 1, Concentration equality and TEAC (Trolox equivalent antioxidant capacity) values calculated by DPPH method for each mushroom species are illustrated. And we found TEAC values for, *Pleurotus ostreatus*, *Lactarius delicious* and *Agaricus bisporus* 0.302, 0.557 and 0.251 µmol /g, respectively.

Thus, we gave results for determination of antioxidant capacity of these fungal extracts as % inhibition in Figure 1. According to these results we obtained different antioxidant properties for the each fungal species. Although *Agaricus bisporus* generally showed the highest activity, we didn't observe a linear inhibition with increasing concentration. However, *Pleurotus ostreatus* and *Lactarius delicious* indicated a linear inhibition with increasing concentration.

And as a result, IC_{50} values that were calculated with DPPH method were found 4.51, 60.32, 21.35 and 8.33 mg/mL for trolox, *Pleurotus ostreatus*, *Lactarius delicious* and *Agaricus bisporus* respectively. Considering the IC_{50} values, in spite of the low concentration, It was observed that *Agaricus bisporus* has more potent antioxidant activity because of scavenging the same amount of free radical.

Onbaşılı *et al.* (2015) studied about antimicrobial, antioxidant activities and chemical composition of *Lactarius deliciosus* (L.) collected from Kastamonu province of Turkey. They found IC_{50}value ›17 for *Lactarius deliciosus* and we found that IC_{50} value of *Lactarius deliciosus* similarly.

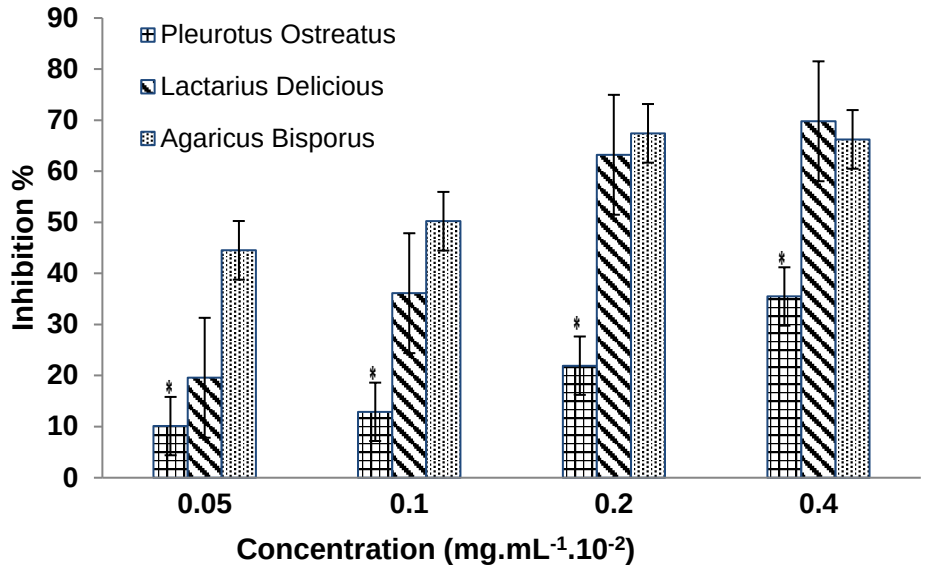

Figure 1. Antioxidant capacity of *Pleurotus ostreatus*, *Agaricus bisporus* and *Lactarius delicious* in different concentrations (measured by DPPH assay). The calculated results are given as mean ± SEM (standard error of the mean). *The statistical significance was accepted at $P<0.05$, (n=4).

Table 1. Calculated of TEAC (trolox equivalent antioxidant capacity) coefficients of *Agaricus bisporus, Pleurotus ostreatus* and *Lactarius delicious* by DPPH method

	Concentration (10^{-6}M)	Inhibition %	Concentration Equation	R^2	TEAC** c=(4.0–32.0) x10^{-6} M
Trolox	4	15.07	y=2.383x10^6c+7.097	0.995	-
	8	28.41			
	16	44.58			
	32	83.32			
	Concentration (mg.mL^{-1})*				
Pleurotus ostreatus	5	10.35	y=0.719c*+6.627	0.994	0.302
	10	13.08			
	20	21.90			
	40	35.12			
Agaricus bisporus	5	19.59	y=1.327c*+21.667	0.705	0.557
	10	35.75			
	20	62.21			
	40	68.61			
Lactarius delicious	5	43.94	y=0.598c*+45.016	0.523	0.251
	10	49.51			
	20	66.30			
	40	65.15			

Mushroom extract concentrations (c*): 5.0; 10.0;20.0;40.0 mg/mL (g/L)
TEAC of mushrooms (**): µmol of Trolox equiv per gram mushroom extract (µM of Trolox equiv./g)

Determination of metal contents

There have been many studies on the mechanism of antioxidative activities of phenolic compouns and flavonoids. At the same time there are many studies on the metal complexation with phenolic compounds such as flavanoids. Rice –Evans *et a.l* (1996) found a relationship between antioxidative activity and structure in flavanoids and they reported metal ions such as copper, iron, zinc, sodium and potasium effects. Nathan *et al.* (2009) showed that both Fe^{2+} and Cu^{+} perform Fenton-like reactions with H_2O_2, polyphenol compounds containing metal binding catechol and gallol groups have very different activities, depending on the metal ion. It is clear that iron-binding are important factors contributing to overall antioxidant activity for polyphenol compounds. And Khokhar *et al.* (2003) said that binding of iron to the flavonoid antioxidants can suppress the accessibility of the iron to oxygen molecules.

This study is a macro dimension work which is discussed in order to demonstrate statistically the effects of different antioxidant properties and the effect of accumulated metals in the fungal species to oxidation mechanism. Eleven metals (Na, Mg, K, Cu, Mn, Zn, Fe, Ca, Ni, Pb and Cr) and two metal oxides (Al_2O_3 and SiO_2) were determined in three mushroom species. Element concentrations and percent of the compounds of the mushroom species are presented in Table 2. According to the results, the most abundant elements were Na, Mg and K, respectively. These are followed by Fe and Ca. And the other minor ones were Cu, Ni, Mn and Pb.

The heavy metal concentration in the mushrooms are mainly affected by acidic and organic matter content of their ecosystem and soil .Toxic heavy metal (such as Pb, Cr, Cu etc.) concentrations of the investigated three mushrooms in this study were found at relatively low levels compared to those of the essential elements and therefore the results presented here were acceptable to human consumption at nutritional and toxic levels (Turkekul et al., 2004; Ayaz *et al.*, 2011).

In this study, the highest copper and manganese contents were found 30 mg/kg and 12mg/kg respectively. And These copper and manganese levels in mushrooms are in good agreement with other studies (Demirbaş, 2000; Işıloğlu *et al.*, 2001).

Table 2. Levels of elementel contents and metal oxides for three different mushroms species, dry weight (as µg /g) and (wt. %). Data are expressed as mean value ± standart deviation (SD).

Compound (wt.%)	*Pleurotus ostreatus*	*Agaricus bisporus*	*Lactarius delicious*
Al_2O_3	0.04 ±0.001	0.07 ±0.001	0.12 ±0.001
SiO_2	0.03 ±0.001	0.01 ±0.001	0.01 ±0.001
Element (mg/kg)			
Na	96000 ±9500	87000 ±8600	14980 ±480
Mg	8830 ±210	8500 ±200	2600 ±140
K	3162 ±29	4057 ±30	3623 ±26
Cr	6 ±1.0	8 ±1.0	5 ±1.0
Mn	11 ±1.0	12 ±1.0	12 ±1.0
Fe	128 ±4.0	121 ±4.0	99 ±3.0
Ni	15 ±1.0	12 ±1.0	6 ±1.0
Cu	30 ±3.0	25 ±2.0	20 ±2.0
Zn	5 ±1.0	7 ±1.0	3 ±1.0
Ca	89 ±7.0	83 ±6.0	19 ±3.0
Pb	1 ±0.5	3 ±1.0	2 ±1.0

In the previous works Mendi *et al.* (2004) studied about determination of trace elements on some wild edible mushroom samples from Kastamonu, Turkey. They gave concentration results of nine trace elements in eight mushroom species and found iron, manganese and zinc contents of mushrooms were higher than ours results. But they found that the similar metal contents order for *Agaricus bisporus* and *Lactarius delicious* in terms of metal contents.

Statistically correlation coefficients were found ($r>0.977$) at ($p<0.05$) significant level between metal concentrations for all mushrooms. Positive correlations were obtained between sodium and chromium (r = 0.68), sodium and nickel (r = 0.97), sodium and copper (r = 0.91), magnesium and chromium (r = 0.72), magnesium and iron (r = 0.98), magnesium and copper (r = 0.88), iron and nickel (r = 0.99), iron and copper (r = 0.95) and copper and zinc (r = 0.50) and negative correlations were found between potasium and copper (r=-0,51), manganese and iron (r = -0.68), manganese and nickel (r = -0.75) and manganese and copper (r = -0.86).

On the other hand, we showed the distribution of some element species in mushrooms species in Figure 2. Although Fe and Ca concentrations were higher in *Lactarius delicious, Pleurotus ostreatus*, they were lower in *Agaricus bisporus*. Similarly Cu concentration followed by the same sort of, but according to mushroom spices the difference was quite small. In this study though the most abundant elements were Na and Mg for each mushroom spices, they were less in *Agaricus bisporus* than the others. In the case of heavy metal oxide, the most abundant was Al_2O_3 in *Agaricus bisporus.*

In an other work Özyürek et al. studied about antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms (*Terfezia boudieri* Chatin, *Boletus edulis* Bull., *Lactifluus volemus* (Fr.) Kuntze) in different regions of Anatolia-Turkey (Özyürek *et al.*, 2014).

Ayaz *et al.* (2011) studied about the nutritional content of eight edible mushrooms (*Boletopsis leucomelaena* (Pers.) Fayod, *Hydnum repandum* L., *Laetiporus sulphureus* (Bull.) Murrill, *B. edulis, Armillaria mellea* (Vahl) P. Kumm., *Macrolepiota procera* (Scop.) Singer, *Lactarius piperatus* (L.) Roussel and *L. quietus* (Fr.) Fr.) collected from East Black Sea region in Turkey.

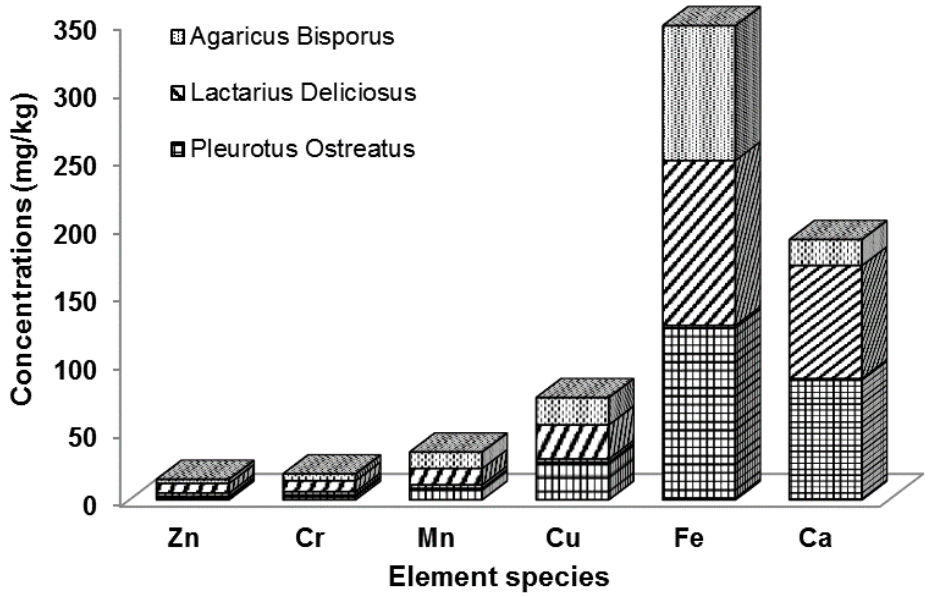

Figure 2. Distribution of element species (Ca, Fe, Cu, Mn, Cr and Zn) in *Agaricus bisporus*, *Lactarius delicious* and *Pleurotus ostreatus*.

Based on the results obtained, the methanolic extract of *Pleurotus eryngii* (DC.) Quél. collected from city center revealed the highest DPPH radical scavenging activity and reducing power, while the highest total phenolics and total antioxidant status was determined in *P. eryngii* collected from Pulumur (Çıkçıkoğlu *et al.*, 2012).

Kumamoto *et al.* (2001) and Rice –Evans *et al.* (1996) found that some ions were able to inhibit antioxidant activity like iron. Kumamoto *et al.* (2001) examined antioxidative activity of (-)-epigallocatechin gallate (EGCG) in the presence of thirteen kinds of metal ions. The antioxidative activity of EGCG was increased by Cu^{+2} and Mn^{+2}, and on the contrary inhibited by Fe^{+2}. And our results were compliance with these studies.

Conclusions

Considering together IC_{50} and metal contents, we saw that *Agaricus bisporus* has got the highest antioxidant activity and lower Fe and Ca concentrations than the others. According to our study a relationship was found between the increase of Fe, Ca, Na, and Mg concentrations and antioxidant activity in the opposite direction. But we didn't compare relation between phenolic antioxidants and metal contents in the mushroom species. Therefore we are going to study polyphenolic contents of the mushroom species to make a definite judgment in an other work.

Abbreviations

AAS	Atomic absorption spectrometry
C_0	Concentration of control solutions
C_1	Concentration of sample solutions
DPPH	1, 1diphenyl-2-picryl hydrazyl
EGCG	(-)-epigallocatechin gallate
IC_{50}	The half maximal inhibitory concentration
SEM	Standard error of the mean
SD	Standart deviation
TEAC	Trolox Equivalent Antioxidant Capacity
XRF	X-ray fluorescence spectrometry

References

Ayaz, F.A., Torun, H., Özel, A., Col, M., Duran, C., Sesli, E., & Colak, A. (2011). Nutritional value of some wild edible mushrooms from the Black Sea region (Turkey). *Turkish Journal of Biochemistry,* 36(3),213-221.

Babu, D.R. & Rao, G.N. (2013). Antioxidant properties and electrochemical behavior of cultivated commercial Indian edible mushrooms. *Journal of Food Science and Technology,* 50(2), 301-308.

Bakır, T., Beker, B.Y., Sönmezoğlu, İ., İmer, F. & Apak, R. (2013). Antioxidant and prooxidant effects of a-tocopherol in a linoleic acid-copper(II)-ascorbate system. *European Journal of Lipid Science and Technology*, 115, 372-376.

Baysal, E., Yigitbasi, O.N., Colak, M., Toker, H., Simsek, H. & Yilmaz, F. (2007). Cultivation of Agaricus bisporus on some compost formulas and locally available casing materials. Part I: Wheat straw based compost formulas and locally available casing materials. *African Journal of Biotechnology*, 6(19), 2225-2230.

Carvalho, M.L., Pimentel, A.C. & Fernandes, B. (2005). Study of heavy metals in wild edible mushrooms under different pollution conditions by X-Ray Fluorescence Spectrometry. *Analytical Sciences*, 21, 747-750.

Cocchi, L., Vescovi, L., Petrini, L.E. & Petrini, O. (2006). Heavy metals in edible mushrooms in Italy. *Food Chemistry*, 98, 277-284.

Çıkçıkoğlu Yıldırım N., Turkoglu S., Yıldırım N. & Kaplan O. (2012). Antioxidant Properties of Wild Edible Mushroom Pleurotus eryngii Collected from Tunceli Province of Turkey. *Journal of Nanomaterials and Biostructures* 7, 1647-1654.

Demirbaş, A. (2000). Accumulation of heavy metals in some edible mushrooms from Turkey. *Food Chemistry*, 68, 415-419.

Frankel, E.N. & Meyer, A.S. (2000). The problems of using onedimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, 80,1925-1941.

Gülçin, I. (2012). Antioxidant activity of food constituents: an overview. *Archives of toxicology*, 86(3), 345-391.

Huang, D., Ou, B. & Prior, L. (2005).The Chemistry behind Antioxidant Capacity Assays. *Journal Agricultural and Food Chemistry*, 53(6), 1841-1856.

Isildak, O., Turkekul, I., Elmasta,s M. & Tuzen, M. (2004). Analysis of heavy metals in some wild-grown edible mushrooms from the middle black sea region, Turkey. *Food Chemistry*, 86, 547-552.

Işıloğlu M., Yılmaz F., & Merdivan, M. (2001). Concentrations of trace elements in wild edible mushrooms. *Food Chemistry*, 73, 169-175.

Khokhar, S., Richard, K. & Apenten, O. (2003). Iron binding characteristics of phenolic compounds: some tentative structure–activity relations. *Food Chemistry*, 81, 133-140.

Kumamoto, M., Sonda, T., Nagayama, K. & Tabata, M. (2001). Effects of pH and Metal Ions on Antioxidative Activities of Catechins. *Bioscience, Biotechnolology and Biochemistry*, 65,126-132.

Lee D. H., Kima J. H., Park J. S., Choi Y. J. & Lee J. S. (2004). Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom Tricholoma giganteum, *Peptides* 25, 621-627.

Mendil D., Uluozlu Ö.D. , Hasdemir E. & Çağlar A. (2004). Determination of trace elements on some wild edible mushroom samples from Kastamonu, Turkey. *Food Chemistry*, 88, 281-285.

Mukherjee, S., Pawar, N., Kulkarni, O., Nagarkar, B., Thopte, S., Bhujbal, A. & Pawar, P. (2011). Evaluation of free-radical quenching properties of standard Ayurvedic formulation Vayasthapana Rasayana. *BMC Complementary and Alternative Medicine*, 11,38-43.

Nathan, R., Julia, P. & Brumaghim, J.L. (2009). A Review of the Antioxidant Mechanisms of Polyphenol Compounds Related to Iron Binding. *Cell Biochemistry and Biophysics*, 53,75-100.

Onbaşılı D., Yuvalı Çelik G., Katırcıoğlu H. & Narin İ. (2015). Antimicrobial, Antioxidant Activities and Chemical Composition of *Lactarius deliciosus* (L.) Collected from Kastamonu Province of Turkey. *Kastamonu Üni., Orman Fakültesi Dergisi*, 15 (1), 98-103.

Ouzouni P.K., Petridis D., Koller W.D. and Riganakos K.A. (2009). Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. *Food Chemistry*, 115(4), 1575-1580.

Özyürek M., Bener M., Güçlü K. & Apak R. (2014). Antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms. *Food Chemistry* 157, 323-331.

Pedraza-Chaverrí, J., Gil-Ortiz, M., Albarrán, G., Barbachano-Esparza, L., Menjívar, M., & Medina-Campos, O. N. (2004). Garlic's ability to prevent in vitro Cu 2+-induced lipoprotein oxidation in human serum is preserved in heated garlic: effect unrelated to Cu 2+-chelation. *Nutrition Journal*, 3(1), 10.

Radulescu, C., Stihi, C., Busuioc, G. & Gheboianu, A.I. (2010). Studies Concerning Heavy Metals Bioaccumulation of Wild Edible Mushrooms from Industrial Area by Using Spectrometric Techniques. *Bulletin of Environmental Contamination and Toxicology*, 84,641-646.

Rajalingam, P., Mayakrishnan, V., Abdullah, N., Sabaratnam, V. & Kuppusamy, U.R. (2013). In-vitro antioxidant properties of different varieties of mushrooms grown on rice grains. *Agro Food Industry Hi Tech*, 24(5), 56-58.

Rice-Evans, C.A., Miller, N.J. & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20, 933-956.

Turkekul, I., Elmastas, M., & Tüzen, M. (2004). Determination of iron, copper, manganese, zinc, lead, and cadmium in mushroom samples from Tokat, Turkey. *Food Chemistry*, 84(3), 389-392.

Yılmaz, A., Yıldız, S., Tabbouche, S., Kılıç, A.O. & Can, Z. (2016). Total Phenolic Content, Antioxidant and Antimicrobial Properties of Pleurotus ostreatus Grown on Lime (Tilia tomentosa) Leaves. *H*acettep*e Journal of Biology and Chemistry*, 44(2), 119-124.

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

HEMŞİRELİK BÖLÜMÜ ÖĞRENCİLERİNDE BEL/BOY ORANI VE TİP 2 DİYABET RİSKİ İLİŞKİSİNİN DEĞERLENDİRİLMESİ

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Öz:

Bu araştırmada hemşirelik bölümü öğrencilerinin tip 2 diyabet riski ile bel/boy oranı ve tip 2 diyabet riski ilişkisinin değerlendirilmesi amaçlanmıştır. Vücut ağırlığı, boy uzunluğu, bel ve kalça çevresi ölçümleri tekniğine uygun olarak yapılmış ve Finlandiya tip 2 diyabet riski anketi uygulanmıştır. Öğrencilerin %67.1'inin 10 yıllık tip 2 diyabet riskinin %1, %28.5'inin 10 yıllık diyabet riskinin %4, %2.2'sinin 10 yıllık tip 2 diyabet riskinin %16 ve %2.2'sinin 10 yıllık tip 2 diyabet riskinin %33 olduğu saptanmıştır. Tip 2 diyabet riski ile bel/boy oranı ve beden kütle indeksi arasındaki ilişki orta düzeyde olup bel/boy oranı, tip 2 diyabet riski ile korelasyon katsayısı en yüksek olan ölçümdür. Gençlerde tip 2 diyabet riskinin girişimsel olmayan yöntemlerle belirlenebilmesi riskin azaltılmasında etkili olabilir.

Anahtar Kelimeler: Diabetes mellitus, Obezite, Abdominal, Öğrenciler, Hemşirelik

Abstract:

THE ASSESMENT OF RELATION BETWEEN WAIST/HEIGHT RATIO AND TYPE 2 DIABETES RISK AMONG NURSING STUDENTS

In this study it's aimed to evaluate type 2 diabetes risk and the relation between type 2 diabetes and waist/height ratio in nursing students. Body weight, height, waist and hip circumferences were performed by following the appropriate techniques and Finnish type 2 diabetes risk score questionnarie was applied. The ratio of students whose ten years risk of type 2 diabetes risk were %1, %4, %16 and %33 assesed as %67.1, %28.5, %2.2 and %2.2, respectively. While type 2 diabetes risk correlated with both waist/height ratio and body mass index at medium level, waist/height ratio had the highest correlation coefficient with type 2 diabetes. The assesment of type 2 diabetes risk of youth with noninasive techniques could be effective for decreasing the risk.

