Antioxidant Activity of Kohlrabi Leaf and Tuber

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Abstract

Ethanol, methanol, acetone and water extract of the leaves and tuber of fresh kohlrabi were evaluated their total phenolic contents and antioxidant activities. Total phenolic contents of leaf and tuber extracts ranged from 16.473-27.582 and 7.3223-8.303 mg GAE/g extract, respectively. The leaf extracts showed higher activity than tuber extracts at all antioxidant activity assays. The EC_{50} values of DPPH scavenging activity varied within 9.04−12.94 µg/mL for ethanol, methanol and acetone extracts of leaf while they varied within 16.69−21.93 µg/mL for tuber, respectively. However the leaf water extract was most effective extract with 80 %, 70 % and 65 % activities at 750 µg/mL extract concentration in terms of ferrous ion chelating ability, β-carotene bleaching activity and superoxide radical scavenging activity, respectively. The results obtained in this study indicate that kohlrabi leaf extracts are more potential source than tuber extracts as natural antioxidant.

Keywords: *Brassica olerecea* var. *Gongylodes*, kohlrabi, DPPH, superoxide radical scavenging, metal chelating

1. *Introduction*

Routine or habitual consumption of fruits and vegetables confers significant benefits to human health. Epidemiological data as well as *in vitro* studies strongly suggest that foods containing phytochemicals with anti-oxidation potential have strong protective effects against major disease risks including cancer and cardiovascular diseases (Kaur and Kapoor, 2002). Because of this, vegetables and fruits have had conferred on them the status of functional foods (Hasler, 1998).The principal function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body. The occurrence of such oxidative damage may be a significant causative factor in the development of many chronic diseases (Ismail *et al*. 2004).

Many epidemiological studies have correlated the intake of a diet rich in vegetables and fruits with a reduced risk of incidence of chronic diseases, such as cancer and cardiovascular disease. In particular, several epidemiological studies report an inverse correlation between consumption of *Brassicaceae* and risk of cancer. The *Brassicaceae* family includes more than 350 genera and 3500 species, for the majority cool season annuals, characterized by short cycle and wide adoptability; for this reason they are suited for cultivation in different seasons and in a variety of environments (Heimler *et al*. 2006). Cabbage, kohlrabi, broccoli, cauliflower, Brussels sprouts and kale are the members of *Brassicaceae* family. *Brassica* vegetables are rich in bioactive compounds including polyphenols, glucosinates, sulphur containing compounds and carotenoids (β-carotene, lutein, zeaxanthin), and are found to have positive effects on human health when consumed regularly. Also these vegetables are rich in potassium, magnesium, calcium, phosphorus, and vitamins C, E, K (Heimler *et al*. 2006; Hagen, *et al*. 2009; Jahangir *et al*. 2009). The content of bioactive compounds of in *Brassicaceae* vegetables varies with genotype, environmental stress, growth conditions and storage processing and cooking methods (Baenas *et al*. 2012). Kohlrabi (*Brassica olerecea* var. *Gongylodes)* is form cabbage of mustard family (*Brassicaceae*), and first described in the 16th century. It is European origin. The flesh resembles that of the turnip but is sweeter and milder. Kohlrabi is not widely grown commercially but is popular in some regions as kitchen garden vegetable.

The younger leaves may be eaten as greens; the thickened stem is served as a cooked vegetable ("Kohlrabi," 2013). Kohlrabi has been widely consumed in England, Germany, Holland, Belgium, though it is new vegetable for Turkey. Some *Brassica oleracea* varieties, namely cauliflower (Kaur and Kapoor, 2002; Podsedek, 2007; Koksal and Gulcin, 2008; Cabello-Hurtado *et al*. 2012; Soengas, *et al*. 2012), broccoli (Podsedek, 2007; Soengas, *et al*. 2012; Kaur *et al*. 2007; Guo *et al*. 2001)[1], kale (Ayaz *et al.* 2008; Korus, 2011; Zhou and Yu, 2006) and several cabbages (Ismail *et al*. 2004; Podsedek, 2007; Roy *et al.* 2007; Kusznierewicz *et al.* 2008; Jaiswal *et al.* 2012) have already been studied for their antioxidant capacity in different experimental models, but no studies are available on antioxidant potential of kohlrabi leaf and tuber according to our knowledge. Thus the aim of this study is to determine the total antioxidant activity of the fresh kohlrabi leaf and tuber and to compare to each other. For this purpose, the antioxidant activity of kohlrabi extracts was evaluated using various experimental assays including β-carotene bleaching test, DPPH radical scavenging assay, ferrous ion chelating ability, superoxide radical scavenging test and reducing power assay.