Keywords: Diabetes mellitus, Obesity, Abdominal, Students, Nursing

Giriş

Uluslararası Diyabet Atlası'na göre 2015 yılında dünya genelinde 20-79 yaş grubunda 415 milyon diyabetlinin olduğu ve 2040 yılında bu sayının 642 milyona ulaşacağı öngörülmektedir. Yaş, ailesel diyabet öyküsü, hipertansiyon, obezite ve düşük fiziksel aktivite düzeyi ise önemli tip 2 diyabet risk faktörlerindendir (International Diabetes Federation, 2015). Türkiye'de yapılan TURDEP-I ve TURDEP-II çalışma sonuçlarına göre diyabet prevalansı %7.2'den %13.7'ye yükselmekle birlikte yaşam tarzı etmenleriyle yakından ilişkili olan obezitedeki artış bunu etkileyen en önemli faktörlerden biridir (Satman, et al., 2013; Satman, et al., 2002). Yaşam tarzı etmenlerinden beslenme ile fiziksel aktivite düzeyindeki değişiklikler nedeniyle adölesan ve gençlerde tip 2 diyabet riskinin giderek arttığı bilinmektedir (Mekary, Giovannucci, Willett, van Dam, & Hu, 2012; Vasconcelos, de Araujo, Damasceno, de Almeida, & de Freitas, 2010). Gençlerde tip 2 diyabet riskindeki artış aile öyküsü ve etnik köken gibi değiştirilemeyen faktörlerin yanısıra teknoloji kullanımına bağlı olarak fiziksel aktivite düzeyindeki azalış, enerji ve yağ oranı yüksek diğer besin ögeleri bakımından yetersiz olan fakir hızlı hazır besin tüketimindeki artış sonucu obezite prevalansındaki artışla ilişkilendirilmektedir (Lima, et al., 2016). Bununla birlikte yetişkinlerde tip 2 diyabetin genellikle başlangıcından 7 yıl sonra tanısının konulduğu bildirilmektedir (Huang & Goran, 2003; Bi, et al., 2012). Dolayısıyla adölesan ve gençlerin de tip 2 diyabet risk gruplarından olduğu öngörülebilir. Uluslararası Diyabet Federasyonu (International Diabetes Federation-IDF) diyabetten korunma stratejisi olarak üç temel adım belirlemiştir. Bunlardan birincisi risk gruplarının belirlenmesi, ikincisi riskin ölçülmesi ve üçüncüsü ise tip 2 diyabet gelişiminin önlenmesine yönelik müdahaledir. IDF birinci adım olan risk gruplarının belirlenmesine yönelik olarak Finlandiya Tip 2 Diyabet Risk Anketi (FINDRISC) gibi risk ölçeklerinin kullanımını önermektedir (Alberti, Zimmet, & Shaw, 2007). Uzun süreli bir izlem çalışmasında 18-30 yaş grubu bireylerin tip 2 diyabet gelişim riskindeki artışın abdominal obezite ile ilişkili olduğu bildirilmektedir (Reis, et al., 2013). Abdominal obezitenin tip 2 diyabetin yanısıra kardiyovasküler hastalıklar ve metabolik sendrom için de önemli bir risk faktörü olduğu ve IDF'in metabolik sendrom tanı kriterlerinde yer aldığı bilinmektedir (International Diabetes Federation, 2006). Beden Kütle İndeksi (BKİ), obezitenin genel değerlendirilmesinde kullanılmakla birlikte abdominal obezite ve viseral adipozitenin değerlendirilmesinde yeterli olmamaktadır (Bastien, Poirier, Lemieux, & Despres, 2014). Bel çevresi ve bel/kalça oranı ise abdominal obezitenin belirlenmesinde sıklıkla kullanılan antropometrik ölçümlerdir. Bel çevresi ölçümü, columna vertebralis dışında önemli kemikler ve kasları kapsamadığından hem karın içi yağlanmayı hem de deri altı yağ doku miktarını yansıtabilmektedir. Bu nedenle bel çevresi ölçümü karın içi yağlanma ve kronik hastalık riski değerlendirmesinde sıklıkla kullanılmaktadır (Heyward & Wagner, 2004; Köksal & Küçükerdönmez, 2008). Ancak bel çevresi ölçümü de boya bağlı farklılıkların değerlendirilmesinde yetersiz kalabilmekte, bel/kalça oranı ise bireylerin ağırlık azalışını yansıtmayabilmektedir (Browning, Hsieh, & Ashwell, 2010). Bu durumda abdominal obezitenin belirlenmesinde vücut ağırlığındaki değişimi daha iyi yansıtabilen bir ölçüm olarak bel/boy oranı ön plana çıkmaktadır. Bel/boy oranının abdominal obezite ve hastalık riskleri ile olan ilişkisi ilk kez 1990'lı yıllarda öne çıkmıştır. Bel/boy oranı ile ilgili son yıllarda yapılan çalışmalar, bel/boy oranının BKİ, bel çevresi ve bel/kalça oranına göre kardiyometabolik risk ile tip 2 diyabet riskinin belirlenmesinde daha iyi bir ölçüm olduğu üzerinde durmaktadır (Ashwell, Gunn, & Gibson, 2012; Savva, Lamnisos, & Kafatos, 2013; Ashwell & Gibson, 2014; Bohr, Laurson, & McQueen, 2016). Tip 2 diyabet risk faktörleri ve risk grupları ile ilgili yapılan bir çalışmada abdominal obezite ve sedanter yaşam tarzı risk faktörleriyle ilgili olarak hemşirelerde tip 2 diyabet riskinin diğer sağlık profesyonellerine kıyasla daha yüksek olduğu belirlenmiştir (Almeida, Zanetti, Almeida, & Damasceno, 2011). Bu araştırma, hemşirelik bölümü öğrencilerinde tip 2 diyabet riski ve bel/boy oranı ile tip 2 diyabet riski ilişkisinin değerlendirilmesi amacıyla yürütülmüştür.

Materyal ve Metot

Araştırma Planı

Bu araştırma Yakın Doğu Üniversitesi, Sağlık Bilimleri Fakültesi, Hemşirelik Bölümü öğrencileri evrenini oluşturan 19-24 yaş aralığında olan 460 öğrenciden gönüllü olarak çalışmaya katılmayı kabul eden 417 öğrenci ile Mart-Mayıs 2015 tarihlerinde yürütülmüştür. Teke tek görüşme tekniğiyle bazı demografik özellikleri (yaş, cinsiyet, vb.) ile tip 2 diyabet riskini kapsayan

anket formu uygulanmış ve antropometrik ölçümleri yapılmıştır.

Antropometrik Ölçümler

Bu araştırmada vücut ağırlığı, boy uzunluğu, bel çevresi, kalça çevresi ölçümleri kullanılmıştır. Vücut ağırlığı, 0.1 g'a duyarlı dijital tartı kullanılarak; boy uzunluğu, frontal düzlemde baş, sırt, kalça ve topuklar duvara değecek şekilde durarak mezura ile ölçülmüştür. Bel çevresi, en alt kaburga kemiği ile iliak çıkıntı ortasındaki en düşük çevre ölçümü alınarak ve kalça çevresi, kalça üzerindeki en geniş çevre ölçümü alınarak ölçülmüştür (Heyward & Wagner, 2004). Bel çevresi ve bel/kalça oranı kronik hastalıklar için risk değerlendirmesi amacıyla kullanmaktadır. Buna göre yetişkin bireylerin bel çevresi kesim noktaları erkekler için ≥94 cm ve kadınlar için ≥80 cm riskli, erkekler için >102 cm ve kadınlar için >88 cm yüksek riskli olarak tanımlanmıştır. Bel/kalça çevresi risk kesim noktası ise erkekler için ≥1.0 ve kadınlar için ≥0.85 olarak bildirilmiştir (World Health Organisation, 2000). Antropometrik ölçmümler ve kardiyovasküler riskin belirlenmesine yönelik Türkiye'de yapılan bir çalışmada en iyi ölçüm sıralamasının bel/boy oranı, bel çevresi, BKİ ve bel/kalça oranı olduğu saptanmıştır (Can, Bersot, & Gönen, 2008). Türk yetişkinlerde kardiyometabolik risk için kesim noktası 2009 yılında yapılan çalışmada 0.59, 2013 yılında yapılan çalışmada ise 0.5 olarak belirlenmiştir (Can, et al., 2009; Meseri, Ucku, & Unal, 2013). Buna göre 0.5 ve üzeri artmış kardiyovasküler riskle ilgilidir ve bu çalışmada kesim noktası olarak bu değer kabul edilmiştir. Yetişkin bireyler için BKİ, vücut ağırlığının (kg) boy uzunluğunun karesine (m^2) bölünmesi ile elde edilmektedir. BKİ değerleri Dünya Sağlık Örgütü (World Health Organisation-WHO)'nün 2004 yılı sınıflamasına göre zayıf (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), kilolu (25.0-29.0 kg/m^2) ve obez (≥30.0 kg/m^2) olarak değerlendirilmiştir (World Health Organisation, 1998).

Tip 2 Diyabet Riski

FINDRISC, Finlandiya popülasyonunun (35-64 yaş) 10 yıllık diyabet insidansını tahmin etmek için geliştirilmiştir. IDF tarafından Türkçe'nin de yer aldığı 15 farklı dile çevrilmiştir. Anket bireylerin yaş, BKİ, bel çevresi, egzersiz alışkanlıkları, sebze ve meyve tüketimi, yüksek kan basıncı öyküsü, yüksek kan glikoz düzeyi öyküsü ve ailede diyabet öyküsünün sorgulandığı sekiz sorudan oluşmaktadır. Elde edilen toplam puan ile bireylerin gelecek 10 yıl içerisinde tip 2 diyabet gelişimi açısından risk derecesi belirlenebilmektedir. Buna göre (<7 puan)-düşük risk/10 yıllık risk %1; (7-11 puan)-hafif yüksek risk/10 yıllık risk %4; (12-14 puan)-orta risk/10 yıllık risk %16; (15-20 puan)-yüksek risk/10 yıllık risk %33, (>20 puan)-çok yüksek risk/10 yıllık risk %50 şeklinde tanımlanmaktadır (Lindström & Tuomilehto, 2003).

İstatistiksel Değerlendirme

Nicel verilerin; aritmetik ortalama ($\bar{x}$), standart sapma (S), ortanca ve alt-üst değerleri saptanmıştır. Dağılımın çarpık olduğu durumlarda ortalama değeri dağılımın yaygınlığından etkilendiğinden böyle olduğu zaman ortanca değeri değerlendirilmiştir. Nitel veriler veya nitel veriye dönüştürülmüş nicel veriler, sayı ve yüzde olarak belirtilmiştir. Cinsiyetler arası karşılaştırmalarda Mann Whitney U Testi, tip 2 diyabet risk gruplarına göre niceliksel karşılaştırmalarda Kruskal-Wallis Testi, niteliksel karşılaştırmalarda Kikare Testi kullanılmıştır. Gerekli istatistik uygulamalar Statistical Package for the Social Sciences (SPSS) 18.0 istatistik programı ile gerçekleştirilmiştir.

Bulgular ve Tartışma

Bu çalışmada öğrencilerin tip 2 diyabet riski puan ortalamaları düşük risk grubu kesim noktası olan 7'nin altındadır ve kadın öğrencilerin puan ortalaması erkek öğrencilere göre daha yüksektir ($p>0.05$) (Tablo 1). Öğrencilerin %67.1'i düşük risk grubunda olup 10 yıllık tip 2 diyabet riskinin %1, %28.5'i hafif risk grubunda olup 10 yıllık diyabet riskinin %4, %2.2'si orta risk grubunda olup 10 yıllık tip 2 diyabet riskinin %16 ve %2.2'si yüksek risk grubunda olup 10 yıllık tip 2 diyabet riskinin %33 olduğu saptanmıştır (Şekil 1). Üniversite öğrencilerinin FINDRISC ile tip 2 diyabet riskinin değerlendirildiği bir çalışmada ise düşük, hafif, orta ve yüksek risk grubundaki öğrencilerin oranının sırasıyla %72, %24.7, %2.8 ve %0.5 olduğu, tip 2 diyabet riski düşük olan kadın öğrencilerin oranının erkek öğrencilere kıyasla daha düşük olduğu belirlenmiştir (Colak, 2015). Bu çalışmada da öğrencilerin cinsiyete göre tip 2 diyabet risk grupları dağılımları incelendiğinde düşük risk grubunda yer alan kadın öğrencilerin oranı (%65.5) bu grupta yer alan erkek öğrencilerin oranına (%77.0) kıyasla daha düşüktür ($p>0.05$) (Şekil 1). Puanlaması FINDRISC'den farklı olmakla birlikte ortak parametrelerin değerlendirildiği Hindistan Diyabet Risk Skoru değerlendirmesine

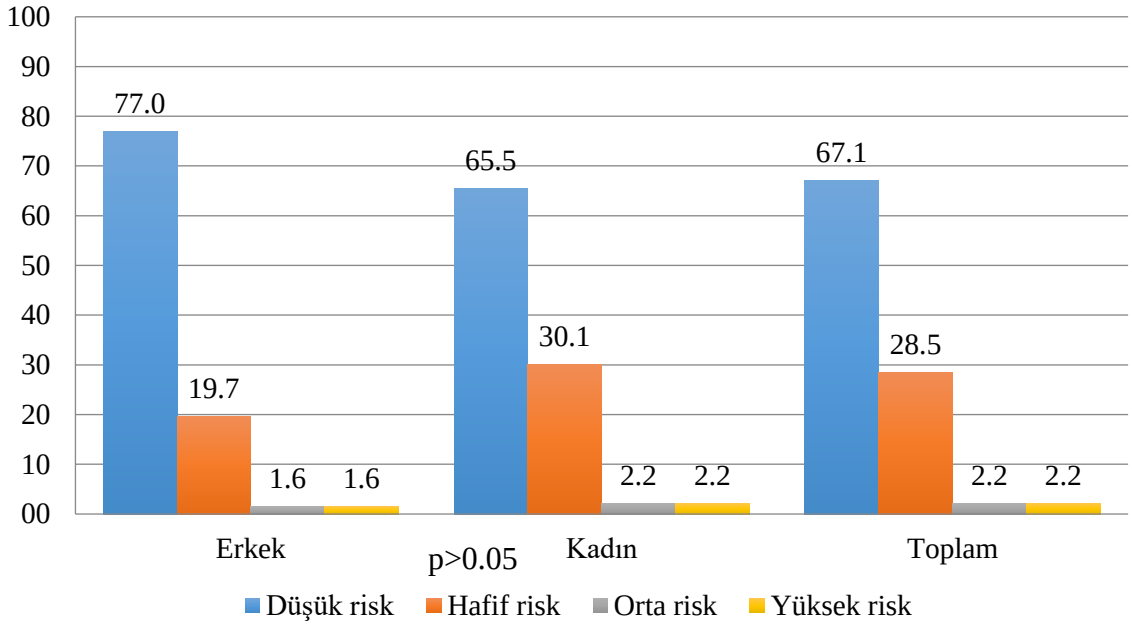
göre ise tıp öğrencileri ile yapılan çalışmada öğrencilerin tip 2 diyabet riskinin %6'sında yüksek, %44.3'ünde orta ve %49.7'sinde düşük olduğu bildirilmiştir (Garg, Jagadamba, Shankar, & Kutty, 2014). Aynı diyabet risk skorlamasının kullanıldığı Hindistan'daki üniversite öğrencileriyle yapılan diğer bir çalışmada da öğrencilerin %31'inin düşük, %68'inin orta ve %1'inin yüksek risk grubunda yer aldığı belirlenmiştir (Bhatia, et al., 2014). Brezilya'da üniversite öğrencilerinin tip 2 diyabet risk faktörlerinin belirlenmesiyle ilgili bir çalışmada ise BKİ'nin ≥25 kg/m^2 olması ve abdominal obezitenin sedanter yaşam tarzıyla ilgili olarak üniversite öğrencilerinde tip 2 diyabet riskini artıran faktörlerden olduğu belirlenmiştir (Lima, Araujo, de Freitas, Zanetti, de Almedia, & Damasceno, 2014). Dolayısıyla gençler bu çalışmada olduğu gibi çeşitli ülkelerde düşük oranda da olsa değişen yaşam tarzıyla ilişkili olarak tip 2 diyabet riski altındadırlar. Bununla ilgili olarak bu çalışmada bel çevresi, bel/kalça oranı ve bel/boy oranı ölçümleri önerilen değer aralıklarında olanların oranı en fazla düşük risk grubundadır ($p<0.001$) (Tablo 2). Ayrıca tip 2 diyabet riski düşük olan grup, BKİ, bel çevresi, bel/kalça oranı ve bel/boy oranı ortalamaları en düşük olan gruptur ($p<0.05$). Bunun yanında orta ve yüksek risk grupları antroprometrik ölçüm değerleri ortalaması önerilen değerlerin üzerindedir (Tablo 3). Bir va olgu-kontrol çalışmasında artmış BKİ, bel çevresi ve bel/boy oranının tip 2 diyabet riskindeki artışla ilgili olduğu bildirilmiştir (Radzeviciene & Ostrauskas, 2013). Diğer taraftan bir meta-analiz çalışması sonucuna göre abdominal obezite ve kardiyometabolik risk ile ilgili antropometrik ölçümlerden bel/boy oranı, BKİ ve bel çevresine kıyasla tip 2 diyabet riskinin belirlenmesinde daha önemlidir (Kodama, et al., 2012). Tablo 4'te antropometrik ölçümler ve tip 2 diyabet arasındaki ilişki korelasyon katsayısı ile gösterilmiştir. Buna göre tüm antropomerik ölçümler ve tip 2 diyabet riski arasında ilişki olduğu ve tip 2 diyabetle ilişki düzeyi yüksekten düşüğe doğru sırasıyla bel/boy oranı, BKİ, bel çevresi ve bel/kalça oranı şeklinde olduğu belirlenmiştir. Bel/boy oranı ve BKİ ile tip 2 diyabet riski arasındaki ilişki orta düzeyde iken bel çevresi ve bel/kalça oranı ile tip 2 diyabet riski arasındaki ilişki zayıf düzeydedir (Tablo 4). Uzunlamasına retrospektif bir çalışmaya göre ise bel çevresi ve bel/boy oranı tip 2 diyabet gelişimiyle ilişkili iken bel/boy oranının tip 2 diyabet riski yüksek olanların belirlenmesinde daha faydalı bir ölçüm olabileceği belirlenmiştir (Son, et al., 2016). İran'da yapılan bir uzunlamasına izlem çalışmasında erkek bireylerde tip 2 diyabet riskinin belirlenmesinde bel/boy oranının BKİ'ye göre daha iyi bir ölçüm olduğu bildirilmiştir (Hadaegh, Zabetian, Harati, & Azizi, 2006). İran'da yapılan başka bir çalışmada, bel çevresi ve bel/boy oranının BKİ'ye kıyasla tip 2 diyabet riskinin belirlenmesinde daha etkili olduğu saptanmıştır (Tilaki-Hajian & Heidari, 2015). Çin, Malezya ve Hindistan olmak üzere üç Asya kökenli etnik gruplarla yürütülen bir çalışmada tip 2 diyabet riskinin belirlenmesinde BKİ'ye kıyasla abdominal obeziteyle ilgili ölçümlerin daha iyi sonuç verdiği ve bu ölçümlerden bel/boy oranının en iyi sonuç veren olduğu bildirilmiştir (Alperet, Lim, Heng, Ma, & van Dam, 2016). Sri Lanka'da Güney Asya kökenli etnik bir grupla yürütülen çalışmada da bel/boy oranının BKİ, bel çevresi ve bel/kalça oranına kıyasla tip 2 diyabet ve metabolik sendrom riskinin belirlenmesinde etkili olduğu belirlenmiştir (Jayawardana, Ranasinghe, Sheriff, Matthews, & Katulanda, 2013). Özetle, bel/boy oranının abdominal obeziteyle ilgili hastalık risklerinin belirlenmesinde BKİ ve bel çevresine göre daha etkili olabileceği bildirilmektedir (Ashwell & Gibson, 2016). Bu bağlamda bel/boy oranı, BKİ değerleri normal olup insülin direnci ve hiperlipidemi belirtilerinin gözlendiği metabolik obezite riskinin belirlenmesinde de etkili olabilmektedir. Tip 2 diyabet riskinin belirlenmesi amacıyla geliştirilen FINDRISC'in insülin direnci belirteçleri ile ilşikili olduğu ve insülin direncinin saptanmasında kullanılabileceği bildirilmiştir (Schwarz, et al., 2009). FINDRISC'in aynı zamanda koroner kalp hastalıkları, inme ve toplam mortalite riskinin belirlenmesinde de geçerli iyi bir gösterge olabildiği bildirilmiştir (Silventoinen, Pankow, Lindström, Jousilahti, Hu, & Tuomiletho, 2005). Yapılan başka bir çalışmada ise FINDRISC'in tip 2 diyabet riskinin yanısıra metabolik sendrom riskinin belirlenmesinde de iyi bir gösterge olabileceği gösterilmiştir (Janghorbani, Adineh, & Amini, 2013). Yunanistan'da yapılan bir çalışmada ise FINDRISC'in tip 2 diyabet ve metabolik sendromun belirlenmesinde geçerli bir ölçek olduğu bildirilmiştir (Makrilakis, et al., 2011). Dolayısıyla bu çalışmada hemşirelik bölümü öğrencilerinde tip 2 diyabet riskinin yanısıra kardiyovasküler riskin de belirlendiği söylenebilir.

Tablo 1. Öğrencilerin cinsiyete göre tip 2 diyabet riski puanları

Table 1. Type 2 diabetes risk score of students according to gender

	Erkek (n:61) $\bar{x}$ **±S (ortanca) (alt-üst)**	**Kadın (n:356)** $\bar{x}$ **±S (ortanca) (alt-üst)**	**Toplam (n:417)** $\bar{x}$ **±S (ortanca) (alt-üst)**
Tip 2 diyabet riski puanı	4.5 ±3.31(4.0) (0.0-17.0)	5.1 ±3.63 (5.0) (0.0-20.0)	5.0 ±3.59 (4.0) (0.0-20.0)

p>0.05



Şekil 1. Öğrenilerin cinsiyete göre tip 2 diyabet risk grupları dağılımı

Figure 1. The percantages of type 2 diabetes risk group of students

Tablo 2. Öğrencilerin tip 2 diyabet riskine göre BKİ, bel çevresi, bel/kalça ve bel/boy oranı dağılımı

Table 2. The ratio of body mass index, waist circumference, waist/hip ratio and waist/height ratio of students according to type 2 diabetes risk

Tip 2 diyabet riski	**Düşük risk**		**Hafif risk**		**Orta risk**		**Yüksek risk**		**Toplam**		**p**
BKİ (kg/m²)	**s**	**%**	**s**	**%**	**s**	**%**	**s**	**%**	**s**	**%**	
<18.5	24	8.6	11	9.2	-	-	-	-	35	8.4	
18.5-24.9	215	76.8	65	54.6	3	33.4	1	11.1	284	68.1	<0.001
25.0-29.9	39	13.9	40	33.6	4	44.4	4	44.4	88	21.1	
≥30.0	2	0.7	3	2.5	2	22.2	4	44.4	10	2.4	
Bel (cm)											
E:<94 K:<80	240	84.7	73	61.4	4	44.4	2	22.2	319	76.5	
E:94-102 K:80-88	30	10.7	20	16.8	1	11.2	4	44.4	55	13.2	<0.001
E:>102 K:>88	10	3.6	26	21.8	4	44.4	3	33.4	43	10.3	
Bel/Kalça											
E:<1.0K:<0.8	255	91.1	94	79.0	4	44.4	4	44.4	62	85.9	<0.001
E: ≥1.0K:≥0.8	25	8.9	25	21.0	5	55.6	5	55.6	355	14.1	
Bel/Boy											
<0.5	245	87.5	81	68.1	4	44.4	4	44.4	335	80.3	<0.001
≥0.5	35	12.5	38	31.9	5	55.6	5	55.6	82	19.7	
Toplam	280	100.0	119	100.0	9	100.0	9	100.0	417	100.0	

Tablo 3. Öğrencilerin tip 2 diyabet riskine göre antropmetrik ölçüm değerleri

Table 3. Anthropometric values of students according to type 2 diabetes risk

Tip 2 diyabet riski	Düşük risk $\bar{x}$ ±S (ortanca) (alt-üst)	Hafif risk $\bar{x}$ ±S (ortanca) (alt-üst)	Orta risk $\bar{x}$ ±S (ortanca) (alt-üst)	Yüksek risk $\bar{x}$ ±S (ortanca) (alt-üst)	Toplam $\bar{x}$ ±S (ortanca) (alt-üst)
BKİ (kg/m²)	21.9 ±2.84 (21.6)[a] (16.2-33.0)	23.4 ±3.57 (23.0) (17.0-35.0)	26.5 ±4.74 (27.8) (19.5-34.9)	28.1 ±3.85 (27.3) (21.0-33.5)	22.6 ±3.35 (22.1) (16.2-35.0)
Bel çevresi (cm)	73.9 ±9.64 (73.0)[b] (58.0-116.0)	78.9 ±12.89 (79.0) (55.0-108.0)	86.6 ±16.5 (87.0) (64.0-110.0)	92.3 ±13.5 (85.0) (80.0-117.0)	76.0-11.48 (75.0) (55.0-117.0)
Bel/Kalça	0.76 ±0.07 (0.76)[c] (0.56-1.09)	0.79 ±0.09 (0.78) (0.61-1.08)	0.84 ±0.12 (0.87) (0.67-1.05)	0.85 ±0.12 (0.86) (0.67-1.04)	0.77 ±0.08 (0.77) (0.56-1.09)
Bel/Boy	0.44 ±0.05 (0.44)[d] (0.35-0.68)	0.47 ±0.06 (0.47) (0.33-0.62)	0.52 ±0.09 (0.49) (0.36-0.65)	0.55 ±0.09 (0.50) (0.47-0.71)	0.45 ±0.06 (0.44) (0.33-0.71)

[a,b,c,d]: Düşük risk grubu diğer risk gruplarından istatistiksel olarak farklı ($p<0.05$)

Tablo 4. Antropometrik ölçümlerle tip 2 diyabet riski arasındaki korelasyon

Table 4. The correlation between anthropometric measurements and type 2 diabetes risk

		BKİ	Bel çevresi	Bel/Kalça	Bel/Boy	Tip 2 diyabet riski
BKİ	**r**	1				
	p					
	S	417				
Bel çevresi	**r**	**0.739**	1			
	p	**0.000**				
	S	417	417			
Bel/Kalça	**r**	**0.477**	**0.784**	1		
	p	**0.000**	**0.000**			
	S	417	417	417		
Bel/Boy	**r**	**0.752**	**0.946**	**0.731**	1	
	p	**0.000**	**0.000**	**0.000**		
	S	417	417	417	417	
Tip 2 diyabet riski	**r**	**0.421**	**0.366**	**0.237**	**0.429**	1
	p	**0.000**	**0.000**	**0.000**	**0.000**	
	S	417	417	417	417	417

Sonuç

Hemşirelik bölümü öğrencilerinde tip 2 diyabet riski grupları dağılımına ve antropometrik ölçüm değerlerine göre obezite ve tip 2 diyabet riski önemsenmelidir. Antropometrik ölçümlerden bel/boy oranı ve BKİ ile tip 2 diyabet riski ilişkisi orta düzeyde olup, bel/boy oranı ilişki düzeyi en yüksek ölçüm olarak belirlenmiştir. Sonuç olarak insülin direnci ve tip 2 diyabet riskinin belirlenmesinde ilk basamak olarak biyokimyasal tetkiler gibi girişimsel işlemlerden önce girişimsel olmayan risk tarama ölçekleri ve antropometrik ölçümler, gençlerde risk taraması için kullanılabilen kullanışlı değerlendirmeler olup riskin azaltılmasında etkili olabilir. Kesitsel araştırmaların yanısıra uzunlamasına izlem çalışmaları ile gençlerin antropometrik ölçümleri ve hastalık riskleri daha kapsamlı irdelenebilir.

Kaynaklar

Alberti, K.M., Zimmet, P. & Shaw, J. (2007). International Diabetes Federation: a consensus on type 2 diabetes prevention. *Diabetic Medicine,* 24, 451-463.

Almeida, V.C., Zanetti, M.L., Almeida, P.C. & Damasceno, M. M. (2011). Occupation and risk factors for type 2 diabetes: A study with health workers. *Revista Latino-Americana de Enfermagem*, 19(3), 476-484.

Alperet, D.J., Lim, W.Y., Heng, D.M., Ma, S. & van Dam, R.M. (2016). Optimal antropometric measures and thresolds to identify undiagnosed type 2 diabetes in tthree major Asian ethnic groups. *Obesity*, 24(10), 2185-2193.

Ashwell, M. & Gibson, S. (2014). A proposal for primary screening tool: 'Keep your waist circumference to less than half your height'. 12, 207-212.

Ashwell, M. & Gibson, S. (2016). Waist-to-height ratio as an indicator of 'early health risk': Simpler and more predictive than using a 'matrix' based on BMI and waist circumference. *BMJ Open,* 6(3), 1-6.

Ashwell, M., Gunn, P. & Gibson, S. (2012). Waist-to-height ration is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obesity Reviews,* 13, 275-286.

Bastien, M., Poirier, P., Lemieux, I. & Despres, J. (2014). Overview of Epidemiology and Contribution of Obesity to. *Progress in Cardiovascular Diseases,* 56, 369-381.

Bhatia, T., Oka, M., Dharamdasani, V., Bhattaccharjee, S., Fortwengel, G., Limaye, V., & Limaye, D. (2014). Type 2 diabetes mellitus: Risk evaluation and advice in undergraduate students in Mumbai. *International Journal of Pharmaceutical Science Invention,* 3(4), 37-40.

Bi, Y., Wang, T., Xu, M., Li, M., Lu, J., Zhu, X., & Ning, G. (2012). Advanced research on risk factors of type 2 diabetes. *Diabetes/Metabolism Research and Reviews,* 28(Suppl 2), 32-39.

Bohr, A.D., Laurson, K. & McQueen, M.B. (2016). A novel cutoff for the waist-to-height ratio predicting metabolic syndrome in young American adults. *BMC Public Health,* 16, 295-303.

Browning, L.M., Hsieh, S.D. & Ashwell, M. (2010). A systematic review of waist-t0-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0.5 could be a suitable global boundary value. *Nutrition Research Reviews,* 23, 247-269.

Can, A.S., Bersot, T.P. & Gönen, M. (2008). Anthropometric indices and their relationship with cardiometabolic risk factors in a sample of Turkish adults. *Public Health Nutrition,* 12(4), 538-546.

Can, A.S., Yıldız, E.A., Samur, G., Rakıcıoğlu, N., Pekcan, G., Özbayrakçı, S., Palaoğlu K. E., Gönen, M., & Bersot, T.P. (2009). Optimal waist:height ration cut-off point for cardiometabolic risk factors in Turkish adults. *Public Health Nutrition,* 13(4), 488-495.

Colak, M. (2015). Physical activity levels and type 2 diabetes risk scores of university students. *Studies on Ethno Medicine*, 9(3), 401-409.

Garg, S., Jagadamba, A., Shankar, V. & Kutty, K. (2014). Risk of type 2 diabetes mellitus in medical students. *International Journal of Compherensive Medical Physiology and Research,* 1(1), 6-9.

Hadaegh, F., Zabetian, A., Harati, H. & Azizi, F. (2006). Waist/height ratio as a better

predictor of type 2 diabetes compared to body mass index in Tehranian adult men-a 3.6 year prospective study. *Experimental and Clinical Endocrinology & Diabetes*, 114, 310-315.

Heyward, V.H. & Wagner, R.R. (2004). *Applied body composition assesment.* Champaign: Human Kinetics. ISBN 13: 9780736046305

Huang, T.K. & Goran, M.I. (2003). Prevention of type 2 diabetes in young people: a theoretical perspective. *Pediatric Diabetes*, 4, 38-56.

International Diabetes Federation. (2006). *The IDF consensus worldwide definition of the Metabolic Syndrome.* Brussels: International Diabetes Federation.

International Diabetes Federation. (2015). *International Diabetes Federation Diabetes Atlas.* Brussels: International Diabetes Federation.

Janghorbani, M., Adineh, H. & Amini, M. (2013). Evaluation of the Finnish Diabetes Risk Score (FINDRISC) as a screening tool for the metabolic syndrome. *The Review of diabetic Studies, 10*(4), 283-292.

Jayawardana, R., Ranasinghe, P., Sheriff, M. H., Matthews, D.R. & Katulanda, P. (2013). Waist to height ratio: A better antropometric marker of diabetes and cardio-metabolic risks in South Asian adults. *Diabetes Research and Clinical Practice*, 99, 292-299.

Kodama, S., Horikawa, C., Fujihara, K., Heianza, Y., Hirasawa, R., Yachi, Y., Sugawara, A., Tanaka, S., Shimano, H., Tada lida, K., Saido, K., & Sone, H. (2012). Compariosns of the strength of associations with future type 2 diabetes risk among antropometric obesity indicators, including waist-to-height ratio: A meta-analysis. *American Journal of Epidemiology*, 176(11), 959-969.

Köksal, E. & Küçükerdönmez, Ö. (2008). Şişmanlığı saptamada güncel yaklaşımlar. In A. Baysal, & M. Baş, *Yetişkinlerde ağırlık yönetimi* (pp. 35-70). İstanbul: Express Baskı. ISBN 9789759205812

Lima, A.C., Araujo, M.F., de Freitas, R.W., Zanetti, M.L., de Almedia, P.C. & Damasceno, M.M. (2014). Risk factors for type 2 diabetes mellitus in collage students: Association with sociodemographic variables. *Revista Latino-americana de Enfermagem*, 22(3), 484-490.

Lima, C.L., Oliveria, P.S., Ferreira, T.M., Silva, E.C., Ferreira, J.D., Andrade, R.S., Macedo, Y.D., Gomes, W.L., Melo, E.G., Lucena, A.L.R., Cunha, A.I.L., & Costa, M.M.L. (2016). Risk factors for type II diabetes mellütus: An Integrative Review. *INternational Archives of Medicine*, 9(308), 1-11.

Lindström, J. & Tuomilehto, J. (2003). The diabetes risk score. *Diabetes care*, 26(3), 725-731.

Makrilakis, K., Liatis, S., Grammatikou, S., Perrea, D., Stathi, C., Tsiligros, P., & Katsilambros, N. (2011). Validation of the Finnish diabetes risk score (FINDRISC) questionnaire for screening for undiagnosed type 2 diabetes, dysglycaemia and the metabolic syndrome in Greece. *Diabetes & Metabolism*, 37, 144-151.

Mekary, R.A., Giovannucci, E., Willett, W.C., van Dam, R.M. & Hu, F.B. (2012). Eating patterns and type 2 diabetes risk in men: breakfast omission, eating frequency, and snacking. *American Journal of Clinical Nutrition*, 95(5), 1182-1189.

Meseri, R., Ucku, R. & Unal, B. (2013). Waist:height ratio: a superior index in estimating cardiovascular risks in Turkish adults. *Public Health Nutrition*, 17(10), 2246-2252.

Radzeviciene, L. & Ostrauskas, R. (2013). Body mass index, waist circumference, waist-hip ratio, waist-height ration and risk for type 2 diabetes in women: A case-control study. *Public Health*, 127, 241-246.

Reis, J.P., Loria, C.M., Lewis, C.E., Powell-Wiley, T.M., Wei, G.S., Carr, J.J., Terry, J. G., & Liu, K. (2013). Association Between Duration of Overall and Abdominal. *Journal of the American Medical Association*, 310(3), 280-288.

Satman, I., Omer, B., Tutuncu, Y., Kalaca, S., Gedik, S., Dinccag, N., Karsidag, K., Genc, S., Telci, A., Canbaz, B., Turker, F., Yilmaz, T., Cakir, B., & Tuomilehto, J. (2013). Twelve-year trends in prevalance and risk factors of diabetes and prediabetes in

Turkish adults. *European Journal of epidemiology*, 28, 169-180.

Satman, I., Yılmaz, T., Sengül, A., Salman, S., Salman, F., Uygur, S., et al. (2002). Population-based study of diabetes and risk characteristics in Turkey. *Diabetes Care*, *25*(9), 1551-1556.

Savva, C.S., Lamnisos, D. & Kafatos, A.G. (2013). Predicting cardiometabolic risk: waist-to-height ratio or BMI. *A meta-analysis*, 6, 403-419.

Schwarz, P.E., Li, J., Reimann, M., Schutte, A.E., Bergmann, A., Hanefeld, M., Bornstein, S. R., Schulze, J., Tuomilekto, J., & Lindst-röm, J. (2009). The Finnish diabetes risk score is associated with insulin resistance and progression towards type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism*, 94, 920-926.

Silventoinen, K., Pankow, J., Lindström, J., Jousilahti, P., Hu, G. & Tuomiletho, J. (2005). The validty of the Finnish Diabetes Risk Score for the prediction of the incidance of coronary hert disease and stroke, and total mortality. *European Journal of Cardiovascular Prevention and Rehabilitation*, 12, 451-458.

Son, Y.J., Kim, J., Park, H.J., Park, S.E., Park, C.Y., Lee, W.Y., Oh, K.W., Park, S.W., & Rhee, E,J. (2016). Association of waist-height ratio with diabetes risk: A 4-year longitudinal retrospetive study. *Endocrinology and Metabolism*, 31, 127-133.

Tilaki-Hajian, K. & Heidari, B. (2015). IS waist cirumference a better predictor of diabetes than body mass index or waist-to-height ratio in Iranian adults? *International Journal of Preventive Medicine*, 6, 5-10.

Vasconcelos, H.C., de Araujo, M.F., Damasceno, M.M., de Almeida, P.C. & de Freitas, R.W. (2010). Risk factors for type 2 diabetes mellitus among adolescents. *Revista da Escola de Enfermagem da USP*, 44(4), 881-887.

World Health Organisation. (1998). *Obesity, preventing and managing the global epidemic.* Geneva: World Health Organisation. http://apps.who.int/iris/handle/10665/63854

World Health Organisation. (2000). *Obesity: Preventing and managing the global epidemic.* Geneva: World Health Organisation. ISBN 9241208945

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

DOES THE REALISTIC CONTACT AND DAILY USE CONDITIONS LIMIT THE USE OF POLYCARBONATE BABY BOTTLES FOR MIGRATION AND RESIDUE LEVEL OF BISPHENOL-A

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Abstract:

Bisphenol-A (BPA) which is a plastic monomer acting as endocrine disruptor, can cause developmental effects and change the metabolism even at low concentrations. This study was carried out to make (i) a general assessment by performing residue analysis on the polycarbonate baby bottles, (ii) determining the factors affecting the migration of BPA to the food and (iii) determining the effect of repetitive procedures on PC baby bottles in daily use. Amount of residual BPA ranged from 0.60 ±0.05 ppm to 6.23 ±0.24 ppm and BPA migration could not be detected in any of contact conditions. Estimated daily intakes of BPA via infant formula in PC baby bottle were calculated as 1.8 µg kg^{-1} bw day^{-1} for a 3 month-old infant and 1.22 µg kg^{-1} bw day^{-1} for a 6 month-old infant which was lower than but very close to the latest value of t-TDI (4 µg kg^{-1} bw day^{-1}) determined by EFSA. Sterilization procedures changed the amount of residual BPA significantly. Although it is concluded that the use of brand-new PC baby bottles does not pose a risk to health, sterilization procedures can lead to the higher amounts of migrated BPA which can be toxically important.

Keywords: Residual Bisphenol-A, BPA, HPLC, Method validation, Tolerable daily intake, Dietary exposure

Introduction

Polycarbonate (PC) is a widely used plastic in the production of food packaging or various materials in contact with food. PC material can be used in a number of consumer products including baby bottles and reusable water bottles (Maia *et al.,* 2009; Üçüncü, 2007; Robertson, 2006; Kawamura *et al.,* 1998). PC is derived from the bisphenol ester of carbonic acid and occurs as a result of condensation polymerization of Bisphenol A [2,2-bis(4-hydroxyphenyl) propane] with carbonyl chloride or diphenyl carbonate. However, the unreacted residual Bisphenol-A (BPA) can be found in the material and migrate to the food from polycarbonate material.

BPA is a plastic monomer containing two phenol groups having high reactivity (Kawamura *et al.,* 1998), and probably the most prominent regulated compound whose use in food contact materials was put into question in the recent years. Hundreds of toxicological studies were published on adverse effects of BPA, which was reported to act as an endocrine disruptor and causes developmental effects and changes in metabolism at low concentrations (Geueke, 2014). According to the report of NTP, there is a clear evidence of adverse developmental effects at "high" doses of BPA (>5 mg kg^{-1} bodyweight (bw) day^{-1}) in laboratory animals, but there is scientific controversy over the interpretation of the "low" dose findings (NTP, 2008). BPA is attracting great attention due to being a chemical widely exposed by people as well as suspicious effects on reproductive and developmental systems in laboratory studies on animals. The studies on humans revealed that amount of BPA in the blood of people with health problems such as frequently recurrent miscarriages, abnormal karyotype in fetuses, obesity, endometrial hyperplasia, and polycystic ovarian syndrome was higher than the people without these cases (Vandenberg *et al.,* 2007; Sugiura-Ogasawa *et al.,* 2005; Takeuchi and Tsutsumi, 2002; Yamada *et al.*, 2002). Therefore, it is necessary to be careful about residue levels and migration values in the PC material in contact with food and to make essential controls.

The data concerning the adverse effects of BPA on health have led to increase interest towards the migration of BPA from PC materials and there have been many studies on this issue. It is noteworthy that the majority of studies are related with PC baby bottles. As a result of literature review; amount of residual BPA in PC baby bottles may range from 5-10 ppb to 347 ppm (Biedermann-Brem *et al.,* 2008; Ehlert *et al.,* 2008; Wong et al., 2005; Biles *et al.,* 1997; Mountfort *et al.,* 1997). In migration tests carried out under different conditions (temperature, incubation time), the amount of migrated BPA from PC baby bottles to distilled water was determined up to 13 ppb level (Maragou *et al.,* 2008; Davis *et al.,* 2007; Kawamura *et al.,* 1998; Hanai, 1997). These studies focused on the effect of temperature on BPA migration, but a simulated study on the daily use of the baby bottles has not been conducted, yet.

PC material can degrade as a result of repeated washing and sterilization procedures in the long term usage and this situation can lead to an increase in the amount of residual BPA in the PC material or in the migration levels of BPA in the food (Biederman-Brem and Grob, 2009; Sajiki and Yonekube, 2004; Brede *et al.,* 2003). But in the literature there are some discrepancies concerning the effect of repetitive processes on BPA migration and residual amounts of BPA in PC material. It was stated that BPA migration did not increase with repetitive processes of sterilization with boiling water and brushing with detergent (Maragou *et al.,* 2008) and process of sterilization with microwave (Ehlert *et al.,* 2008); whereas, it was reported that the residual amount of BPA in PC material increased with process of sterilization with microwave (Biederman-Brem and Grob, 2009), process of sterilization with boiling water and process of brushing with detergent (Brede *et al.,* 2003).

The aim of this study was (i) to make a general evaluation by performing residue analysis on the brand-new polycarbonate baby bottles, (ii) to determine the factors affecting the migration of BPA to the food by applying realistic contact conditions, and (iii) to investigate effects of repetitive processes of brushing with detergent, sterilization with boiling water, sterilization with microwave oven, and sterilization with baby bottle sterilizer on residual BPA.

Legal status of BPA

In Europe in 2002, BPA was authorized to be used as a monomer and an additive for the manufacturing of plastic materials and articles intended to come in contact with foodstuffs together with a specific migration limit of 0.6 mg kg^{-1} food (Commission Directive, 2002/72/EC).

This Directive was amended in 2011, with a temporary ban on the use in the manufacturing of polycarbonate infant feeding bottles as from 1 March 2011 and the marketing of these feeding bottles as from 1 June 2011 (Commission Directive, 2011/8/EU). Since May 2011, Directive 2002/72/EC is replaced by Regulation No 10/2011/EU, which has maintained the ban of BPA in polycarbonate infant feeding bottles and kept the current restriction for BPA as a monomer with a specific migration limit (SML) of 0.6 mg kg^{-1} food but removed its authorization as an additive in plastic food contact materials and articles (Commission Regulation, No 10/2011).

BPA was evaluated for the first time in 1986 by the Scientific Committee for Food (SCF) for its use in plastic materials and articles intended to come into contact with foodstuffs. At that time, the Committee allocated a tolerable daily intake (TDI) of 50 µg kg^{-1} bw day^{-1} (EFSA, 2002).

EFSA re-evaluated BPA in 2002 for its use in food contact materials. The Panel's conclusions were based on the then available, extensive database on repeated-dose toxicity, reproductive and developmental toxicity of BPA in rodents and on comparison of toxicokinetics in primates including humans and rodents. The Panel concluded to derive a temporary TDI (t-TDI) of 10 µg BPA kg^{-1} bw day^{-1} (EFSA, 2008; EFSA, 2006; EFSA, 2002).

EFSA completed its first full risk assessment of BPA in 2006 and revised a TDI of 50 µg kg^{-1} bw day^{-1} for this substance. This is based on the no-observed-adverse-effect-level (NOAEL) of 5 mg kg^{-1} bw day^{-1} that has been identified in two multi-generation reproductive toxicity studies in rodents, where the critical effects were changes in body and organ weights in adults and offspring rats and liver effects in adult mice, respectively (EFSA, 2006).