2. *Material and Methods*

2.1 Plant Materials.

Kohlrabi samples are planted at Trakya University's greenhouse, Havsa, TURKEY. White kohlrabi tuber and leaves were stored at 4 °C until used. Fresh kohlrabi tuber and leaves were grinded one by one. For water extraction; 15 g plant material was extracted in 150 ml boiling distilled water with magnetic stirrer for 15 min. The extract was filtrated and then freeze-dried (Armfield; FT 36, England). For ethanol, methanol and acetone extractions; 50 g fresh plant material was extracted 500 ml of ethanol, methanol and acetone in three steps (as 200, 200, 100), at 30°C and 400 rpm for 5 hours. After filtration, the residual solvent was removed by rotary evaporator at 40°C (Buchi R-200, Switzerland). All samples were stored at 4°C until used.

2.2 Total Phenolic Compound Assays.

Total phenolics were determined by Folin-Ciocalteu reagent at 760 nm in a UV spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) (Singleton and Rossi, 1965). Results for phenolic compounds content were expressed as mg gallic acid equivalence (GAE) per g extract.

2.3 DPPH Radical Scavenging Activity.

The free radical scavenging activity for extracts of kohlrabi was assessed by discoloration of an ethanolic solution of DPPH radical. The degree of discoloration indicates the free radical efficiency of the extracts (Blois, 1958). Briefly 1 ml portions of aqueous solutions of kohlrabi extracts and standards of five different concentrations (100, 250, 500, 750, 1000 µg/ml) were mixed with 4 ml of ethanolic DPPH solution (0.1 mM). After 30 min in darkness, absorbance at 517 nm was measured (Shimadzu UV-1601) and EC_{50} values were obtained from the resulting inhibition curves.

2.4 Metal Chelating Activity.

1 ml of kohlrabi extracts and standard solutions at different concentrations (100, 250, 500, 750, 1000 µg/ml) were spiked with 3.7 ml deionized water, 0.1 ml 2 mM $FeCl_2$ and 0.2 ml 5mM ferrozine solution. After reaction for 30 min at room temperature, the absorbance (at 562 nm) of the resulting solutions was recorded (Shimadzu UV-1601). The lower absorbance sings the higher the ferrous ion chelating ability of samples (Dinis *et al*., 1994).

2.5 Determination of Antioxidant Activity with β-carotene Bleaching Test.

Evaluation of antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was conducted as described by Jayaprakasha *et al*. (2001).

1 ml of β-carotene solution (200 µg/ml chloroform) was pipetted into a round-bottom flask containing 200 µl Tween 40 and 20 µl linoleic acid. The mixture was than evaporated by means of a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 50 ml of distilled water. 3 ml aliquot of the β-carotene emulsion and 750 µl of the kohlrabi extracts and standards at different concentrations (100, 250, 500, 750, 1000 µg/ml) were placed in a test tube and mixed well, then initial absorbance was measured at 490 nm. After this test tubes placed in an incubator at 50°C for 180 min (EnoLab MB-80), again at 490 nm, final absorbance was measured as spectra photo metrically (Shimadzu UV-1601). The control sample consisted of 250 µl distilled water instead of the extracts.

2.6 Superoxide Radical Scavenging Activity.

1 ml of kohlrabi extracts and standards at different concentrations (100, 250, 500, 750, 1000 µg/ml) were added to the mixture of 1 ml 156 µM NBT (at Tris-HCl buffer, pH 8.0) and 1 ml of 468 µM NADH (at Tris-HCl buffer, pH 8.0). The reaction mixture spiked with 100 µl of 60 µM PMS (at Tris-HCl buffer, pH 8.0). After the waiting at 25^{o}C for 5 min, absorbance was measured at 560 nm as spectra photo metrically (Shimadzu UV-1601). Low absorbance value shows high superoxide radical scavenging activity (Nishimiki *et al.*, 1972). Blank absorbance was measured by using 1 ml of distilled water instead of extracts.