Since 2006, EFSA and its scientific panels have considered hundreds of scientific publications in peer-reviewed scientific journals as well as reports from studies submitted by industry. EFSA reviewed new scientific information on BPA in 2008, 2009, 2010 and 2011: on each occasion EFSA's experts concluded that they could not identify any new evidence which would lead them to revise the TDI for BPA of 50 µg kg^{-1} bw day^{-1} (EFSA, 2015).

In the draft opinion dated January 2014, EFSA's Panel on Food Contact Materials, Enzymes, Flavorings and Processing Aids (CEF) identified possible adverse effects on liver and kidney and effects on the mammary gland as being linked to exposure to the chemical. It is therefore recommended for the current TDI to be lowered from its current level of 50 µg kg^{-1} bw day^{-1}) to 5 µg kg^{-1} bw day^{-1} (EFSA, 2014). And t-TDI value was lowered to 4 µg kg^{-1} bw day^{-1} by EFSA CEF Panel as from January 2015 (EFSA, 2015).

Materials and Methods

Chemicals and samples

BPA (Pubchem CID:6623) standard (99% purity) was purchased from Aldrich Chemical Co. (St.Louis, MO). Stock solution for residual BPA analysis was prepared by using methanol and standard working solutions for residual BPA analysis were in the range of 25-400 µg L^{-1}. Stock solutions for migration analyses were prepared by using distilled water, 50% EtOH and 3% acetic acid for migration of BPA. Standard working solutions for migration analyses were in the range of 10-100 µg L^{-1}. Water, used as HPLC solvent, as simulant for the migration tests, and for preparation of BPA standard solutions, was purified with by using a Zeneer Power 1 (Human Corporation, Seoul-Korea).

For residue analysis of BPA, PC baby bottles from eight international brands were supplied from different companies. PC baby bottles from the trademark that was manufactured in Turkey were used for BPA migration analysis.

Identification of the material of baby bottles with FT-IR

The material of the baby bottles was subjected to infrared analysis to confirm that the material was polycarbonate. FT-IR measurements were carried out by using a Perkin-Elmer Spectrum 100 FT-IR spectrometer (Bucks, UK).

Residual BPA analysis

Total residual content of BPA in PC baby bottle was determined according to the method of Biles *et al.,* (1997). 20 mL of dichloromethane was added in to 1 g portion of polymer. Beaker was placed in an ultrasonic water bath at ambient temperature and kept until the polymer was completely dissolved. The dissolved polymer was titrated with 50 mL of methanol. The polymer precipitate was allowed to settle for 10 min. The supernatant was removed and filtered through a polytetrafluoroethylene (PTFE) filter of 0.45 µm

pore size and analyzed by HPLC-FLD. Method validation procedure of residual BPA analysis was performed by examining linearity, limit of detection (LOD), limit of quantitation (LOQ), and repeatability (Rec).

BPA migration analysis

Migration analyses were performed after PC baby bottles were subjected to proper migration test conditions. PC baby bottles were contacted with food simulants by the filling method. Sample from the simulant was taken into the vial and analyzed by HPLC-FLD. Method validation procedure of BPA migration analysis was performed by examining linearity, limit of detection (LOD) and limit of quantitation (LOQ) for each food simulant.

Design for migration analysis

Migration of BPA from PC baby bottles was investigated at three filling temperatures with two storage temperatures and three contact times representing the realistic conditions of the use of baby bottles on three food types to determine the migration potentials of PC baby bottles.

Food simulants indicated in Commission Regulation No 10/2011: distilled water (representing aqueous food), 50% ethanol (representing milk) and 3% acetic acid (v/v) aqueous solution (representing fruit juice) were used as food simulants for the migration testing.

I. Contact conditions: 5°C and 22°C were chosen for representing refrigerator temperature and room temperature, respectively.
II. Contact conditions: 40°C, 60°C and 80°C were chosen for representing hot filling. Baby bottles were filled at these temperature values and then stored at room temperature.

BPA migration analysis was carried out at 0.5, 12 and 24 hours of contact.

Design for repetitive procedures

Following treatments were applied to baby bottles for simulation and residual BPA content of PC material was analyzed before treatment (initial amount of BPA) and at 10^{th}, 30^{th} and 50^{th} cycles of treatments.

Brushing with detergent (APP1): Baby bottles were washed with tap water at 45°C with a standardized procedure and then left for drying for 10 minutes in each cycle.

Sterilization with boiling water (APP2): Baby bottles were sterilized for 5 minutes and then left for drying for 10 minutes in each cycle.

Sterilization with microwave oven (APP3): Baby bottles were sterilized for 5 minutes and then left for drying for 10 minutes in each cycle.

Sterilization in baby bottle sterilizer (APP4): Baby bottles were sterilized for 5 minutes and then left for drying for 10 minutes in each cycle.

Chromatographic parameters

Chromatographic separations were carried out by using an Agilent 1200 Series HPLC system (Santa Clara, United States). HPLC conditions employed were as follows:

Mobile phase: methanol/water (70:30, v/v) was used in isocratic elution mode,

Flow rate for analysis: 0.4 mL/min,

Analytical column: Zorbax Eclipse XDB C_{18} (150mm ×4.6mm i.d.),

Temperature: 25°C,

Injection volume was 10 µl for all the solutions,

Fluorescent detection at Ex: 225, 235 and 285 nm and at Em:315 nm)

Statistical analysis

The data of residual content of BPA were statistically analyzed and presented as the mean ± standard deviation (SD). The coefficient of determination (r^2) was determined by regression/ correlation analysis in the SPSS software (IBM, PASW Statistics 19, USA). Statistical significance was performed using one-way ANOVA and Duncan's multiple range tests ($p \leq 0.05$).

Results and Discussion

Method validation of total residual content

Standard working solutions were prepared in methanol. Calibration curves were obtained by measuring standard working solutions at five levels in the range of 25-400 µg L^{-1} with three replicates per concentration. Linear regression analysis was performed using the analyte peak area vs. analyte concentration ($r^2 > 0.99$) (Figure 1). Limit of detection (LOD) and limit of quantification (LOQ) were determined as 33.75 ppb and 54.15 ppb, respectively (Table 1).

Recovery was determined as 100.41%. Figure 2 shows a chromatogram of residual BPA in sample of PC baby bottle.

Method validation of migration analysis

Method validation was carried out for each food simulant. Standard working solutions were prepared by using distilled water, 50% ethanol, and 3% acetic acid. Calibration curves were obtained by measuring standard working solutions at five levels in the range of 10-100 µg L^{-1} with three replicates per concentration. The coefficient of determination (r^2) of the obtained calibration curve was found as >0.99. Table 1 shows LOD and LOQ values for each food simulant. Figure 3 shows chromatograms of BPA for each food simulant.

Residue analysis

Residue analyses were performed on samples of PC baby bottles supplied from eight companies. As a result of these tests, different amounts of residual BPA were determined (Table 2). While the highest amount of residual BPA was 6.23 ±0.24 ppm, the lowest amount was 0.60 ±0.05 ppm.

Different amounts of residual BPA in PC baby bottles are found in the literature and these values range from 5-10 ppb levels to 141 ppm (Table 3). While considering the results of these studies, baby bottles analyzed from different international companies were not too high and had relatively standard quality in terms of levels of residual BPA.

Migration analysis

As a result of migration analyses performed under contact condition I (5°C and 22°C of storage temperatures) and contact condition II (40°C, 60°C, and 80°C of filling temperatures), the migrated BPA could not be detected in any of the contact conditions. This result showed that amount of migrated BPA under these contact conditions was below the specified limit of detection value of the method for each simulant shown in Table 1.

As a result of literature review, it was observed that BPA migration varied from 3 to 55 µg L^{-1} (LOD was 2 µg L^{-1}) in PC baby bottles filled with boiling water (95°C) and kept overnight at room temperature (Hanai, 1997). In another study, any BPA migration was not specified from PC baby bottles that were contacted with distilled water at 60°C for 30 minutes but 0,5 ppb of BPA was detected when they performed the same test at 95°C of contact temperature (Kawamura *et al*., 1998). Other researchers analyzed the migration of BPA from PC baby bottles filled with boiling water and left at ambient temperature for 45 min. The migration values of BPA ranged between 2.4-14.3 µg L^{-1} (LOD is 2.4 µg L^{-1}) (Maragou *et al*., 2008). In a study conducted in the UK by Central Test Laboratory, BPA migration tests were performed on PC baby bottles. Half an hour contact at 70°C was applied with 10% ethanol and 3% acetic acid. No migration was detected (LOD was 1.1 µg L^{-1} for 10% ethanol and 0.3 µg L^{-1} for 3% acetic acid) (CSL, 2004). Although limit of detection was very low, migration of BPA was not detected in this study as in the present study. In a migration study conducted with five baby bottle brands in America and Canada, BPA migration was not determined from PC baby bottles left at ambient temperature for 24 hours; whereas, 5-8 ppb of BPA migration was reported when the contact temperature was increased to 80°C for 24 hours (Davis *et al.*, 2007).

According to these results, when the LOD value of 10.40 ppb for 50% ethanol (the simulant of infant formula) was considered as the maximum migration value, the daily intakes of BPA estimated via infant formula in PC baby bottle can be calculated as follows: (The food consumption scenario was taken from EFSA, 2006 based on the German DONALD study by Kersting, 1998) (EFSA, 2006).

- ✓ For a 3 month-old infant having a weight of 6.1 kg and consuming 174 mL kg^{-1} bw of infant formula via PC baby bottle, the daily intake of BPA estimated from our results was 1.8 µg kg^{-1} bw day^{-1}.

  10.40 µg L^{-1} (LOD) x 1.061 L (daily consumption amount of infant formula for an infant having a weight of 6.1 kg) / 6.1 kg (weight of infant)= 1.8 µg kg^{-1} bw day^{-1},
- ✓ For a 6 month-old infant having a weight of 7.8 kg and consuming 118 mL kg^{-1} bw of infant formula, the daily intake of BPA estimated from our results was 1.22 µg kg^{-1} bw day^{-1}.

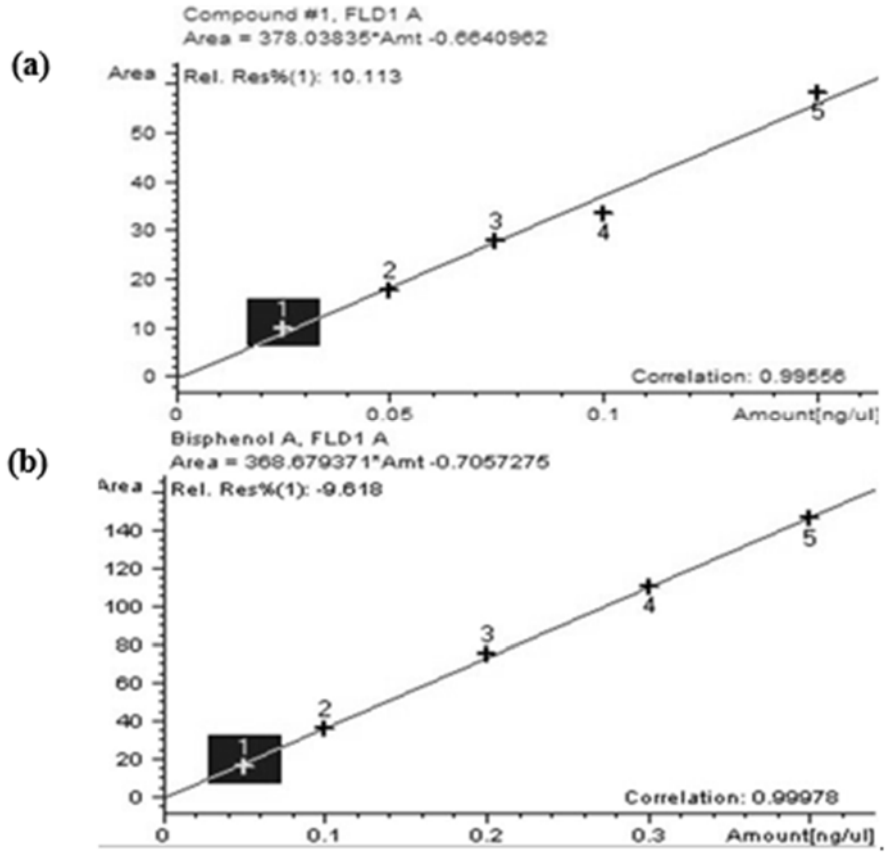

Figure 1. Calibration curves used in BPA residue analysis (a) 25-150µg l^{-1} standard solution (b) 50-400µg l^{-1} standard solution.

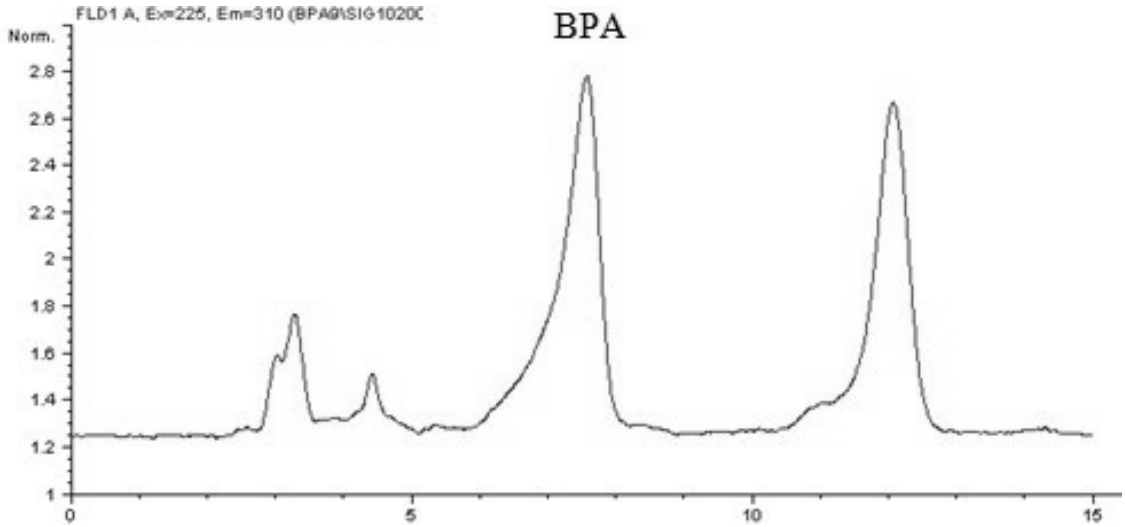

Figure 2. Chromatogram of BPA residue in PC baby bottle sample.

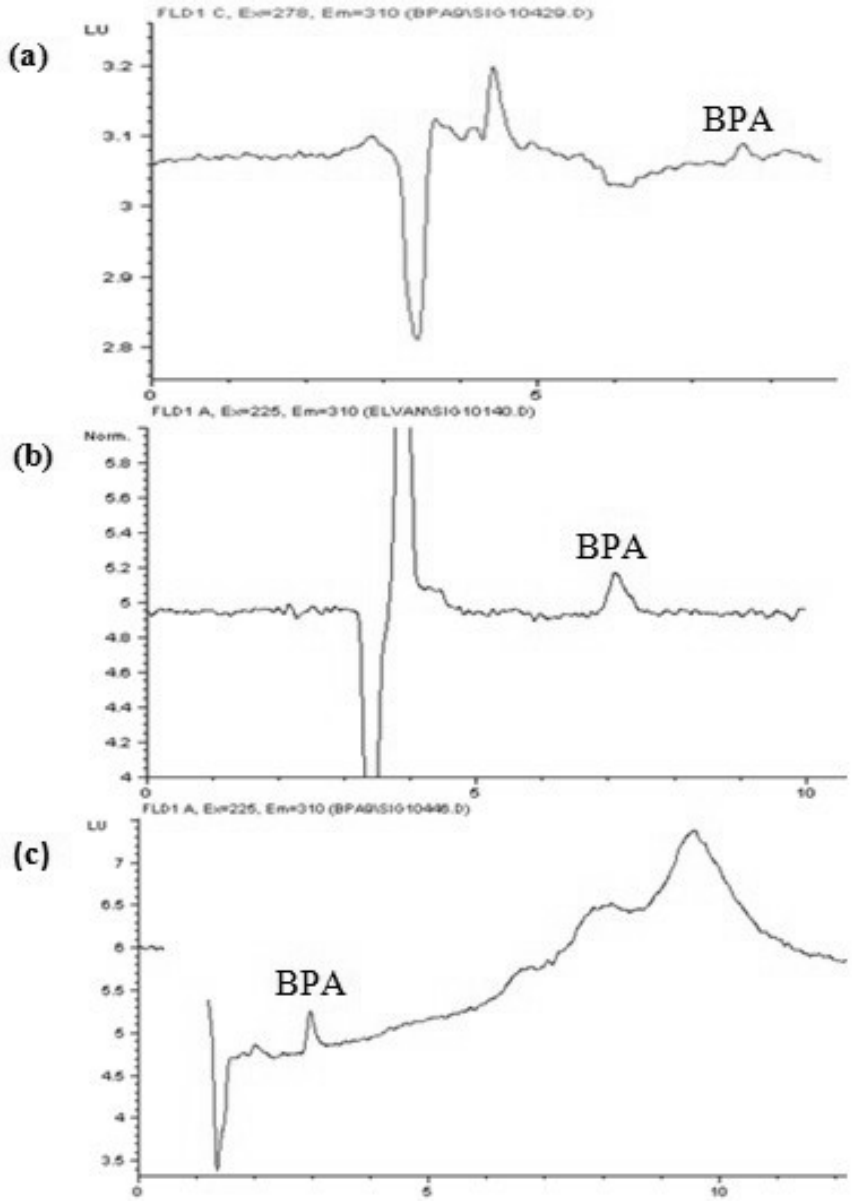

Figure 3. Chromatograms of (a) 12,5 ppb BPA in distilled water, (b) 10 ppb BPA in 50% ethanol and (c) 1 pbb BPA in 3 % acetic acid.

Table 1. Limit of detection and limit of quantification values of BPA migration analysis for different food simulants and residual BPA analysis

	LOD (ppb)	LOQ (ppb)
Distilled water	15.10	26.90
50% ethanol	10.40	11.34
3% acetic acid	1.30	2.00
Residue analysis	33.75	54.15

Table 2. The detected amount of residual BPA in PC baby bottles belong to different firms

Brand number	Residual content (ppm)
1	1.75 ± 0.15^{b}
2	6.23 ± 0.24^{d}
3	0.60 ± 0.05^{a}
4	5.76 ± 1.08^{d}
5	2.20 ± 0.40^{c}
6	2.79 ± 1.36^{c}
7	1.95 ± 0.60^{b}
8	2.10 ± 0.10^{c}

Means within each column followed by different letters are significantly different at $p \leq 0.05$.

Table 3. Residual BPA amounts of PC baby bottles in different studies.

Reference	Material type	Amount of BPA residue
Ehlert *et al.*, 2008	PC baby bottle	1.4-35.3 ppm
Biedermann-Brem *et al.*, 2008	PC baby bottle	6-25 ppb
Wong *et al.*, 2005	PC baby bottle	4.01-141 ppm
Biles *et al.*, 1997	PC baby bottle	7-58 ppm
Mountforth *et al.*, 1997	PC baby bottle	18-139 ppm
Nam *et al.*, 2010	PC baby bottle	16.2-17.6 ppm
IAT, 2007	PC baby bottle	5-10 ppb

10.40 µg L^{-1} (LOD) x 0.920 L (daily consumption amount of infant formula for an infant having a weight of 7.8 kg) / 7.8 kg (weight of infant)=1.22 µg kg^{-1} bw day^{-1}

When we considered that the amount of BPA migrated from analyzed baby bottles to 50% ethanol was equal to the LOD value of 10.40 ppb, this assessment on infant dietary exposure to BPA from PC baby bottles in the present study was lower than the dietary estimates for infants presented in opinion of EFSA (2006) as shown in Table 4 and also was lower than the latest value of t-TDI (4 µg kg^{-1} bw day^{-1}) determined by EFSA (2015).

Effect of repetitive procedures:

As a result of repetitive procedures as it was seen from Table 5, it was found that; brushing with detergent procedure (APP1) did not change the amount of residual BPA in PC material and it varied between 1.30 ±0.05 ppm and 1.50 ±0.48 ppm amongst all cycles. Sterilization with boiling water procedure (APP2) significantly ($p\leq0.5$) affected the residual BPA amount in PC material at each cycle from 1.38 ±0.46 ppm to 10.03 ±0.93 ppm. Sterilization in microwave (APP3) significantly ($p\leq0.5$) affected the residual BPA amount especially at 50^{th} cycle of procedures whereas there was no significant change in first two cycles. It varied from 1.28 ± 0.04 ppm to 3.54 ±0.56 ppm. Sterilization in baby bottle sterilizer procedure (APP4) significantly ($p\leq0.5$) affected the amount of residual BPA for each cycle of the treatment from 1.33 ±0.07 ppm to 11.59 ±0.54 ppm. It can be concluded that brushing process does not give any wearing affect to the bottles to increase the residual BPA amount. In contrast to this all of the sterilization processes leaded to the increase in residual BPA amount in PC baby bottles. PC material can be degraded as a result of contact with high temperature water in boiling water or in microwave sterilization procedures or with saturated water in baby bottle sterilizer. Thus, as it was stated by Bidermann-Brem and Grob (2009), when the tap water boiled the degassing of carbon dioxide causes the pH to increase and form a far more aggressive medium. The high pH combined with a high temperature can lead to degradation of PC material. It was also stated by Sajiki and Yonekubo (2004) that basic pH provokes the degradation of PC material leading

to the increased migration values from the material.

Table 4. Estimates of total dietary exposure to BPA for infants (EFSA, 2006).

Age of consumer	Food/beverage consumed	Dietary exposure to BPA ($\mu g\ kg^{-1}$ bw day^{-1})
3 mounths infant	Infant formula fed with PC bottle	11
6 mounts infant	Infant formula fed with PC bottle and commercial food/beverages	13

Table 5. Effect of repetitive procedures on the amounts of residual BPA

Repetative procedure	Amount of residual BPA (ppm)
APP1 initial	1.30 ± 0.05^{a}
APP1 10^{th} cycle	1.31 ± 0.06^{a}
APP1 30^{th} cycle	1.40 ± 0.02^{b}
APP1 50^{th} cycle	1.50 ± 0.48^{c}
APP2 initial	1.38 ± 0.46^{a}
APP2 10^{th} cycle	7.23 ± 0.41^{b}
APP2 30^{th} cycle	8.56 ± 0.15^{c}
APP2 50^{th} cycle	10.03 ± 0.93^{d}
APP3 initial	1.28 ± 0.04^{a}
APP3 10^{th} cycle	1.12 ± 0.07^{a}
APP3 30^{th} cycle	1.60 ± 0.02^{a}
APP3 50^{th} cycle	3.54 ± 0.56^{b}
APP4 initial	1.33 ± 0.07^{a}
APP4 10^{th} cycle	3.59 ± 0.38^{b}
APP4 30^{th} cycle	4.78 ± 0.53^{bc}
APP4 50^{th} cycle	11.59 ± 0.54^{c}

Means within each column followed by different letters are significantly different at $p \leq 0.05$.
APP1: Brushing with detergent procedure; APP2: Sterilization with boiling water procedure; APP3: Sterilization in microwave procedure; APP4: Sterilization in baby bottle sterilizer procedure

Conclusion

This study showed that PC baby bottles can be produced with different amounts of the residual BPA ranging from 0.60 ± 0.05 ppm to 6.23 ± 0.24 ppm and the BPA residue was inevitable in PC baby bottle production. Migration of BPA from brand-new PC baby bottle to 50% EtOH was far below the specific migration limit of BPA. But for the compounds posing a health risk like BPA, it should be toxicologically evaluated. Although the tolerable daily intake of BPA has been varied too much within the years, it was lowered to 4 $\mu g\ kg^{-1}$ bw day^{-1} by EFSA in the year of 2015. Although the migrated amount of BPA was below its t-TDI value, it was very close to it.