2.7 Determination of Reducing Power.

The method developed by Oyaizu (1986) for reducing power test was used. 1 ml of the kohlrabi extracts and standard solutions at different concentrations were spiked with 2.5 ml of phosphate buffer (0.2 M, pH=6.6) and 2.5 ml of 1 % potassium ferricyanide, and kept in a water-bath at 50^{o}C for 20 min (Clifton). At the end of the 20 min, 2.5 ml of TCA (10 %) were added to the mixture, which was then centrifuged for 10 min at 2500xg (Hettich Rotina 38 R). 2.5 ml reaction mixture was taken from tubes and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$. Absorbance at 700 nm was measured (Shimadzu UV-1601). The higher absorbance, the stronger was the reducing power.

3. *Result and Discussion*

3.1 Plant Material

Water extracts of kohlrabi leaf and tuber, their solid: liquid rate being 1:10 (w/v), were prepared by boiling with distilled water for 15 min. The other kohlrabi extracts were prepared with ethanol, methanol and acetone in shaker for 5 h at room temperature. The extractions yields are shown in the Table 1. The different extraction methods used in antioxidant activity studies were reported. These were to use the solvents like water, methanol, ethanol, acetone, petroleum ether, ethyl acetate, chloroform and dichloromethane or to use solvent mixtures, to keep at room temperature, to boil with the solvent, and to get extractions by using Soxhlet extraction (Nakiboglu *et al.* 2007; Su *et al.* 2007; Silva *et al.* 2007; Tawaha *et al.* 2007).

In this study, ethanol, methanol, acetone and water were preferred as solvent because they have high polarity and low toxicity, also are economical and available. The extraction yields varied within 3.050-8.619 % (Table 1). As seen in Table 1, the extraction yields from kohlrabi leaves and tubers increased in order of Methanol>Ethanol>Acetone>Water. The difference between the extractions yields obtained depended on polarity of used solvents. The yields of various solvents are attributed to polarities of compounds present in kohlrabi leaves and tubers, and such differences have been reported in literature concerning fruit and seeds (Jayaprakasha *et al.* 2001; Hayouni *et al.* 2007).

Table 1: Extraction yields (%) of kohlrabi leaf and tuber in different solvents

	Extraction Yield (%)			
	Ethanol	**Methanol**	**Acetone**	**Water**
Leaf	5.556 ±1.451	8.229±0.987	3.194±1.022	3.050±1.187
Tuber	7.979 ±2.044	8.619±1.132	4.301±1.870	3.185±1.339

3.2 Total Phenolic Compound Assay.

The total phenol contents of kohlrabi extracts are shown in Figure 1. The total phenolic contents of leaf extracts were higher about three times than tuber extracts. Among all the leaf extracts; acetone extract had the highest phenolic content, and followed by ethanol extract, water extract and methanol extract. The total phenolic contents of kohlrabi tuber extracts increased in order of Ethanol>Water>Methanol≥ Acetone. It was observed that phenolic contents of kohlrabi leaf had higher than these of tuber. The amounts of total phenolic compounds were between 16.473 and 27.582 mg/g extract for kohlrabi leaf while 7.323 and 8.303 mg g/extract for tuber.

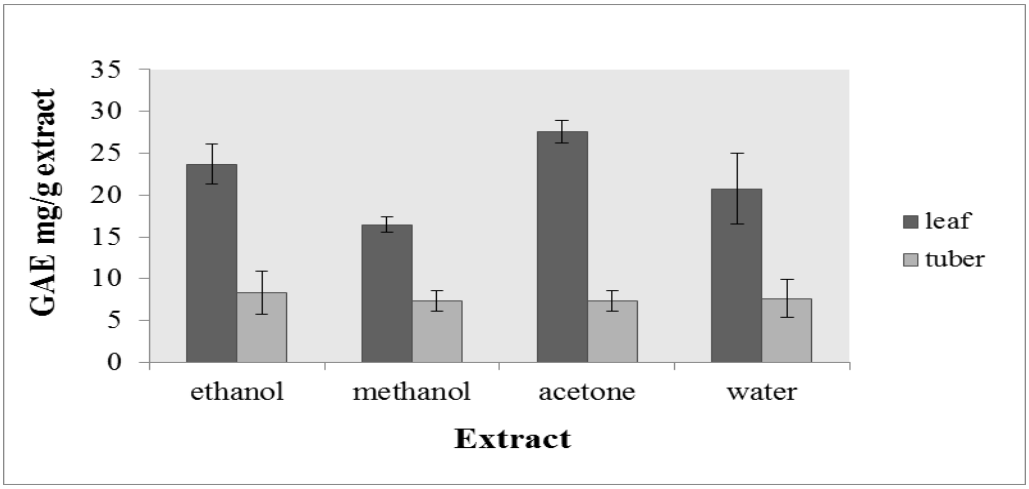

Figure 1: Total phenolic contents of various solvent extracts obtained from kohlrabi leaf and tuber

The total phenolic contents of metabolic extracts of 8 different kinds of fresh broccoli were between 19.60-41.40 GAE mg/g (Kaur *et al*. 2007). Water extracts of white cabbage and Chinese cabbage was 623.3 and 534.3 GAE µg/100 g plant, respectively (Roy *et al.* 2007). In another study done with fresh and edible parts of broccoli, cauliflower and white cabbage, the total phenolic contents were 82.2±8.8, 27.8±1.5 and 15.3±2.1 GAE mg/g, respectively (Podsedek, 2007). Total phenolic content of fresh kale was found to be between 16 % and 67 % in the methanol extract (Ayaz *et al*. 2008). Kohlrabi leaves contains phenolic compounds as high as some broccoli extracts and cauliflower extracts. Also phenolic contents of kohlrabi leaf are higher than that of white cabbage while these of tuber are lower. It was reported that the most significant antioxidants in *Brassica* vegetables were vitamin C and phenolic compounds that contain 80 % of total antioxidant capacity (Kusznierewicz *et al*. 2008). On the other hand polyphenol content in plants can change because of the kind of plant, agricultural process, climatic conditions, light, harvest time and storage (Heimler *et al.* 2006). Also extraction conditions, process and its solvent features may effect on phenolic content.

3.3 DPPH Radical Scavenging Activity.

Free radical scavenging activity of kohlrabi tuber and leaves has been done by DPPH radical which used widely as indicator in antioxidant activity experiments. The antioxidant activities of all fractions were determined in terms of proportion (%) of DPPH radical scavenged by different concentrations of kohlrabi leaf and tuber extracts (Figure 2). All of the fractions were able to scavenge the radical but they were less active than standards including BHA, BHT, and α-tocopherol. As seen in Figure 2, the kohlrabi leaf extracts have shown higher radical scavenging activities than kohlrabi tuber extracts. DPPH radical scavenging activities have linearly increased with the increasing concentrations of kohlrabi leaf and tuber extracts. At 750 and 1000 µg/ml concentrations, DPPH radical scavenging activities of acetone, ethanol and methanol extracts of kohlrabi leaf almost reached a comparable level of standards (Figure 2).