It was seen that as the PC baby bottles were used in daily life with washing and sterilization treatments, the processes of sterilization with boiling water, microwave, and steam sterilizer changed the amount of residual BPA significantly which will lead to higher amounts of migrated BPA. But the washing treatment by brushing with detergent did not change the residual BPA amount significantly.

Although it was concluded that the use of brand-new PC baby bottles will not pose a risk to health, the amount of residual BPA will increase with the effect of sterilization processes which are the necessary treatments for the use of baby bottles for infants under the age of 1. And these processes will lead to the higher amounts of migrated BPA which can be toxically important.

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References

Biedermann-Brem, S. & Grob, K. (2009). Release of Bisphenol A from polycarbonate baby bottles: water hardness as the most relevant factor. *European Food Research Technology*, 228, 679-684.

Biedermann-Brem, S., Grob, K. & Fjeldahl, P. (2008). Release of bisphenol A from polycarbonate baby bottles: mechanism of formation and investigation of worst case scenarios. *European Food Research Technology*, 227, 1053-1060.

Biles, J.E., McNeal, T.P., Begley, T.H. & Hollifield, H.C. (1997). Determination of bisphenol-a in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. *Journal of Agricultural and Food Chemistry*, 45, 3541-3544.

Brede, C., Fjeldal, P., Skjevrak, I. & Herikstad, H. (2003). Increased migration levels of Bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing conditions. *Food Additives and Contaminants*, 20(7), 684-689.

CSL (Central Science Laboratory). (2004). A study of the migration of bisphenol A from polycarbonate feeding bottles into food simulants. Central Science Laboratory Test Report L6BB-1008 for the Boots Group.

Commission Directive. 2002/72/EC. (2002). Relating to plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Communities,* L 220/ 27.

Commission Directive. 2011/8/EU. (2011). Amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. *Official Journal of the European Communities*, L 26/ 11.

Commission Regulation. No 10/2011. (2011). Plastic materials and articles intended to come into contact with food. Official Journal of the European Communities, L 12/1.

Council Directive. 85/572/EEC. (1985) .The list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Communities,* L 372 /14

Davis, M., Fould, J., Freeman, A., Khatter, K., Polyzou, C., Robinson, J., Schade, M., Smith, R. & Vom Saal, F. (2007). Baby's Toxic Bottle: Bisphenol a leaching from popular baby bottles. Columbia: University of Missouri.

Ehlert, K.A., Beumer, C.W.E. and Groot, M.C.E. (2008). Migration of bisphenol A into water from polycarbonate baby bottles during microwave heating. *Food Additives and Contaminants A*, 25(7), 904-910.

EFSA (European Food Safety Authority). (2002). Opinion of the scientific committee on food on Bisphenol A.

EFSA (European Food Safety Authority). (2006). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). *EFSA Journal*, 428, 1-75.

EFSA (European Food Safety Authority). (2008). Toxicokinetics of bisphenol a: scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food (AFC). *EFSA Journal*, 759, 1-10.

EFSA (European Food Safety Authority). (2014). Draft scientific opinion on the risks to public health related to the presence 2 of bisphenol A (BPA) in foodstuffs: EFSA panel on food contact materials, enzymes, flavourings and processing aids.

EFSA (European Food Safety Authority). (2015). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA panel on food contact materials, enzymes, flavourings and processing aids.

Geueke, B., Wagner, C.C. & Muncke, J. (2014). Food contact substances and chemicals of concern: a comparison of inventories. *Food Additives and Contaminants A*, 33, 1438-1450.

Hanai, Y. (1997). Bisphenol-A eluted from nursing bottles. Unpublishing data. Enviromental Science Research Center. Yokohama National University.

IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.

IAT (Institute for Agricult. and Trade Policy), (2007), Toxic Chemical Leaches from Popular Baby Bottles

Kawamura,Y., Koyama,Y., Takeda, Y. & Yamada T. (1998). Migration of bisphenol-A from polycarbonate products. *Journal of the Food Hygienic Society of Japan,* 39, 206-212.

Maia, J., Cruz, J.M., Sendon, R., Bustos J., Sanchez J.J. & Paseiro P. (2009). Effect of detergents in the release of bisphenol a from polycarbonate baby bottles. *Food Research International,* 42, 1410-1414.

Maragou, N.C., Marki, A., Lampi, E.N., Thomaidis, N.S. & Koupparis M.A. (2008). Migration of bisphenol A from poycarbonate baby bottles under real use conditions. *Food Additives and Contaminants*, 25(3), 373-383.

Mountfort, K.A., Kelly, J., Jickells, S.M. & Castle, L. (1997). Investigations in to the potential degradation of polycarbonate baby bottles during sterilization with consequent release of Bisphenol A. *Food Additives and Contaminants*, 14 (6-7), 737-740.

NTP (National Toxicology Program). (2008). NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. Center for the Evaluation of Risks to Human Reproduction. NIH Publication No: 08-5994.

Robertson, G.L. (2006). *Food packaging: principles and practice*. Boca Raton: Taylor and Francis. p. 9-42.

Sajiki, J., Yonekubo, J. (2004). Leaching of Bisphenol A (BPA) from polycarbonate plastic to water containing amino acids and its degradation by radical oxygen species. *Chemosphere,* 55, 861-867.

Sugiura-Ogasawara, M., Ozaki, Y., Sonta, S.I., Makino, T. & Suzumori, K. (2005). Exposure to bisphenol A is associated with recurrent miscarriage. *Human Reproduction,* 20, 2325-2329.

Takeuchi, T. & Tsutsumi, O. (2002). Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. *Biochemical and Biophysical Research Communications*, 291, 76-78.

Üçüncü, M. (2007). Plastikler ve Plastik Esaslı Ambalaj Materyalleri. In Üçüncü, M. (Ed). *Gıda Ambalajlama Teknolojisi*. p. 328-329. İzmir: Meta Basım Matbaacılık Hizmetleri.

Vandenberg, N., Hauser, R., Marcus, M., Olea, N. & Wade, W. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology,* 24, 139-177.

Wong, K.O., Leo, L.W. & Seah, H.L. (2005). Dietary exposure assessment of infants to bisphenol A from the use of polycarbonate baby milk bottles. *Food Additives and Contaminants A*, 22(3), 280-288.

Yamada, H., Furuta, I., Kato, E.H., Kataoka, S., Usuki, Y. & Kobashi, G. (2002). Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. *Reproductive Toxicology,* 16, 735-739.

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

SOME BIOACTIVE PROPERTIES OF WILD AND COMMERCIAL MUSHROOM SPECIES

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Abstract:

In this study, the protein and total phenolic contents of some commercially cultivated (*Agaricus bisporus*, *Pleurotus ostreatus*) and wild mushrooms (*Amanita caesarea*, *Fistulina hepatica*, *Meripilus giganteus*) were determined. Antioxidant and antimicrobial properties of these mushrooms against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Acinetobacter haemolyticus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Candida albicans* were also investigated. The protein contents, total phenolic contents and antioxidant activities of the mushrooms were found in the range of 11.00 - 25.1%, 1.111 - 3.858 mg GAE g^{-1}, and 1.528 - 9.340 µmol $FeSO_4 \cdot 7H_2O$ g^{-1}, respectively. *Meripilus giganteus* had higher protein than all the tested mushrooms. The highest total phenolic content was detected in *Agaricus bisporus* obtained from B company (3.858 mg GAE g^{-1}), whereas the lowest total phenolic content was observed in *Meripilus giganteus* (1.111 mg GAE g^{-1}). Total phenolic and antioxidant properties of mushrooms were found significantly different ($P<0.05$) by Duncan's multiple range test. Methanolic extracts of the tested mushrooms showed no inhibitory activity against bacteria and yeast.

Keywords: Antioxidant, Antimicrobial, Mushroom, Protein, Total phenolic content

Introduction

Most of edible mushrooms such as basidiomycetes are known as high nutritional value food rich in biologically active compounds (Breene, 1990). These mushrooms naturally grow on soils, trunks and on the roots of the trees (Iwalokun *et al.*, 2007). Mushrooms are generally rich in vegetable proteins, vitamins and minerals with low levels of calories, fats and essential fatty acids. (Barros *et al.*, 2007a). Many of these ingredients include molecules with medical activities such as anti-inflammatory, antitumor, antibacterial, antioxidant and antiviral activities (Barros et al., 2007b).

Human body provides its needs in protein from meat and meat products and vegetarian peoples usually get difficulties to recompense their protein supply for a healthy life with their restricted food sources. However, mushroom may constitute an important and natural alternative of essential proteins and vitamins for these cases. Some researchers reported that protein values of mushrooms are higher than some vegetables and fruits as asparagus, potatoes, tomatoes, carrots, and oranges (Jiskani, 2001; Adejumo and Awosanya, 2005).

Mushrooms also contain phenolic compounds that are important scavengers of free radicals (Murcia *et al*. 2002). These phenolic compounds may inhibit atherosclerosis and cancer (Diplock *et al.*, 1998) (Williams *et al.*, 1999) without considerable mutagenic effects (Ishikawa *et al.*, 1984). Undoubtedly, the toxic effects of chemical and synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) may be prevented by the use of mushrooms constituents as natural antioxidant compounds (Lee *et al.*, 2007). For this reason, such types of natural antioxidants gained importance especially in the medical researches. Recently, infections with multi-drug resistant microorganisms have increased due to the irregular overuse of antimicrobials. This situation has forced scientists to search for new antimicrobial compounds in natural sources like mushrooms (Karaman *et al.*, 2003).

Mushroom is considered a valuable food for Turkish people and *Agaricus bisporus* is one of the most commercially cultivated mushroom varieties in Turkey. Moreover, the habit of consumption of wild edible mushrooms is also very common in Turkey.

The objectives of this study were: (i) to determine protein and total phenolic contents of some wild (*Amanita caesarea*, *Fistulina hepatica*, *Meripilus giganteus*) and commercially cultivated mushrooms (*Agaricus bisporus* obtained from four different companies, *Pleurotus ostreatus* obtained from one company) grown in Turkey, (ii) to investigate antioxidant and antimicrobial properties of these mushrooms. This study is one of a few recent researches that compare the bioactive performance of wild and cultivated mushrooms.

Materials and Methods

Mushrooms

Wild mushrooms (*Amanita caesarea*, *Fistulina hepatica*, *Meripilus giganteus*) were collected from the province of Kastamonu, located in the northwest of Turkey, in October 2014. The morphological and ecological characteristics of mushrooms were noticed and photographed in their natural habitats. Cultivated mushrooms, *Pleurotus ostreatus* were obtained from one company and *Agaricus bisporus* were obtained from four different companies, in Trabzon, Turkey. Ethically, the commercial mushroom companies were coded as A, B, C, and D. Mushrooms were identified based on their morphological characteristics and dried for future analysis. Some information about the mushrooms used in this study such as species, habitat, location and edibility are given in Table 1.

Determination of protein content, carbon (C), hydrogen (H), nitrogen (N) values

Each mushroom was dried at 40˚C before the analysis. Dried mushroom samples were crushed and powdered for passing a 40 mm mesh sieve. Protein contents of mushrooms were determined according to Dumas method (Ebeling, 1968). Briefly, 0.5 - 0.7 mg dried mushroom samples were weighed and placed on 5 × 9 mm tin capsules. The capsules were then placed into Costech ECS 4010 elemental analysis instrument and were burned. The values of carbon, hydrogen and nitrogen were determined using Costech ECS 4010 program. Protein contents were determined by multiplying (%) of nitrogen results with conversion factor (4.38) (Crisan and Sands, 1978).

Measurement of total phenolic content

Extraction method

Mushrooms were dried 60°C for 24 hour (Profilo, PFD1350W, Turkey). Four grams of dried sample was extracted with 40 mL methanol by shaking at 150 rpm for 24 h and filtered through Whatman

No. 4 filter paper pore size 20-25 µm. Then solutions were filtrated from hydrophilic polyvinylidene fluoride (PVDF) 0.45 µm for sterilization. The final volume of the solution was adjusted by the level of methanol. Extracts were stored at 4°C for future use.

Total phenolic content

The total phenolic contents of the methanolic extracts were determined according to Folin–Ciocalteu method using gallic acid standard (Slinkard and Singleton, 1977). The Folin assay was based on all phenolic contents including phenolic acids, flavonoids, and anthocyanins in the aquatic solution, which gives a blue color complex whose maximum absorbance can be read at 760 nm. Briefly, 680 µL distilled water, 20 µL methanolic extract and 400 µL of 0.5 N Folin-Ciocalteu regents were mixed in a test tube, vortexed for 2 min, then 400 µL Na_2CO_3 10% (v/v) was added and incubated for 2 hours at room temperature. Following the incubation, absorbance of the mixtures was measured at 760 nm on an ATI-Unicam UV-2 UV-VIS spectrophotometer (Cambridge, U.K.). The concentration of total phenolic compounds was calculated as mg gallic acid equivalents (GAE) g^{-1} of dry weight. Total polyphenol calibration graph was shown in Figure 1.

Table 1. Some properties of mushrooms examined in the study

No	Mushroom species	Habitat and Location	Edibility	Growing Form
1	*Amanita caesarea*	On soil, Kastamonu	Edible	Wild
2	*Fistulina hepatica*	On woods, Kastamonu	Edible*	Wild
3	*Meripilus giganteus*	On woods, Kastamonu	Edible	Wild
4	*Agaricus bisporus* (A)	On compost, Trabzon	Edible	Cultivated
5	*Agaricus bisporus* (B)	On compost, Trabzon	Edible	Cultivated
6	*Agaricus bisporus* (C)	On compost, Trabzon	Edible	Cultivated
7	*Agaricus bisporus* (D)	On compost, Trabzon	Edible	Cultivated
8	*Pleurotus ostreatus*	On compost, Trabzon	Edible	Cultivated

*: It is known as poisonous in the current location

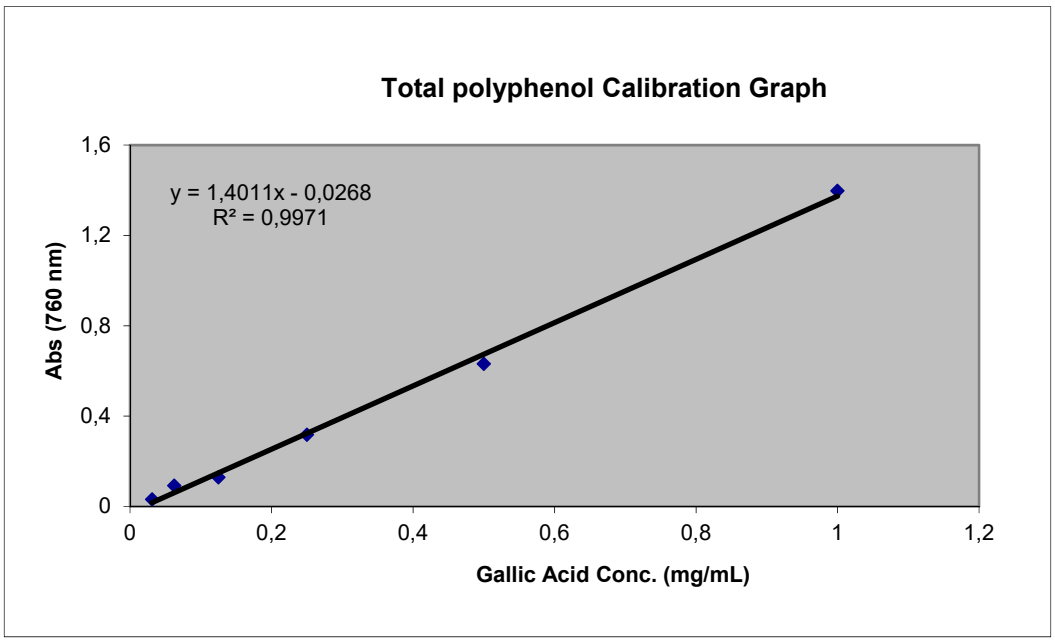

Figure 1. Total polyphenol calibration graph

Determination of antioxidant activity

The antioxidant activity of the mushroom extracts was determined according to Ferric-reducing antioxidant power (FRAP) method. The reducing ability of ferric tripyridyltriazine (Fe-III-TPTZ) complex was used for total antioxidant capacity assay (Benzie and Strain, 1999) with some modifications. Working FRAP reagent was prepared as required by mixing of 300 mM acetate buffer, pH 3.6 with 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. Three milliliters freshly prepared FRAP reagent, 100 µL of samples was mixed and incubated for 4 min at 37 °C, and the absorbance was read at 593 nm against reagent blank containing distilled water. $FeSO_4 \cdot 7H_2O$ was used as positive control. The ferric-reducing antioxidant power of the antioxidants in the extracts was calculated by comparison with $FeSO_4 \cdot 7H_2O$ as µmol $FeSO_4 \cdot 7H_2O$ g^{-1} dry weight of mushrooms.

Antimicrobial activity testing

Mushroom extracts were tested for antimicrobial activity by agar-well diffusion method in accordance with the Clinical & Laboratory Standards Institute (CLSI) (M100-S22; 2012). Tested microorganisms included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Acinetobacter haemolyticus* ATCC 19002, *Klebsiella pneumoniae* ATCC 13883, *Salmonella* typhimurium ATCC 14028, *Proteus mirabilis* ATCC 7002 and *Candida albicans* ATCC 10231. Microorganisms were obtained from Karadeniz Technical University, Department of Medical Microbiology, Faculty of Medicine Trabzon, Turkey.

Several bacterial colonies were suspended in 5 mL of sterile isotonic sodium chloride solution and turbidity was adjusted to 0.5 McFarland standards. The microbial suspension was spread on Mueller Hinton agar using sterile cotton swabs. The wells were made in agar plates using the wide end of a blunted sterile Pasteur pipette. Each well was filled with 100 µL of mushroom extracts. Commercial Ampicillin, Gentamicin, Cefotaxime and Amphotericin B solutions were used as positive control (10 µg for each well) and methanol was tested as negative control. The cultures were incubated at 37 °C for 24 hours. Activity was determined by visual inspection and measurement of the diameter of clear inhibition zones around the agar-wells.

Statistical analysis

Total phenolic content and antioxidant analyses were performed in triplicates. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 23.0). The data related to total phenolic content and antioxidant property were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests. Differences among the means at 5% ($p < 0.05$) level were considered as significant. Pearson correlation coefficient was used to determine the relationship between protein content, total phenolic content and the antioxidant activity in the same sample.

Results and Discussion

Protein content, Carbon (C), Hydrogen (H) and Nitrogen (N) Values of Mushrooms

Carbon, hydrogen, nitrogen values and protein contents of wild and cultivated mushrooms were given in Table 2.

Nitrogen content of mushrooms were ranged from 2.42 to 5.75%. The lowest value was determined in *P. ostreatus.* The amounts given in the references for the desirable nitrogen content at the outset of composting vary between 1.5% and 2.0% computed on dry weight basis (Vedder, 1978; Demirer *et al.*, 2005). However, as a wild mushroom, *M. giganteus* showed higher protein and nitrogen content than that of *A. bisporus* grown commercially. Thus, the nature has met the requirements of *M. giganteus* in the best way. Some researchers reported that the excess or lack of N content in the substrate might be a limiting factor for fungus growth (Carlile *et al.*, 2001).

Protein content of mushrooms ranged from 11.00 to 25.19%. The result can be compared with different mushrooms protein contents that varies between 8.6% and 42.5% (Peksen *et al.*, 2008; Cohen *et al.*, 2014). It was observed a linear relationship between the amount of protein and nitrogen ratio. The previous studies showed that the protein contents of mushrooms were affected by many factors, such as mushroom species, growth conditions, compost mixture, mycelium quality, the part sampled, level of nitrogen available and the location (Al-Momany and Gücel 2012, Yildiz *et al.*, 2015).

Some researchers already reported that the protein contents in the mushrooms were higher than that

of some fruits such as grape (*Vitis vulpina*), hackberry (*Celtis occidentalis*) (Halls, 1977; Johnson *et al*., 1985). Mushrooms are a good source in terms of protein and amino acids compared to foods of plant origin (Kurtzman *et al*., 1993). According to the literature, the amino acid configurations of mushrooms are comparable to some animal proteins (Longvah and Deosthale, 1998; Mattila *et al*., 2001).

Total phenolic content and ferric reducing antioxidant capacity

The total phenolic content and ferric reducing antioxidant capacity of mushrooms are presented in Table 3.

In the study, the total phenolic content ranged from 1.111 to 3.858 mg GAE g^{-1}. While the highest total phenolic content was determined in *A. bisporus* obtained from B company (3.858 mg GAE g^{-1}), the lowest value was detected in *M. giganteus* (1.111 mg GAE g^{-1}). Average of total phenolic content values (2.341 mg GAE g^{-1}) was found higher than some fresh vegetables, such as loquat at a level of 1.994 mg GAE g^{-1} (Lin and Tang, 2007) and carrot, lettuce, white cabbage, and cauliflower at 0.132, 0.134, 0.153, 0.278 mg GAE g^{-1}, respectively (Bahorun *et al*., 2004).

In a recent study; it was reported that the total phenolic content of methanolic extracts of 4 wild mushrooms (*Ganoderma lucidum*, *Morchella esculenta*, *Lentinula edodes* and *Hericium erinaceus*) varied from 5.81 to 26.40 mg g^{-1} dw (Yildiz *et al*., 2005). The composition of phenolic contents of mushrooms generally depends on genetic, environmental and other factors. It was recorded that the phenolic structure in mushrooms might be affected by a number of factors such as composition of growth media, mushroom species, time of harvest, the types and ratios of substrate supplements (Heleno *et al*., 2010).

In this study, the second highest phenolic content (3.101 mg GAE g^{-1}) was determined in *F. hepatica*. However, Heleno *et al*., (2010) reported that the total phenolic content of the same mushroom grown in Portuguese was 4.44 mg GAE g^{-1}. *F. hepatica*, also known as beefsteak fungus or ox tongue because of the color and texture of the edible fruiting body, is a cosmopolitan fungus (Keleş *et al*., 2011). As one of the wild edible species; *F. hepatica* exhibited a relatively high phenolic content. This result can be considered as a satisfactory situation. The presence of such a high total phenolic contents in these mushrooms makes them important natural source of phenolic compounds. These kinds of mushrooms are very precious because of their use as a foodstuff and in medical applications (Yildiz *et al*., 2005). The phenolic compounds were reported as natural antioxidants. They are stopping the free radical reactions; therefore, arise of many diseases such as cancer, and lung diseases are being prevented (Nizamlıoğlu and Nas 2010). In addition, total phenolic contents of the mushrooms used in this study were found significantly different ($P< 0.05$) from each other by Duncan's multiple range test.

Table 2. Carbon (C), hydrogen (H), nitrogen (N) values and protein contents of wild and cultivated mushrooms*

Mushroom species	H (%)	C (%)	N (%)	Protein (%)
Amanita caesarea	6.41	38.95	3.46	15.15
Fistulina hepatica	6.28	40.72	2.67	11.70
Meripilus giganteus	6.36	43.30	5.75	25.19
Agaricus bisporus (A)	6.31	36.94	3.68	16.11
Agaricus bisporus (B)	5.98	38.23	4.22	18.48
Agaricus bisporus (C)	6.33	38.44	4.03	17.65
Agaricus bisporus (D)	6.18	39.46	3.98	17.43
Pleurotus ostreatus	6.37	38.15	2.42	11.00

*: The values were expressed on dry weight basis.

FRAP activities ranged from 1.528 to 9.340 µmol $FeSO_4 \cdot 7H_2O$ g^{-1}. The average FRAP activity for the mushrooms used in this study (5.778 µmol $FeSO_4 7H_2O$ g^{-1}) was found higher than that of some fresh vegetables such as carrot, lettuce, tomato, white cabbage (0.60, 0.68, 0.78, 1.56 µmol Fe^{2+} g^{-1}; respectively) (Bahorun *et al.*, 2004).

The results showed that FRAP activity for the mushrooms used in this study (5.778 µmol $FeSO_4 7H_2O$ g^{-1}) were higher than some fresh wild edible mushrooms (*Lactarius deliciosus*, *L. sanguifluus*, *L. semisanguifluus*, *Russula delica*, *Suillus bellinii*) grown in the island of Lesvos, Greece (0.271– 0.523 µmol Fe^{2+} g^{-1}, respectively) studied by Kalogeropoulos *et al.*, (2013).

Ferric reducing antioxidant capacity among the mushrooms used in this study was significantly different ($P< 0.05$) by Duncan's multiple range test. Extraction conditions are the basis factors to improve the efficiency of antioxidative natural resources. In previous studies, it was reported that solvent type, concentration, extraction time, temperature and particle size etc. influenced the total phenolic content and antioxidant activity of extracts (SengYim *et al.*, 2009; Slawinska *et al.*, 2013).

Antimicrobial activity

Antimicrobial activity of some mushrooms has been determined against some microorganisms (Barros *et al.*, 2007b) (Vazirian *et al.*, 2014). However, the methanolic extracts of mushrooms used in this study showed no inhibitory activity against the tested bacteria or yeast. Moreover, Öztürk *et al*. (2011) also reported that methanolic extract of *Agaricus bisporus* did not show any antibacterial activity against Gram-negative bacteria. However, Vamanu *et al*., (2011) found out that *P. ostreatus* (ethanol and methanol extraction) was able to inhibit *Escherichia coli, Bacillus cerreus*, *Listeria innocua* and other gram positive and negative bacteria and fungi. Giri *et al*., (2012) also presented that *F. hepatica* (methanol extract) was able to inhibit *Proteus vulgaris*, *Escherichia coli*, and *P. ostreatus* inhibited *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Escherichia coli*. It can be said that there is no antimicrobial effect in this study because it is studied at low concentration.

Correlation between protein content, total phenolic content and antioxidant activity

The statistical test results showed that there was a positive correlation (r = 0.885) between total phenolic content and values of ferric reducing antioxidant capacity. In general, it was found a linear correlation between higher antioxidant activity and larger amount of total phenolic compounds in the mushroom extracts.

Table 3. Total phenolic content and ferric reducing antioxidant capacity of mushrooms

Mushroom species	Total phenolic content (mg GAE g^{-1})*	FRAP** (µmol $FeSO_4 \cdot 7H_2O$ g^{-1})*
Amanita caesarea	2.979 ± 0.039^{e}	5.228 ± 0.063^{d}
Fistulina hepatica	3.101 ± 0.009^{f}	8.141 ± 0.008^{f}
Meripilus giganteus	1.111 ± 0.017^{a}	3.088 ± 0.031^{b}
Agaricus bisporus (A)	2.662 ± 0.035^{d}	7.148 ± 0.013^{e}
Agaricus bisporus (B)	3.858 ± 0.130^{g}	9.340 ± 1.069^{g}
Agaricus bisporus (C)	2.442 ± 0.078^{c}	7.608 ± 0.014^{ef}
Agaricus bisporus (D)	1.300 ± 0.052^{b}	4.144 ± 0.012^{c}
Pleurotus ostreatus	1.276 ± 0.065^{b}	1.528 ± 0.042^{a}

* Means having the different superscript letters are significantly different ($P<0.05$) by Duncan's multiple range test.

**FRAP: Ferric Reducing Antioxidant Capacity

Conclusions

In this study, protein and total phenolic contents, antioxidant and antimicrobial properties of some cultivated (*A. bisporus*, *P. ostreatus*) and wild mushrooms (*A. caesarea*, *F. hepatica*, *M. giganteus*) were investigated. Protein content of mushrooms varied from 11.00 to 25.19% and total phenolic amounts ranged from 1.111 to 3.858 mg GAE g^{-1} while antioxidant activities were determined as 1.528 - 9.340 µmol $FeSO_4 \cdot 7H_2O$ g^{-1}.

As a wild mushroom, *M. giganteus* had a higher protein and nitrogen content than that of other commercially cultivated and wild mushrooms studied in this research. The natural conditions met the requirements of the *M. giganteus* in the best way. While the highest total phenolic content was detected in *A. bisporus* obtained from B company (3.858 mg GAE g^{-1}), the lowest value was determined in *M. giganteus* (1.111 mg GAE g^{-1}).

The results of this study indicated that methanolic extracts of the mushrooms possessed the antioxidant activity and phenolic capacity. The antioxidant activity of mushroom extracts highly depends on the type of mushroom and extraction process. Bioactive properties of commercial mushrooms can be different from each other because of the differences in compost types, growth conditions, chemicals types and quantities used for hygiene during the cultivation process.

Because of total phenolic, antioxidant properties of mushrooms were found significantly different ($P<0.05$) from each other by Duncan's multiple range test. In order to obtain the better results from mushrooms extracts, different solvent types, concentrations and different extraction methods can be tested.

Abbreviations

A; **B**; **C**; **D**, The code names of the commercial companies that supplied the *Agaricus bisporus* mushrooms; **C**, Carbon; **FRAP**, Ferric-reducing antioxidant power; **H**, hydrogen; **N**, nitrogen; **P**, significant level; **r**, correlation coefficients.

References

Adejumo, T., & Awosanya, O. (2005). Proximate and mineral composition of four edible mushroom species from South Western Nigeria. *African Journal of Biotechnology*, 4, 1084-1088.

Al-Momany A.M., & Gücel, S. (2012). Chemical compositions and nutritional value of three edible mushrooms widely consumed in Cyprus. *Jordan Journal of Agricultural Sciences*, 7, 540-548.

Bahorun, T., Luximon-Ramma, A., Crozier, A., & Aruoma, O.I. (2004). Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. *Journal of the Science of Food and Agriculture*, 84, 1553-1561.

Barros, L., Baptista, P., Estevinho, L.M, &, Ferreira, I.C. (2007)a. Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of *Lactarius* sp. mushrooms. *Journal of Agricultural and Food Chemistry*, 55, 8766-8771.

Barros, L., Calhelha, R.C., Vaz, J.A., Ferreira, I.C., Baptista, P., & Estevinho, L.M. (2007)b. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *European Food Research and Technology*, 225, 151-156.

Benzie, I.F., & Strain, J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method in Enzymology*, 299, 15-27.

Breene, W.M. (1990). Nutritional and medicinal value of specialty mushrooms. *Journal of Food Protection*, 53, 883-899.

Carlile, M.J., Watkinson, S.C., & Gooday, G.W. (2001). The Fungi. Academic Press, ISBN: 9780127384467.

Cohen, N., Cohen, J., Asatiani, M.D., Varshney, V.K., Yu, H.T., Yang, Y.C., Li, Y.H., Mau, J.L., & Wasser, S.P. (2014). Chemical composition and nutritional and medicinal value of fruit bodies and submerged cultured mycelia of culinary-medicinal higher Basidiomycetes mushrooms. *International Journal of Medicinal Mushrooms*, 16, 273-291.

Crisan, E.V., & Sands, A. (1978). Nutritional value. In: S.T. Chang and W.A. Hayes (Eds). *The biology and cultivation of edible mushrooms* (p. 137-165). London: Academic Press Inc.

Demirer, T., Röck-Okuyucu B., & Özer, I. (2005). Effect of different types and doses of nitrogen fertilizers on yield and quality characteristics of mushrooms (*Agaricus bisporus* (Lange) Sing) cultivated on wheat straw compost. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 106, 71-77.

Diplock. A., Charuleux. J.L., Crozier-Willi, G., Kok, F., Rice-Evans, C., Roberfroid, M., Stahl, W., & Vina-Ribes, J. (1998). Functional food science and defence against reactive oxidative species. *British Journal of Nutrition*, 80, 77-112.

Ebeling, M.E. (1968). The Dumas method for nitrogen in feeds. *Journal Association of Official Analytical Chemists*, 51, 766-770.

Giri, S., Biswas, G., Pradhan, P., Mandal, S.C., & Acharya, K. (2012). Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India. I *International Journal of PharmTech Research*, 4, 1554-1560.

Halls, L.K. (1977). Southern fruit-producing woody plants used by wildlife. USDA Forest Service General Technical Report, Southern Forest Experiment Station(SO-16).

Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., & Ferreira, I.C. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, 119, 1443-1450.

Ishikawa, Y., Morimoto, K., & Hamasaki, T. (1984). Flavoglaucin, a metabolite of *Eurotium chevalieri*, its antioxidation and synergism with tocopherol. *Journal of the American Oil Chemists' Society*, 61, 1864-1868.

Iwalokun, B., Usen, U., Otunba, A., & Olukoya, D. (2007). Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *African Journal of Biotechnology*, 6, 1732-1739.

Jiskani, M. (2001). Energy potential of mushrooms. T*he DAWN Economic and Business Review*, 15-21.

Johnson, R.A., Willson, M.F., Thompson, J.N., & Bertin, R.I. (1985). Nutritional values of wild fruits and consumption by migrant frugivorous birds. *Ecology*, 66, 819-827.

Kalogeropoulos, N., Yanni, A.E., Koutrotsios, G., & Aloupi, M. (2013). Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece. *Food and Chemical Toxicology*, 55, 378-385.

Karaman, I., Şahin, F., Güllüce, M., Öğütçü, H., Şengül, M., & Adıgüzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *Journal of Ethnopharmacology*, 85, 231-235.

Keleş, A., Koca, I., & Gençcelep, H. (2011). Antioxidant properties of wild edible mushrooms. *Journal of Food Processing & Technology*, 2, 130-136.

Kurtzman, R., Chang, S., Buswell, J., & Chiu, S. (1993). Analysis, digestibility and the nutritional value of mushrooms. Mushroom Biology and Mushroom Products. Eds. Chang, S.T., Buswell, J.A. and Chiu, S.W. Chinese University Press, Shatin, NT Hong Kong.

CLSI. (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

Lee, I.K., Kim, Y.S., Jang, Y.W., Jung, J.Y., &Yun, B.S. (2007). New antioxidant polyphenols from the medicinal mushroom *Inonotus obliquus*. *Bioorganic & Medicinal Chemistry Letters*, 17, 6678-6681.

Lin, J.Y., & Tang, C.Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chemistry*, 101, 140-147.

Longvah, T., & Deosthale, Y. (1998). Compositional and nutritional studies on

edible wild mushroom from northeast India. *Food Chemistry*, 63, 331-334.

Mattila, P., Könkö, K., Eurola, M., Pihlava, J.M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M., & Piironen, V. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, 49, 2343-2348.

Murcia, M.A., Martínez-Tomé, M., Jiménez, A.M., Vera, A.M., Honrubia, M., & Parras, P. (2002). Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. *Journal of Food Protection*, 65, 1614-1622.

Nizamlıoğlu, N., & Nas, S. (2010). The phenolic compounds in vegetables and fruit; structures and their importance. *Electronic Journal of Food Technologies*, 5, 20-35.

Öztürk, M., Duru, M.E., Kivrak, Ş., Mercan-Doğan, N., Türkoglu, A., & Özler, M.A. (2011). In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms. *Food and Chemical Toxicology*, 49, 1353-1360.

Peksen, A., Yakupoglu, G., & Kibar, B. (2008). Some chemical components of *Lactarius pyrogalus* from diverse locations. *Asian Journal of Chemistry*, 20, 3109-3114.

SengYim, H., Yee Chye, F., KhengHo, S., & Wai Ho, C. (2009). Phenolic profiles of selected edible wild mushrooms as affected by extraction solvent, time and temperature. *Asian Journal of Food and Agro-Industry*, 2, 392-401.

Slawinska, A., Radzki, W., & Kalbarczyk, J. (2013). Antioxidant activities and polyphenolics content of *Flammulina velutipes* mushroom extracts. *Herba Polonica*, 59, 26-36.

Slinkard, K., & Singleton, V.L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28, 49-55.

Vamanu, E., Ene, M., Vamanu, A., Smarandache, D., Sârbu, I., Popa, O., Băbeanu, N., Nita, S., & Veaceslav, B. (2011). Antioxidant and antibacterial properties of the extracts from *Pleurotus ostreatus* EVFB1 and EVFB4. *Romanian Biotechnological Letters*, 16, 40-46.

Vazirian, M., Faramarzi, M.A., Ebrahimi, S.E.S., Esfahani, H.R.M., Samadi, N., Hosseini, S.A., Asghari, A., Manayi, A., Mousazadeh, S.A., & Asef, M.R. (2014). Antimicrobial effect of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (higher Basidiomycetes) and its main compounds. *International Journal of Medicinal Mushrooms*, 16, 77-84.

Vedder, P.J.C. (1978) Modern mushroom growing. London: Educaboek.

Williams, G., Iatropoulos, M., & Whysner, J. (1999). Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food and Chemical Toxicology*, 37, 1027-1038.

Yildiz, A., Yeşil, Ö.F., Yavuz, Ö., & Karakaplan, M. (2005). Organic elements and protein in some macrofungi of south east Anatolia in Turkey. *Food Chemistry*, 89, 605-609.

Yildiz, O., Can, Z., Laghari, A.Q., Şahin, H., & Malkoç, M. (2015). Wild edible mushrooms as a natural source of phenolics and antioxidants. *Journal of Food Biochemistry*, 39, 148-154.

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REVIEW ARTICLE DERLEME MAKALESİ

BLACK CUMIN *(Nigella sativa)* AND ITS ACTIVE COMPONENT OF THYMOQUINONE: EFFECTS ON HEALTH

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Abstract:

Nigella sativa has the richest historical past of plants in the healthcare field. It has been used as a food preservative and to enhance flavour in many countries of the world for thousands of years and has also been used as a spice, and *Nigella sativa* seed and oil has been consumed for the treatment of many diseases in the world for many years. Today, it is believed to have antihypertensive, antihyperlipidemic, antidiabetic, anticancer, antioxidant, antimicrobial, antitumour, antibacterial, anti-inflammtory and immune-system effects through its components. And therefore *Nigella sativa* and its effects on health are discussed in this review.

Keywords: *Nigella sativa*, Black cumin, Thymoquinone, Nutrition, Health

Introduction

Nigella sativa is a plant that is grown worldwide – primarily in the Middle East, Mediterranean regions, Southern Europe, India, Pakistan, Syria, Saudi Arabia, and Turkey. For centuries, medicinal plants have taken part in the treatment of many diseases in various medicinal branches and also in traditional medicine (S. Ahmad & Beg, 2013). *Nigella sativa* has been widely used for more than two thousand years as a curative and preventive substance against many diseases in Central Asia and some other Asian countries (BAYRAM; Razavi & Hosseinzadeh, 2014). *Nigella sativa* is acknowledged to be a miraculous plant due to its rich history and religious background (S. Ahmad & Beg, 2013) and *N. sativa* is given even greater importance especially in Islamic countries, due to its many different beneficial properties (Razavi & Hosseinzadeh, 2014).

Nigella sativa has been widely used from the past to the present for various purposes, including as a painkiller, and for anthelmintic, as an appetiser, and for carminative, sudorific, digestive, diuretic, emmenagogue, guaiacol, antifebrile, galactagouge and cathartic uses. *Nigella sativa* is reported to decrease asthenia and depression, and to increase body resistance (Razavi & Hosseinzadeh, 2014).

It has also been highlighted that the active substances of *N. sativa* have antibacterial, antifungal, antidiabetic, immunomodulator, anti-inflammatory, analgesic, antiviral, antioxidant, anticonvulsant, antihypertensive, anticancer and antihyperlipidemic effects (Entok et al., 2014; Leong, Rais Mustafa, & Jaarin, 2013; Shafiq, Ahmad, Masud, & Kaleem, 2014; Singh et al., 2014). Due to these effects, *N. sativa* seed and oil have been used globally in the treatment of many diseases such as asthma, diarrhoea, dysentery, dyspepsia, fever, icterus, apoplexy, hemorrhoids and cardiovascular, digestive, immune-system, liver, respiratory and kidney diseases (Forouzanfar, Bazzaz, & Hosseinzadeh, 2014). However, scientific evidence is required to explain and corroborate the mechanism of action for the given positive effects of *N. sativa*. This review presents the effects of *Nigella sativa* and its active component, thymoquinone, on various diseases such as cardiovascular disease in particular and Type 2 Diabetes Mellitus (DM), obesity and cancer, with supportive studies in humans and animals.

History of *Nigella sativa*

It has been highlighted that *N. sativa* has the richest and most mystical history among all the plants used in medicine. The *N. sativa* oil sample that was discovered in the remnants of Tutankhamun's tomb is indicative of its use since ancient times. It is reported that *N. sativa* seed and oil were used by Hippocrates to strengthen the liver, to solve problems related to the digestive system, to treat snake and scorpion stings, abscesses, skin rashes, infections in the head, and the common cold. It has also been suggested that in later years, Penedius Dioscorides used *N. sativa* oil to relieve headache and toothache, to clear nasal congestion and to destroy enterozoa. *N. sativa* oil is also stated to have been used for treatment by Ibni Sina to stimulate the metabolism and to relieve asthenia and lethargy (Botnick et al., 2012; Salem, 2005; Tembhurne, Feroz, More, & Sakarkar, 2014). Religious statements also highlight the important properties of black cumin. The oil obtained from this plant is known to have been used by Cleopatra, the Queen of Egypt, for health and beauty (Lord, Sekerovic, & Carrier, 2014; Paarakh, 2010). Today, the black cumin seed and oil are assumed to be an indispensable source in alternative medicine for the treatment and prevention of various diseases (Botnick et al., 2012; Lord et al., 2014).

Nigella sativa and its Chemical Composition

The black cumin plant is generally grown in Western Asia, in Middle Eastern countries and in the Konya region in Turkey (Lord et al., 2014). Black cumin, which is usually used as a spice, is grown as twelve different types; the most widely used type in agriculture and trade is *N. sativa*, on which, as indicated, many studies have been conducted worldwide (Yakup, 2007). Black cumin, *N. sativa*, which belongs to the Ranunculacea (Buttercup) family, is also known as black seed (Güllü & Gülcan, 2013). It is a rather pilous annual herbaceous plant with a height of approximately 20–30 cm. Its flower is five-leaved and is light or dark blue. The part that is used for nutrition is the seed, which consists of many white, trigonal and bitter grains with a special aroma inside the capsule (Salem, 2005). The chemical compound of the *N. sativa* seed, with a bitter taste, differs depending on the harvest season and type of plant, as well as on the climate and region where it grows (Bulca, 2015; Güllü & Gülcan, 2013; Heshmati & Namazi, 2015; Yakup, 2007). The *N. sativa* seed,

depending on the region, contains volatile (0.40%–0.45%) and non-volatile (32%–40%) oils, protein (16.00%–20.85%), carbohydrates (31.0%–33.9%), fibre (5.50–7.94%), alkaloids, tannins, saponins, minerals such as iron, calcium, potassium, magnesium, zinc and copper (1.79%–3.44%), vitamin A and C, thiamine, niacin, pyridoxine and folate (Al-Mahasneh, Ababneh, & Rababah, 2008; Güllü & Gülcan, 2013; Salama, 2010; Sultan et al., 2009). *Nigella sativa* is also rich in unsaturated and essential fatty acids and studies indicate that the volatile oil content ranges from 0.4% to 2.5% (Hosseinzadeh & Parvardeh, 2004; Ramadan & Moersel, 2004; Sultan et al., 2009). Volatile oil contains active basic components such as thymoquinone, dithymoquinone and thymohydroquinone (Güllü & Gülcan, 2013; Kaya, Kara, & Özbek, 2003). Studies indicate that *N. sativa* seeds and its components have a positive effect on health. Thymoquinone is the most studied of the black cumin components and researchers have aimed to clarify the mechanisms by which thymoquinone plays a role in the prevention and treatment of disease (S. Ahmad & Beg, 2013; Akash et al., 2011; Ilaiyaraja & Khanum, 2010; Paarakh, 2010; Randhawa & Alghamdi, 2011).

Thymoquinone: the Basic Active Substance of *Nigella sativa*

Nigella sativa can be used in various forms, as a powder, oil or extract in traditional treatment (Heshmati & Namazi, 2015; Heshmati, Namazi, Memarzadeh, Taghizadeh, & Kolahdooz, 2015). Thymoquinone, which is one of the most important bioactive components of *N. sativa* and is responsible for its many biological effects, was first synthesised in 1959 and it was reported that thymoquinone exists as a volatile oil in a proportion of 18.4%–24.0% (Ali & Blunden, 2003; Burits & Bucar, 2000; Yüncü, Şahin, Bayat, & İbrahim, 2013). Other analyses have indicated that the concentration of thymoquinone is 52.6 mg/100 g and 20.13 mg/100 g (Tüfek, Altunkaynak, Altunkaynak, & Kaplan, 2015).