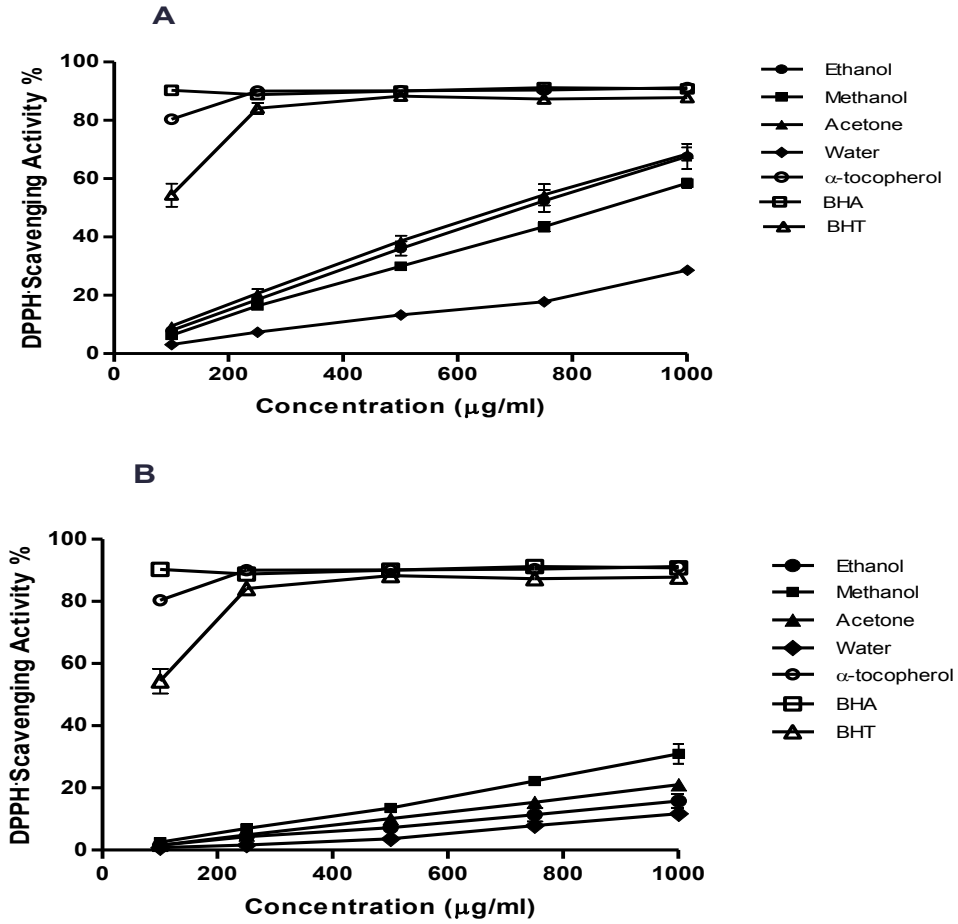

Figure 2: DPPH radical scavenging activity of kohlrabi leaf (A) and tuber (B)

DPPH radical scavenging activities of all of the kohlrabi tuber extracts and leaf water extracts were found to be lower than these of the standards whereas activities of leaf extracts were observed to be close to these of some *Brassicaceae* vegetables. DPPH radical scavenging activities of methanol extracts obtained from eight different kinds of broccoli were between 57.78% and 70.12% (Kaur *et al.* 2007). DPPH scavenging activities of lyophilized fresh white cabbage and Chinese cabbage were determined to be 32 % and 8 %, respectively (Roy *et al.* 2007). In another study done with the leaves and tuber of fresh broccoli, their methanol extracts had more scavenging activity values than 43 %, whereas acetone extracts didn't show any activity (Guo *et al.* 2001). In a study done with kale, it was reported that all fractions of methanol extract of fresh kale showed DPPH radical scavenging activity but all of them were less than standard (Ayaz *et al.* 2008).

The EC_{50} value is the effective concentration which is required to decrease the initial DPPH concentration by 50% and lower EC_{50} value reflects better protective action. The EC_{50} values of all extracts were calculated by using the results of DPPH scavenging activity between 100 and 1000 µg/mL extract concentrations. As seen in Table 2, the EC_{50} values of DPPH scavenging activity varied within 9.04−12.94 µg/mL for ethanol, methanol and acetone extracts of kohlrabi leaf while they varied within 16.69−21.93 µg/mL for kohlrabi tuber, respectively. These values for water extracts of kohlrabi leaf and tuber were 20.50 and 228.3 µg/mL, respectively.

Table 2: EC_{50} values of DPPH scavenging activities of kohlrabi leaf and tuber extracts

	EC_{50} value (µg/ml)			
	Ethanol	**Methanol**	**Acetone**	**Water**
Leaf	11.11±1.04	12.94±1.11	9.04±0.95	20.50±1.74
Tuber	16.69±1.22	21.93±1.34	17.57±1.24	228.3±2.35

In a study done with white cabbages in England, Germany, Belgium and Poland; the EC_{50} values of DPPH radial scavenging activity of methanol extracts of fresh cabbage were found to be between 3.31-5.42 µmol/g (Kusznierewicz *et al.* 2008). It was reported that broccoli (81.45 mg/mg dry weight) and Italian kale (92.95 mg/mg dry weight) had the lowest EC_{50} value among *Brassicaceae* family vegetables including white cabbage, Savoy cabbage, broccoli, Italian kale, green cauliflower, cauliflower and Brussels sprouts (Heimler *et al.* 2006).