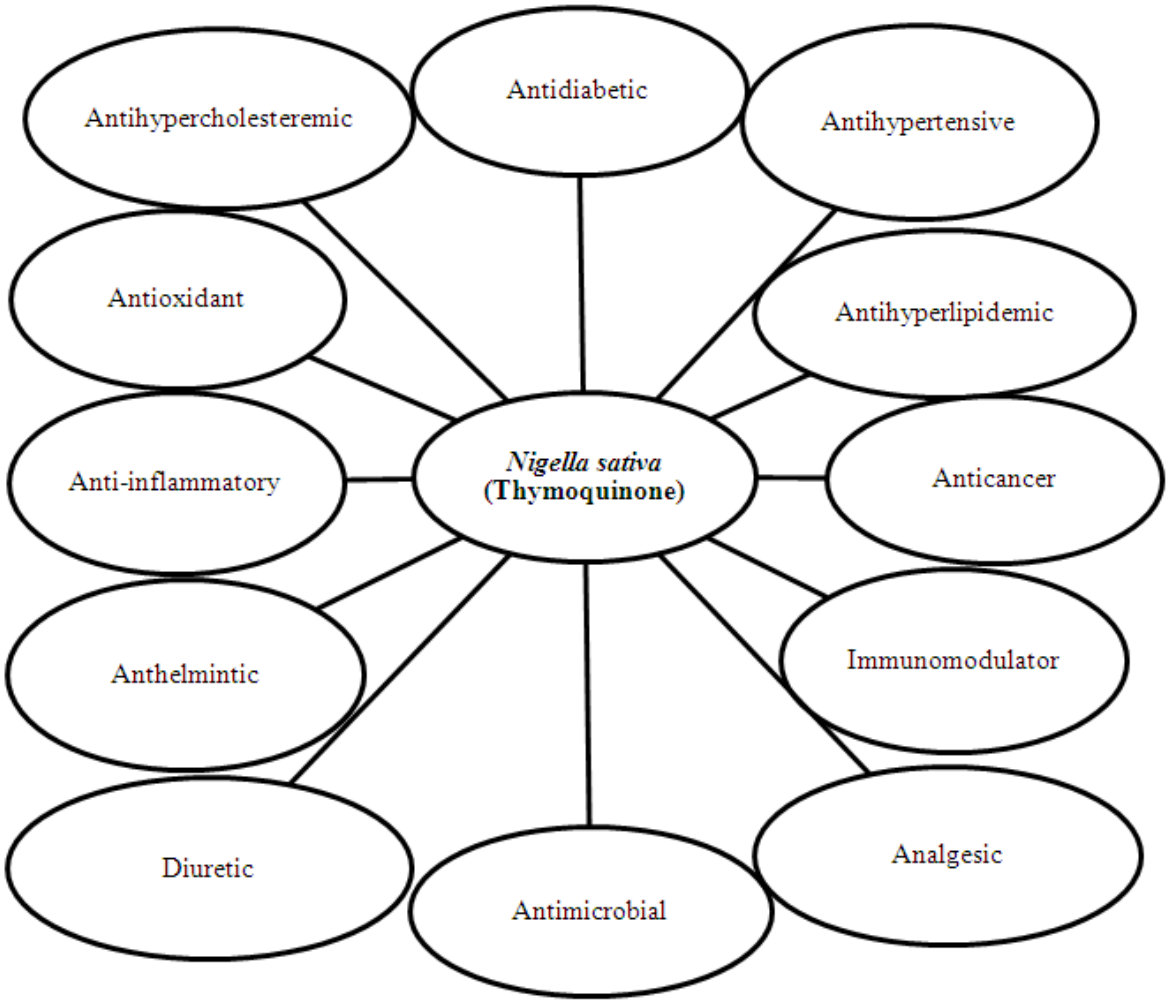
Effects of *Nigella sativa* on Health

A wide range of studies have been conducted concerning the biological activities and curative properties of black cumin (S. Ahmad & Beg, 2013). *Nigella sativa* is used in the treatment of many diseases in many countries globally. Its beneficial effects on health, especially against diseases such as cancer, diabetes and cardiovascular disease have been highlighted (Bamosa, 2015; Entok et al., 2014; Leong et al., 2013; Shafiq et al., 2014; Singh et al., 2014). The effects of *N. sativa* on health are shown in Figure 1.

Cardiovascular Health Benefits of *Nigella sativa*

Cardiovascular disease is among the top causes of death worldwide and is thus deemed to be an important public health concern. Therefore, current strategies are being developed for its prevention and treatment. Changes in lifestyle and increases in the prevalence of obesity especially increase the incidence of cardiovascular disease. Hypertension, atherosclerosis, a high cholesterol level and other metabolic diseases are among the contributory factors of cardiovascular disease. Weight control and restoring the lipid profile play an important role in the prevention of the disease. Dieting has an important role in both these aims and complementary and alternative treatments have also gained in popularity in recent years (Mahdavi, Namazi, Alizadeh, & Farajnia, 2015). Although *N. sativa* is stated to have positive effects on lipid profile and cardiovascular disease, other studies present different results in terms of metabolic indicators (S Bourgou, Pichette, Marzouk, & Legault, 2010; Datau, Surachmanto, Pandelaki, & Langi, 2010; Dehkordi & Kamkhah, 2008; Houcher, Boudiaf, Benboubetra, & Houcher, 2007; Shafiq et al., 2014; Shahzad & Nasiruddin, 2011).

Figure 1. Health benefits of *Nigella sativa*.



The effects that Nigella sativa has on cardiovascular diseases are as follows:

Antioxidant Properties of *Nigella sativa*

Analysis of the antioxidant content of *N. sativa* has indicated that *N. sativa* has a higher content of volatile-oil antioxidants than non-volatile oil antioxidants (Sultan et al., 2009). When the antioxidant capacities of *N. sativa* grown in different regions (Egypt and Samsun, for instance) were analysed, they were found to be more active than synthetic antioxidants (Yakup, 2007). It is proposed that the *N. sativa* extract grown in Tunisia can be used as a natural antioxidant in *in vitro* and *ex vitro* environments, and as a food additive to prevent the organoleptic deterioration that occurs due to free radicals (Soumaya Bourgou, Pichette, Marzouk, & Legault, 2012). Another study on antioxidant capacity focused on the positive effects of thymoquinone, the basic bioactive component of volatile oil, and demonstrated that the antioxidant effect of thymoquinone plays a large part in the mechanism of action of the volatile oil (S Bourgou et al., 2010). According to many studies, the potential antihyperglycemia and antihyperlipidemia properties of *N. sativa* are based on its antioxidant content (Al-Mahasneh et al., 2008; Bamosa, Kaatabi, Lebda, Elq, & Al-Sultan, 2010; Ragheb et al., 2008). Thymoquinone and dithymoquinone are amongst the main antioxidant components of *N. sativa*. The intake of *N. sativa* in all forms develops the antioxidant defence capacity of the body.

Some studies indicate that *N. sativa* decreases lipid peroxidation and increases antioxidant enzymes (Al-Mahasneh et al., 2008; Ragheb et al., 2008). It is thought that a decrease in oxidative stress renews the pancreatic beta-cells, maintains the integrity of beta-cells, increases the number and volume of islet cells, decreases insulin resistance and increases insulin secretion, and aids glycation end-product inhibition. The glycaemic improvement resulting from the mentioned positive effects of *N. sativa* is thought to relieve lipid dysfunction, especially in diabetics. Furthermore, a decrease in free radicals affects lipid metabolism directly and indirectly; antioxidant components can improve enzyme functions in lipid metabolism by protecting cells against lipid peroxidation (Bamosa et al., 2010; Heshmati & Namazi, 2015).

Many studies on humans and animals have shown that *N. sativa* and its active component thymoquinone have positive effects by decreasing levels of serum lipids, total cholesterol (TC), triglycerides (TG) and low-density lipoproteins (LDL), whereas other studies have suggested that *N. sativa* and thymoquinone have no effect (Bamosa, Ali, & al-Hawsawi, 2002; Nader, El-Agamy, & Suddek, 2010; Ragheb et al., 2008). However, no definite results have demonstrate that they increase the level of high-density lipoproteins (HDLs), which play an active role in decreasing the risk of cardiovascular disease, in particular (Razavi & Hosseinzadeh, 2014).

In a study that examined the effect of *N. sativa* seed and oil on its anti-atherogenic potential in rabbits that were fed with a hypercholesteremic diet, 25 rabbits were divided into five groups. Four groups were determined as hypercholesteremic and the other group as negative normal. One of the hypercholesteremic groups was separated as a positive control group, and was fed with a diet containing 1% cholesterol for 3 weeks. During the final 8 weeks, 1 g/kg *N. sativa* powder, 0.5 g/kg *N. sativa* oil or 10 mg/day simvastatin were added, respectively, to the diet of each the groups except the positive control group. It was found that weight, plasma TC and LDL increased considerably, whereas there was no significant different in the HDL level in the group fed on a diet with 1% cholesterol, compared to the negative control group. On the other hand, plasma TC, TG and LDL levels considerably decreased in the groups whose diets contained *N. sativa* oil and seed, compared to levels in the positive group (Al-Naqeep, Al-Zubairi, Ismail, Amom, & Esa, 2011). Similar to these results, intake of 10 mg/mL thymoquinone by gavage positively affected blood lipids in rats that were fed an atherogenic diet for 30 days (S. Ahmad & Beg, 2013). In a study conducted to examine the effects of different doses of *N. sativa* supplement on the serum lipid profile in rats, 15 rats were separated as a control group and 60 rats were fed with *N. sativa* supplement. Either 100 mg, 200 mg, 400 mg and 600 mg per kg were given daily to rats for four weeks, which resulted in a significant decrease in total cholesterol level (Kocyigit, Atamer, & Uysal, 2009). In another study, individuals with type 2 DM were separated into three groups and were given *N. sativa* at a rate of 1, 2, 3 g/day for twelve weeks. Individuals who were given *N. sativa* at minimum level (1 g/day) and a maximum level (2 g/day and 3 g/day) were compared. After twelve weeks, plasma TG, TC and LDL cholesterol levels were significantly lower in individuals given 2 g/day *N. sativa*, and the most positive effects occurred in those who took 2 g/day. This study, therefore, determined that increasing the amount of *N. sativa* has no positive effective on individuals' lipid profiles (Kaatabi, Bamosa, Lebda, Al Elq, & Al-Sultan, 2012). In a follow-up study conducted on premenopausal women, individuals were given either a placebo or 1,600 mg *N. sativa* powder and the lipid profile of the groups was then examined. No significant changes in the lipid profile values of the placebo group were observed, compared to the initial level after 12 weeks. Additionally, no significant decrease in LDL and TG levels were found, compared to the initial level in the group treated with black cumin; however, the total cholesterol level was considerably lower. Significant decreases in the blood pressure were also observed, which is a risk factor for cardiovascular disease (Latiff, Parhizkar, Dollah, & Hassan, 2014).

In a study in which menopausal women were given 1 g/day *N. sativa* powder after breakfast for two months, the weight of women decreased compared with that of the control group, although the difference was not statistically significant; however, their TC, TG, LDL and HDL levels considerably improved (Ibrahim et al., 2014). Therefore, it has been claimed that intake of *N. sativa* in different forms can be used as a supportive for drugs that decrease the lipid profile (Razavi & Hosseinzadeh, 2014).

Antihyperlipidemic and Antihypercholesteremic Effects of *Nigella sativa*

Hypercholesterolemia is reflected by an increase in TG, TC, LDL, HDL and very low-density lipoprotein (VLDL) levels (S. Ahmad & Beg, 2013). An increase in the HDL level and a decrease in the LDL level in the circulation have positive effects on reducing the risk of cardiovascular disease (Mani & Rohatgi, 2015). *Nigella sativa* and its important active component thymoquinone demonstrate an antihypercholesteremic effect by decreasing the level of HMG-CoA reductase enzyme, which is the rate limiting enzyme in cholesterol synthesis, to cause protective effects on dyslipidemia. Moreover, *Nigella sativa* is reported to display antihyperlipidemic properties by stimulating paraoxonase enzyme (PON1), which functions as an antioxidant, due to its LDL protective property against oxidation and its ability to neutralise radicals including hydrogen peroxide, to increase the activity of arylesterase, the protein indicator of the PON1 enzyme (S. Ahmad & Beg, 2013; Türkoğlu et al., 2008).

It is claimed that the positive effects of *N. sativa* and its essential active component thymoquinone on cholesterol stem from their regulatory roles in antioxidant and gene metabolism. Their antioxidant properties are particularly important for the prevention of free radical formation due to diets with a high level of saturated fat and cholesterol, and for the prevention of oxidative stress and hypocholesteremia, because *N. sativa* is stated to have a protective role especially in LDL oxidation (S. Ahmad & Beg, 2013; Türkoğlu et al., 2008).

Antihypertensive Effects of Nigella sativa

Another important risk factor for cardiovascular disease is hypertension. Arabs have used *N. sativa* seed together with honey or garlic for the treatment of hypertension in traditional medicine. It has been suggested that *N. sativa* extract reduces blood pressure in dogs. It has also been claimed that another antihypertensive effect of *N. sativa* oil might result from its diuretic effect (Salama, 2010).

An increase in oxidative stress is associated with the pathogenesis of hypertension. Blood pressure increases, depending on the imbalance between the antioxidant defence mechanism and free-radical production. An excessive increase in reactive oxygen products reduces the bioavailability of nitric oxide in endothelium dysfunction and increases the total peripheral resistance (Leong et al., 2013).

In human and animal studies, *N. sativa* and its active component thymoquinone is reported to contribute to a reduction in blood pressure and to reduce hypertension via various mechanisms, such as by antioxidant properties, calcium-channel blockage, and diuretic and hypotensive (soothing heartbeat) functions (A. Ahmad et al., 2013; Keyhanmanesh, Gholamnezhad, & Boskabady, 2014).

In a study conducted on 70 healthy individuals with an age range of 34–63 years, a body weight range of 55–75 kg, a systolic blood pressure range of 110–140 mm Hg and a diastolic blood pressure range of 60–90 mm Hg, individuals were divided into two groups – control and intervention. The intervention group was provided with 2.5 mL *N. sativa* oil after meals, every 12 hours twice a day (5 mL/day total) for eight weeks. A significant decrease in diastolic and systolic blood pressure resulted in the group that was given *N. sativa* oil (Fallah Huseini et al., 2013). In another randomised controlled double-blind dose-response study that lasted 8 weeks, on 119 men between 35 and 50 years with mild hypertension, individuals were divided into three groups and were given a placebo, 100 mg or 200 mg *N. sativa* extract A significant decrease in the systolic and diastolic blood pressure was observed in the intervention group compared to initial levels and to those in individuals with the placebo; furthermore, extract usage decreased the diastolic and systolic blood pressure, depending on its dose. These results indicate that use of *N. sativa* extract for two months has a positive effect on lowering blood pressure in individuals with mild hypertension (Dehkordi & Kamkhah, 2008).

Type 2 Diabetes Mellitus and Nigella sativa

Diabetes Mellitus is becoming more common worldwide; data from the IDF (International Diabetes Federation) indicate that the number of diabetic individuals will increase from 171 million individuals in 2001, to 366 million by 2030. As a result of the metabolic dysfunction in DM, there is a higher risk of cardiovascular disease, dyslipidemia, infection, mortality and morbidity. Various treatment methods such as diet, changes in life style, biochemical and herbal treatment are used together or separately to control diabetes. Many countries mostly tend to use herb treatments

for diabetes, which are preferred as an alternative, and complementary medicine. Individuals generally tend to use herbs due to the side-effects of chemical medicines. The World Health Organisation (WHO) aims to convince researchers to research the positive and negative side effects of the potential therapeutic effects of herbs (Heshmati & Namazi, 2015).

The effect mechanisms of *N. sativa* and its use in Type 2 DM are as follows:

Anti-diabetic Effect of *Nigella sativa*

Nigella sativa and its active component thymoquinone have been shown to have positive effects in controlling glucose levels and lipid profiles in diabetics (Heshmati & Namazi, 2015). Although the molecular mechanism of thymoquinone on insulin secretion has not been completely clarified, it is reported that thymoquinone causes an increase in glucose use by increasing the serum concentration, and decreasing a high levels serum glucose, and decreasing blood glucose by preventing gluconeogenesis (Benhaddou-Andaloussi et al., 2008; Heshmati & Namazi, 2015; Kaatabi et al., 2015).

Insulin Secretion

Thymoquinone and other antioxidant components in *N. sativa* can increase insulin secretion by improving the energy metabolism of mitochondria and might also reduce liver injury, according to a study conducted on diabetic rats. The compounds also cause an increase in the insulin concentration by promoting the intracellular insulin receptor pathways (Heshmati & Namazi, 2015). It has been suggested that thymoquinone and the other antioxidant components in *N. sativa* can activate the mitogen-activated protein kinases (MAPKs) and protein kinase B (PKB) pathways, which function in insulin sensitivity (Le et al., 2004).

Gluconeogenesis

Nigella sativa decreases gluconeogenesis, which contributes to hyperglycemia in diabetic individuals. Thymoquinone can reduce the expression of gluconeogenic enzymes (glucose 6-phosphatase and fructose 1,6-biphosphatase) and the production of hepatic glucose (Heshmati & Namazi, 2015). Furthermore, *N. sativa* prevents gluconeogenesis by activating the protein kinases activated by adenosine monophosphate (AMPK) in liver and muscles (Heshmati et al., 2015).

Glucose Absorption

It has been shown that liquid intake of *N. sativa* extract reduces glucose absorption and inhibits the glucose carrier in diabetic rats. Another potential mechanism of action is that polyphenol components can suppress the properties of glucose absorption transport (Heshmati & Namazi, 2015). Furthermore, thymoquinone is reported to have a curative effect in decreasing the oxidative stress that results from hyperglycaemia and protects β-cell integrity. As a consequence, the clinical use of thymoquinone can be effective in protecting β-cells against oxidative stress (Kaatabi et al., 2015). A double-blind placebo controlled study with 114 participants consisting of 63 men and 51 women, studied the effect of *N. sativa* supplement on glycaemic control and antioxidant capacity in Type 2 diabetic patients that used hypoglycaemic drugs; the intervention group was given 2 g/day *N. sativa* for one year. The study revealed considerable decreases in fasting blood glucose and HbA1c levels of individuals; there were significant differences between groups and significant increases were observed in total antioxidant capacity, superoxide dismutase and glutathione levels of the group that took *Nigella sativa* powder compared to the control group. At the end of the treatment, insulin resistance decreased and β-cell activity increased compared to the level in individuals at the start of treatment (Kaatabi et al., 2015).

In a study in which individuals with Type 2 DM were given *N. sativa* powder at a dose of 1, 2 and 3 g/day, significant decreases were observed in insulin resistance and β-cell function indicators such as postprandial blood sugar, HbA1c, HOMA-IR, only in individuals who were supplied with 2 g *N. sativa* powder per day. It was reported that the three different doses did not negatively affect the renal or hepatic functions of individuals during the study. The most effective does was determined to be 2 g and this might also confer positive benefits with hypoglycaemic agents (Bamosa et al., 2010). In another study, Type 2 DM patients were given 2 g/day *N. sativa* powder for one year and it was shown that HbA1c values decreased significantly in the intervention group (Bamosa, 2015). In another study that treated 60 individuals with insulin resistance who took hypoglycaemic drugs with 2.5 mL *N. sativa* oil twice daily for 60 weeks, significant improvements were observed in TC, LDL and prepandial blood sugar levels. In addition, *N. sativa* oil is effective as an additional treatment in

individuals with insulin resistance. *N. sativa* is reported to have important effects especially in diabetic and dyslipidemic individuals (Najmi, Haque, Naseeruddin, & Khan, 2008) and has also been shown to play an important role in the prevention of diabetic neuropathy in diabetic rats that were supplied with 50 mg/day thymoquinone for 8 weeks (Omran, 2014).

Anti-obesity effects of Nigella sativa

Obesity is one of the most common health problems in all age groups worldwide. In recent years, herbal supplement use has assumed a place among the complementary diet-based and alternative treatment methods that are commonly used for weight loss (Hasani-Ranjbar, Jouyandeh, & Abdollahi, 2013). *Nigella sativa* and its active substance thymoquinone show anti-obesity effects, due to their positive effects against cardiovascular disease, cancer, insulin sensitivity and their immune-modular effects (A. Ahmad et al., 2013; Vanamala, Kester, Heuberger, & Reddivari, 2012). Although weight loss can occur in individuals, depending on decreases in insulin resistance, *N. sativa* can improve the lipid profile and blood glucose levels in individuals with DM after weight loss (Bamosa et al., 2010).

A 25% decrease in food intake was observed in rats that were fed with *N. sativa* oil by intragastric gavage for four weeks. Therefore, *N. sativa* oil might possess anorectic effects, and can cause a decrease in food intake and body weight, and possibly also improvements in lipid peroxidation and insulin sensitivity (Le et al., 2004). Another study demonstrated significant decreases in the body weight of diabetic rats following treatment with 300 mg/day *N. sativa* extract for 30 days (Fararh, Ibrahim, & Elsonosy, 2010).

Human studies in which the effect of *N. sativa* on obesity has been examined are limited. In a randomised controlled double-blind single study, 50 obese men were given 3 g/day *Nigella sativa* powder for 3 months and significant decreases in body weight, and the waist and hip circumference of individuals were found (Datau et al., 2010). However, *N. sativa* given to individuals with normal weight did not cause any significant change in the body weight (Qidwai, Hamza, Qureshi, & Gilani, 2009). It is claimed that the consumption of *N. sativa* might be effective against obesity if used for a long period and in large amounts (Nader et al., 2010). Despite these results, more controlled intervention studies are required to understand better the effects of *Nigella sativa* on weight loss.

Anticancer Effects of Nigella sativa

Cancer is considered to be one of the health concerns that has rapidly become widespread. In 2013, half a million people died of cancer in America, and this rate is even higher in undeveloped countries and 8.2 million people died of cancer worldwide (WHO, 2015).

Nigella sativa and its active component thymoquinone are claimed to exhibit anticancer activity by causing the death of cancer cells or by preventing genetic changes in normal cells (Shafiq et al., 2014). Thymoquinone is considered to have antioxidant, anticancirogenic and antimutagenic properties. Many studies have shown that *N. sativa* and thymoquinone have antioxidant properties, and that they increase the activities of antioxidant enzymes such as superoxide, dismutase, catalase and glutathione peroxidase. Because oxidative stress has an effective role in the formation and development of different cancer types and thymoquinone increases the activity of the antioxidant enzymes mentioned above, the positive effects of *N. sativa* against cancer types possibly occurs via antioxidant effects (Badary, Taha, Gamal El-Din, & Abdel-Wahab, 2003; Soumaya Bourgou et al., 2008; Khader, Bresgen, & Eckl, 2010; Randhawa & Alghamdi, 2011).

Over time, many different mechanisms of *N. sativa* seed function and its extract have been examined in different cancer types in both *in vivo* and *in vitro* studies. *Nigella sativa* has been shown to kill various cancerous cell types, and to cause an increase in macrophage cell number and activation (Ait Mbarek et al., 2007; Chehl, Chipitsyna, Gong, Yeo, & Arafat, 2009; Darakhshan, Pour, Colagar, & Sisakhtnezhad, 2015; El-Mahdy, Zhu, Wang, Wani, & Wani, 2005; Shoieb, Elgayyar, Dudrick, Bell, & Tithof, 2003; Yi et al., 2008; Yüncü et al., 2013).

Lung cancer

According to data from the American Cancer Association in 2012, lung cancer caused approximately 20% of total cancer deaths (1.59 million people), and this rate reached 27% in 2014 (Society, 2015). *Nigella sativa* supplement and *N. sativa* seed extract demonstrated cytotoxicity properties against lung sarcoma cells (Rooney & Ryan, 2005). In particular, thymoquinone ob-

tained from *N. sativa* extract showed important anticancer properties against lung cancer cell numbers, and prevented cell proliferation by approximately 90% (Shafiq et al., 2014).

Breast cancer

Although breast cancer is the primary causes of the death of females in undeveloped countries, it is the second most-important cause of death in developed countries (Shafiq et al., 2014). The liquid and alcoholic extracts of *N. sativa* have been shown to be effective in inactivating a breast cancer cell line (MCF-7), and have an effect on life span. Therefore, these extracts are proposed to represent a promising treatment for breast cancer (Farah & Begum, 2002; Shafiq et al., 2014).

Colon cancer

Colon cancer causes the second-highest number of deaths among different cancers. According to statistics, more than half million people (690,000) per annum die from this type of cancer (Society, 2015). It has been suggested that *N. sativa* can reduce DNA damage and prevent carcinogenesis in colon tissues exposed to toxic agents. A study that analysed the relationship between *N. sativa* and colon cancer demonstrated that thymoquinone inhibits the formation of 5-lipoxygenase products such as 5-hydroxeicosa-tetraenoic acids, which are necessary for colon cancer cells (El-Mahmoudy et al., 2002). It has been shown that the effect of thymoquinone depends on the type of colon cancer cell; for instance, although it affects HCT-116 colon cancer cells by increasing apoptosis, it has no effect on HT-29 colon cancer cells (Abukhader, 2012; Rooney & Ryan, 2005).

The summary of possible mechanisms of action of *N. sativa* in certain diseases are shown in Figure 2.

Figure 2. Possible effect mechanisms of *Nigella sativa* at certain diseases

***Nigella sativa* (Thymoquinone)**	
Mechanism of action	**Disease**
HMG-COA ↓ Reductase activity arylesterase ↑ Antioxidant properties shown in cholesterol metabolism	Dyslipidaemia Metabolic syndrome
Reducing oxidative stress Blocking calcium channels Diuretic effect Hypotension (soothing heartbeat) effects	Hypertension
Increasing insulin sensitivity Blocking gluconeogenesis Decreasing glucose absorption Increasing insulin secretion Increasing β-cell proliferation	Hyperglycaemia Type 2 DM
Dietary intake ↓	Obesity
Increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) Protection against cancer cells Inhibiting cancer cell growth Various types of cancer-cell death Macrophage cell number and activation ↑ Inhibiting metastasis	Cancer

Potential Toxicity of *Nigella sativa*

Nigella sativa and its oil are reported to have a very low toxicity (Ali & Blunden, 2003). The toxicological properties of thymoquinone were assessed *in vitro* and *in vivo*, by treating subjects with thymoquinone at doses between 20 mg/kg and 500 mg/kg (Abukhader, 2012; Ali & Blunden, 2003) and death occurred due to other complications when the amount of thymoquinone reached 500 mg/kg. Another study concerning the potential toxicity of *N. sativa* that was conducted on rats, by treating them with 2.5 mL/kg/day *N. sativa* oil, resulted in no toxicity at the histopathological level (Yüncü et al., 2013).

Conclusions

Nigella sativa and its active component thymoquinone have positive effects on health, and their mechanism of action depends on the type of disease. The effective amount, form of intake (powder, extract, oil), and active component of *N. sativa* change according to the type of disease. Although the degree of effect and mechanism of action of *N. sativa* on some diseases have been demonstrated by *in vivo* and *in vitro* studies, insufficient studies exist in humans. Although at least 2 g *N. sativa* per day should be consumed in order to activate its antihyperlipidemic and antidiabetic effects, it is not realistically possible to propose this for cancer treatments, because the anticancer activities of *N. sativa* have been researched at the cellular level rather than via dose-response studies. Considering suggestions about the consumption amount of black cumin, its interaction with anticancer drugs should also be taken into consideration. We believe that the consumption of one heaped teaspoon of black cumin seed (approximately 2.5 g) daily is beneficial for a healthy diet and to improve lipid profiles and blood glucose levels.

References

Abukhader, M. (2012). The effect of route of administration in thymoquinone toxicity in male and female rats. *Indian Journal of Pharmaceutical Sciences,* 74(3), 195.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A.K., Siddique, N. A., . . . Anwar, F. (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine,* 3(5), 337-352.

Ahmad, S., & Beg, Z.H. (2013). Hypolipidemic and antioxidant activities of thymoquinone and limonene in atherogenic suspension fed rats. *Food Chemistry,*138(2), 1116-1124.

Ait Mbarek, L., Ait Mouse, H., Elabbadi, N., Bensalah, M., Gamouh, A., Aboufatima, R., . . . Dalal, A. (2007). Anti-tumor properties of blackseed (Nigella sativa L.) extracts. *Brazilian Journal of Medical and Biological Research,* 40(6), 839-847.

Akash, M., Rehman, K., Rasool, F., Sethi, A., Abrar, M., Irshad, A., . . . Murtaza, G. (2011). Alternate therapy of type 2 diabetes mellitus (T2DM) with Nigella (Ranunculaceae). *Journal of Medicinal Plants Research,* 5(31), 6885-6889.

Al-Naqeep, G., Al-Zubairi, A.S., Ismail, M., Amom, Z.H., & Esa, N.M. (2011). Antiatherogenic potential of *Nigella sativa* seeds and oil in diet-induced hypercholesterolemia in rabbits. *Evidence-Based Complementary and Alternative Medicine,* 2011, 1-8.

Al-Mahasneh, M.A., Ababneh, H.A., & Rababah, T. (2008). Some engineering and thermal properties of black cumin (*Nigella sativa* L.) seeds. *International Journal of Food Science & Technology,* 43(6), 1047-1052.

Ali, B., & Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytotherapy Research,* 17(4), 299-305.

Badary, O.A., Taha, R.A., Gamal El-Din, A.M., & Abdel-Wahab, M.H. (2003). Thymoquinone is a potent superoxide anion scavenger. *Drug and Chemical Toxicology,* 26(2), 87-98.

Bamosa, A.O. (2015). A review on the hypoglycemic effect of *Nigella sativa* and thymoquinone. *Saudi Journal of Medicine and Medical Sciences,* 3(1), 2.

Bamosa, A.O., Ali, B.A., & al-Hawsawi, Z.A. (2002). The effect of thymoquinone on blood lipids in rats. *Indian Journal of Physiology and Pharmacology,* 46(2), 195-201.

Bamosa, A.O., Kaatabi, H., Lebda, F.M., Elq, A.-M.A., & Al-Sultan, A. (2010). Effect of *Nigella sativa* seeds on the glycemic control

of patients with type 2 diabetes mellitus. *Indian Journal of Physiology and Pharmacology,* 54(4), 344-54.

Bayram, U.Ö.İ. (2012). Çörek Otunun (*Nigella sativa*) Kuzularda Besi Performansı, Bazı Kan ve Rumen sıvısı Parametreleri Üzerine Etkisi. *Kocatepe Veteriner Dergisi,* 5(2), 27-33.

Benhaddou-Andaloussi, A., Martineau, L. C., Spoor, D., Vuong, T., Leduc, C., Joly, E., . . . Arnason, J.T. (2008). Antidiabetic activity of *Nigella sativa* seed extract in cultured pancreatic β-cells, skeletal muscle cells, and adipocytes. *Pharmaceutical Biology,* 46(1-2), 96-104.

Botnick, I., Xue, W., Bar, E., Ibdah, M., Schwartz, A., Joel, D. M., . . . Lewinsohn, E. (2012). Distribution of primary and specialized metabolites in *Nigella sativa* seeds, a spice with vast traditional and historical uses. *Molecules,* 17(9), 10159-10177.

Bourgou, S., Ksouri, R., Bellila, A., Skandrani, I., Falleh, H., & Marzouk, B. (2008). Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. *Comptes Rendus Biologies,* 331(1), 48-55.

Bourgou, S., Pichette, A., Marzouk, B., & Legault, J. (2010). Bioactivities of black cumin essential oil and its main terpenes from Tunisia. *South African Journal of Botany,* 76(2), 210-216.

Bourgou, S., Pichette, A., Marzouk, B., & Legault, J. (2012). Antioxidant, anti-inflammatory, anticancer and antibacterial activities of extracts from *Nigella sativa* (black cumin) plant parts. *Journal of Food Biochemistry,* 36(5), 539-546.

Bulca, S. (2015). Çörek otunun bileşenleri ve bu yağın ve diğer bazı uçucu yağların antioksidan olarak gıda teknolojisinde kullanımı. *Journal of Adnan Menderes University, Agricultural Faculty,* 11(2), 29-36.

Burits, M., & Bucar, F. (2000). Antioxidant activity of Nigella sativa essential oil. *Phytotherapy Research,* 14(5), 323-328.

Chehl, N., Chipitsyna, G., Gong, Q., Yeo, C.J., & Arafat, H.A. (2009). Anti-inflammatory effects of the *Nigella sativa* seed extract, thymoquinone, in pancreatic cancer cells. *HPB,* 11(5), 373-381.

Darakhshan, S., Pour, A.B., Colagar, A.H., & Sisakhtnezhad, S. (2015). Thymoquinone and its therapeutic potentials. *Pharmacological Research,* 95, 138-158.

Datau, E., Surachmanto, E., Pandelaki, K., & Langi, J. (2010). Efficacy of Nigella sativa on serum free testosterone and metabolic disturbances in central obese male. *Acta Medica Indonesiana, 42*(3), 130-134.

Dehkordi, F.R., & Kamkhah, A.F. (2008). Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. *Fundamental & Clinical Pharmacology,* 22(4), 447-452.

El-Mahmoudy, A., Matsuyama, H., Borgan, M., Shimizu, Y., El-Sayed, M., Minamoto, N., & Takewaki, T. (2002). Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. *International Immunopharmacology,* 2(11), 1603-1611.

El-Mahdy, M.A., Zhu, Q., Wang, Q.E., Wani, G., & Wani, A.A. (2005). Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. *International Journal of Cancer,* 117(3), 409-417.

Entok, E., Ustuner, M.C., Ozbayer, C., Tekin, N., Akyuz, F., Yangi, B., . . . Gunes, H. V. (2014). Anti-inflammatuar and anti-oxidative effects of *Nigella sativa* L.: 18FDG-PET imaging of inflammation. *Molecular Biology Reports,* 41(5), 2827-2834.

Fallah Huseini, H., Amini, M., Mohtashami, R., Ghamarchehre, M., Sadeqhi, Z., Kianbakht, S., & Fallah Huseini, A. (2013). Blood Pressure Lowering Effect of *Nigella sativa* L. Seed Oil in Healthy Volunteers: A Randomized, Double-Blind, Placebo-controlled Clinical Trial. *Phytotherapy Research,* 27(12), 1849-1853.

Farah, I.O., & Begum, R.A. (2002). Effect of Nigella sativa (*N. sativa* L.) and oxidative stress on the survival pattern of MCF-7 breast cancer cells. *Biomedical Sciences Instrumentation,* 39, 359-364.

Fararh, K.M., Ibrahim, A.K., & Elsonosy, Y.A. (2010). Thymoquinone enhances the activities of enzymes related to energy metabolism in peripheral leukocytes of diabetic rats. *Research in Veterinary Science,* 88(3), 400-404.

Forouzanfar, F., Bazzaz, B. S.F., & Hosseinzadeh, H. (2014). Black cumin (*Nigella sativa*) and its constituent (thymoquinone): A review on antimicrobial effects. *Iranian Journal of Basic Medical Sciences,* 17(12), 929.

Güllü, E.B., & Gülcan, A. (2013). Timokinon: *Nigella sativa*' nın Biyoaktif Komponenti. *Kocatepe Veterinary Journal,* 6(1), 51-61.

Hasani-Ranjbar, S., Jouyandeh, Z., & Abdollahi, M. (2013). A systematic review of anti-obesity medicinal plants-an update. *Journal of Diabetes & Metabolic Disorders,* 12(1), 28.

Heshmati, J., & Namazi, N. (2015). Effects of black seed (*Nigella sativa*) on metabolic parameters in diabetes mellitus: A systematic review. *Complementary Therapies in Medicine,* 23(2), 275-282.

Heshmati, J., Namazi, N., Memarzadeh, M.-R., Taghizadeh, M., & Kolahdooz, F. (2015). *Nigella sativa* oil affects glucose metabolism and lipid concentrations in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *Food Research International,* 70, 87-93.

Hosseinzadeh, H., & Parvardeh, S. (2004). Anticonvulsant effects of thymoquinone, the major constituent of Nigella sativa seeds, in mice. *Phytomedicine,* 11(1), 56-64.

Houcher, Z., Boudiaf, K., Benboubetra, M., & Houcher, B. (2007). Effects of methanolic extract and commercial oil of Nigella sativa L. on blood glucose and antioxidant capacity in alloxan-induced diabetic rats. *Pteridines,* 18(1), 8-18.

Ibrahim, R.M., Hamdan, N.S., Ismail, M., Saini, S.M., Rashid, S.A., Latiff, L.A., & Mahmud, R. (2014). Protective effects of *Nigella sativa* on metabolic syndrome in menopausal women. *Advanced Pharmaceutical Bulletin,* 4(1), 29-33.

Ilaiyaraja, N., & Khanum, F. (2010). *Nigella sativa* L: a review of therapeutic applications. *Journal of Herbal Medicine and Toxicology,* 4(2), 1-8.

Kaatabi, H., Bamosa, A. O., Badar, A., Al-Elq, A., Abou-Hozaifa, B., Lebda, F., . . . Al-Almaie, S. (2015). Nigella sativa improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: Placebo controlled participant blinded clinical trial. *PloSone, 10*(2), e0113486.

Kaatabi, H., Bamosa, A.O., Lebda, F.M., Al Elq, A.H., & Al-Sultan, A.I. (2012). Favorable impact of Nigella sativa seeds on lipid profile in type 2 diabetic patients. *Journal of Family and Community Medicine, 19*(3), 155.

Kaya, M.S., Kara, M., & Özbek, H. (2003). Çörek otu (Nigella sativa) tohumunun insan hücresel bağışıklık sisteminin CD^{3+}, CD^{4+}, CD^{8+} hücreleri ve toplam lökosit sayısı üzerine etkileri. *Genel Tıp Dergisi,* 13, 109-112.

Keyhanmanesh, R., Gholamnezhad, Z., & Boskabady, M.H. (2014). The relaxant effect of Nigella sativa on smooth muscles, its possible mechanisms and clinical applications. *Iranian Journal of Basic Medical Sciences,* 17(12), 939.

Khader, M., Bresgen, N., & Eckl, P. (2010). Antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. *Journal of Ethnopharmacology,* 127(2), 319-324.

Kocyigit, Y., Atamer, Y., & Uysal, E. (2009). The effect of dietary supplementation of Nigella sativa L. on serum lipid profile in rats. *Saudi Medical Journal,* 30(7), 893-896.

Latiff, L.A., Parhizkar, S., Dollah, M.A., & Hassan, S.T.S. (2014). Alternative supplement for enhancement of reproductive health and metabolic profile among perimenopausal women: a novel role of Nigella sativa. *Iranian Journal of Basic Medical Sciences,* 17(12), 980.

Le, P., Benhaddou-Andaloussi, A., Elimadi, A., Settaf, A., Cherrah, Y., & Haddad, P. (2004). The petroleum ether extracts of Nigella sativa seeds exert insulin sensitizing

and lipid lowering action in rats. *Journal of Ethnopharmacology,* 94(2-3), 251-259.

Leong, X.-F., Rais Mustafa, M., & Jaarin, K. (2013). Nigella sativa and its protective role in oxidative stress and hypertension. *Evidence-Based Complementary and Alternative Medicine, 2013*, 1-9.

Lord, C., Sekerovic, Z., & Carrier, J. (2014). Sleep regulation and sex hormones exposure in men and women across adulthood. *Pathologie Biologie,* 62(5), 302-310.

Mahdavi, R., Namazi, N., Alizadeh, M., & Farajnia, S. (2015). Effects of Nigella sativa oil with a low-calorie diet on cardiometabolic risk factors in obese women: a randomized controlled clinical trial. *Food & function,* 6(6), 2041-2048.

Mani, P., & Rohatgi, A. (2015). Niacin therapy, HDL cholesterol, and cardiovascular disease: is the HDL hypothesis defunct? *Current Atherosclerosis Reports,* 17(8), 1-9.

Nader, M.A., El-Agamy, D.S., & Suddek, G.M. (2010). Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. *Archives of Pharmacal Research (Seoul),* 33(4), 637.

Najmi, A., Haque, S., Naseeruddin, M., & Khan, R. (2008). Effect of *Nigella sativa* oil on various clinical and biochemical parameters of metabolic syndrome. *Int J Diabetes Dev Ctries,* 16, 85-87.

Omran, O. M. (2014). Effects of thymoquinone on STZ-induced diabetic nephropathy: an immunohistochemical study. *Ultrastructural Pathology,* 38(1), 26-33.

Paarakh, P.M. (2010). *Nigella sativa* Linn. A Comprehensive Review. 1(4), 409-429.

Qidwai, W., Hamza, H.B., Qureshi, R., & Gilani, A. (2009). Effectiveness, safety, and tolerability of powdered *Nigella sativa* (kalonji) seed in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults: results of a randomized, double-blind controlled trial. *The Journal of Alternative and Complementary Medicine,* 15(6), 639-644.

Ragheb, A., Elbarbry, F., Prasad, K., Mohamed, A., Ahmed, M.S., & Shoker, A. (2008). Attenuation of the development of hypercholesterolemic atherosclerosis by thymoquinone. *International Journal of Angiology,* 17(04), 186-192.

Ramadan, M.F., & Moersel, J.-T. (2004). Antiradical performance of some common and nontraditional vegetable oils. *Inform,* 15, 553-555.

Randhawa, M.A., & Alghamdi, M.S. (2011). Anticancer activity of Nigella sativa (black seed)—a review. *The American Journal of Chinese Medicine,* 39(06), 1075-1091.

Razavi, B., & Hosseinzadeh, H. (2014). A review of the effects of *Nigella sativa* L. and its constituent, thymoquinone, in metabolic syndrome. *Journal of Endocrinological Investigation,* 37(11), 1031-1040.

Rooney, S., & Ryan, M. (2005). Modes of action of alpha-hederin and thymoquinone, active constituents of *Nigella sativa*, against HEp-2 cancer cells. *Anticancer Research,* 25(6B), 4255-4259.

Salama, R. H. (2010). Clinical and Therapeutic Trials of *Nigella sativa*. *TAF Preventive Medicine Bulletin,* 9(5), 513-522.

Salem, M. L. (2005). Immunomodulatory and therapeutic properties of the N*igella sativa* L. seed. *International immunopharmacology, 5*(13), 1749-1770.

Shafiq, H., Ahmad, A., Masud, T., & Kaleem, M. (2014). Cardio-protective and anti-cancer therapeutic potential of *Nigella sativa*. *Iranian Journal of Basic Medical Sciences,* 17(12), 967.

Shahzad, F., & Nasiruddin, M. (2011). Indigenous herbal product *Nigella sativa* proved effective as an anti-obesity therapy in metabolic syndrome. *International Journal of Medicobiological Research,* 1(3), 173-176.

Shoieb, A.M., Elgayyar, M., Dudrick, P.S., Bell, J.L., & Tithof, P.K. (2003). In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. *International Journal of Oncology,* 22(1), 107-114.

Singh, S., Das, S., Singh, G., Schuff, C., de Lampasona, M.P., & Catalán, C.A. (2014). Composition, in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (*Nigella sativa* L.). *BioMed research international,* 2014.

American Cancer Society (2015). Cancer Facts & Figures 2014 http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014/ (available June, 2016).

Sultan, M.T., Butt, M. S., Anjum, F.M., Jamil, A., Akhtar, S., & Nasir, M. (2009). Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. *Pakistan Journal of Botany*, 41(3), 1321-1330.

Tembhurne, S., Feroz, S., More, B., & Sakarkar, D. (2014). A review on therapeutic potential of Nigella sativa (kalonji) seeds. *Journal of Medicinal Plants Research,* 8(3), 167-177.

Tüfek, N.H., Altunkaynak, M.E., Altunkaynak, B.Z., & Kaplan, S. (2015). Effects of thymoquinone on testicular structure and sperm production in male obese rats. *Systems Biology in reproductive Medicine,* 61(4), 194-204.

Türkoğlu, S., Bulmuş, F. G., Parmaksiz, A., Özkan, Y., & Gürsu, F. (2008). Metabolik sendromlu hastalarda paraoksonaz 1 ve arilesteraz aktivite düzeyleri. *Fırat Tıp Dergisi, 13*(2), 110-115.

Vanamala, J., Kester, A. C., Heuberger, A. L., & Reddivari, L. (2012). Mitigation of obesity-promoted diseases by Nigella sativa and thymoquinone. *Plant Foods for Human Nutrition, 67*(2), 111-119.

WHO. (2015). Cancer. http://www.who.int/mediacentre/factsheets/fs297/en/ (available June, 2016).

Yakup, K. (2007). Samsun yöresinde ve misir ülkesinde yetiştirilen çörekotu (*Nigella sativa* L.) Tohumlarinin antioksidan aktivite yönünden incelenmesi. *Süleyman demirel Üniversitesi Fen Dergisi,* 2(2). 197-203.

Yi, T., Cho, S.-G., Yi, Z., Pang, X., Rodriguez, M., Wang, Y., . . . Liu, M. (2008). Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. *Molecular Cancer Therapeutics,* 7(7), 1789-1796.

Yüncü, M., Şahin, M., Bayat, N., & İbrahim, S. (2013). Çörek otu yağının sıçan karaciğer gelişimine etkisi. *Gaziantep Medical Journal,* 19(3), 180-184.