3.3 Metal Chelating Activity.

Metal chelation may provide important antioxidative effects by retarding metal-catalyzed oxidation reactive oxygen species (singlet oxygen) as for Gulcin *et al.* (2010). As seen in Figure 3, leaf extracts of kohlrabi had higher ferrous ion chelating power than tuber extracts. Leaf water extract with about 80 % activity had the best ferrous ion chelating power among all the extracts; whereas other extracts showed 30-40 % ferrous ion chelating power. Metal chelation capacity of kohlrabi leaf and tuber increased in order of Water > Methanol >Ethanol>Acetone.

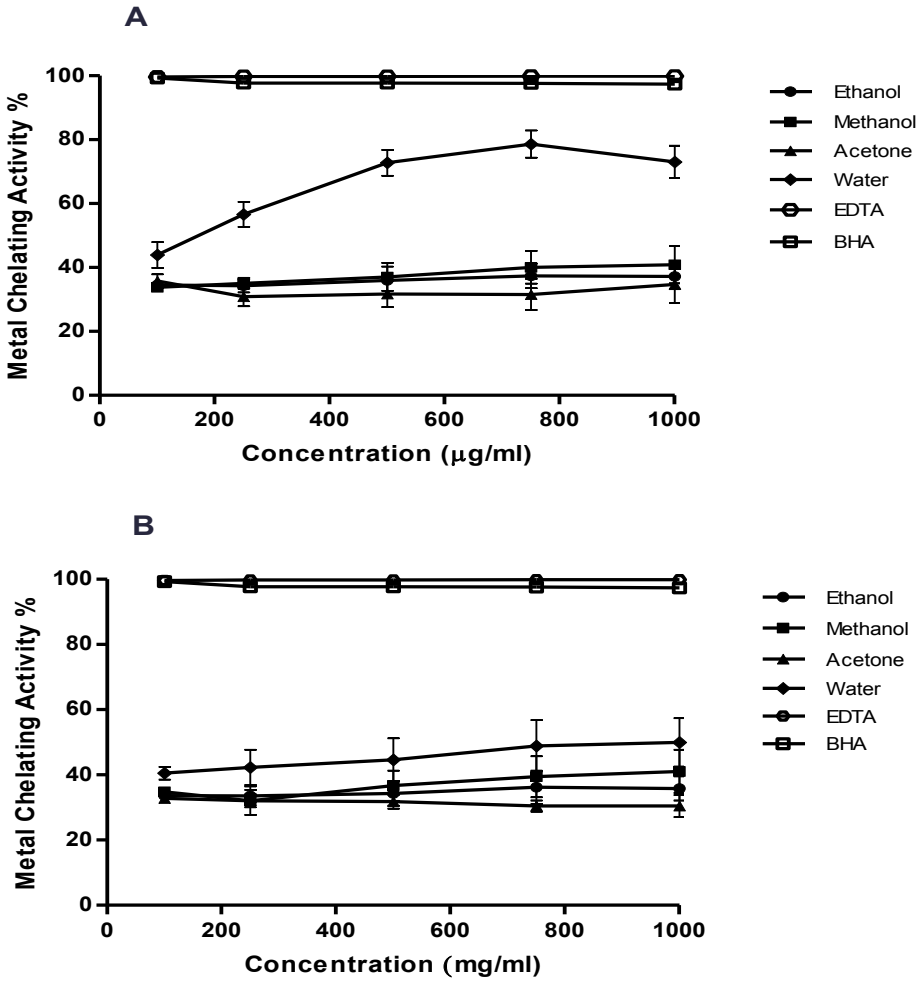

Figure 3: Metal chelating ability of kohlrabi leaf (A) and tuber (B)

According to literature on ferrous ion chelating power in *Brassicaceae* family; metal chelating capacity of water and ethanol extracts of cauliflower was found to be effective (Koksal and Gulcin, 2008). For fresh broccoli leaf and tuber, methanol and water extracts were determined to have higher chelating capacity than that of acetone extracts (Guo *et al.* 2001). In a study done with kale, red cabbage and other plants; kale and red cabbage had the highest chelating capacity.

3.4 β-Carotene Bleaching Method.

The β-carotene undergoes rapid discoloration in the absence of an antioxidant. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromospheres and characteristic orange color, which is monitored spectro photo metrically (Jayaprakasha *et al.* 2001).

All extracts of kohlrabi leaf were capable of inhibiting the free radical-induced decay of β-carotene with effectiveness close to BHT and α-tocopherol under the conditions examined. Figure 4 shows that total antioxidant activity of leaf extracts increases with increasing extract concentration.

β-Carotene bleaching activities of water extracts of kohlrabi leaf changed 45-85 % between 100 and 1000 µg/mL extract concentrations while these of ethanol, methanol and acetone extracts changed between 15-45 %. As shown in Figure 4, kohlrabi tuber extracts didn't show any total antioxidant activity.

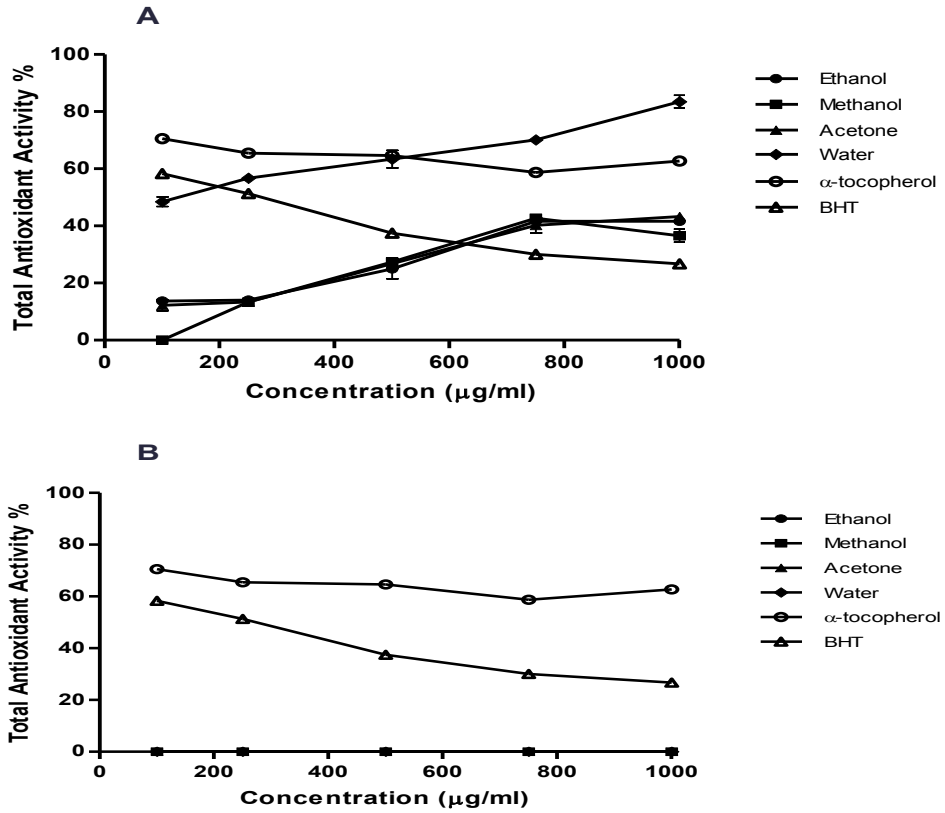

Figure 4: β-caroten bleaching activity of kohlrabi leaf (A) and tuber (B)

In similar studies, methanol extracts of eight different kinds of broccoli showed β-Carotene bleaching activity between 47.23 % and 65.34 % (Kaur *et al.* 2007). β-carotene bleaching activities of ethanol extracts of fresh Brussels sprout, cabbage and cauliflower were determined to be 72.5 %, 68.5 %, 47.8 %, 13.5 % while these of water extracts were observed to be 78.4 %, 73.8 %, 69.3%, 19.5 %, respectively.[1] In another study, ethanol extracts of fresh kale, cabbage and swamp cabbage showed inhibition rates of 50.2 %, 59.3 %, 60.3 %, respectively.[3]

3.5 Superoxide Radical Scavenging Activity.

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidant thus indicates the consumption of superoxide anion in the reaction mixture (Muruhan *et al.* 2013). As seen in Figure 5, leaf extracts of kohlrabi showed a better superoxide radical scavenging activity than tuber extracts of kohlrabi.

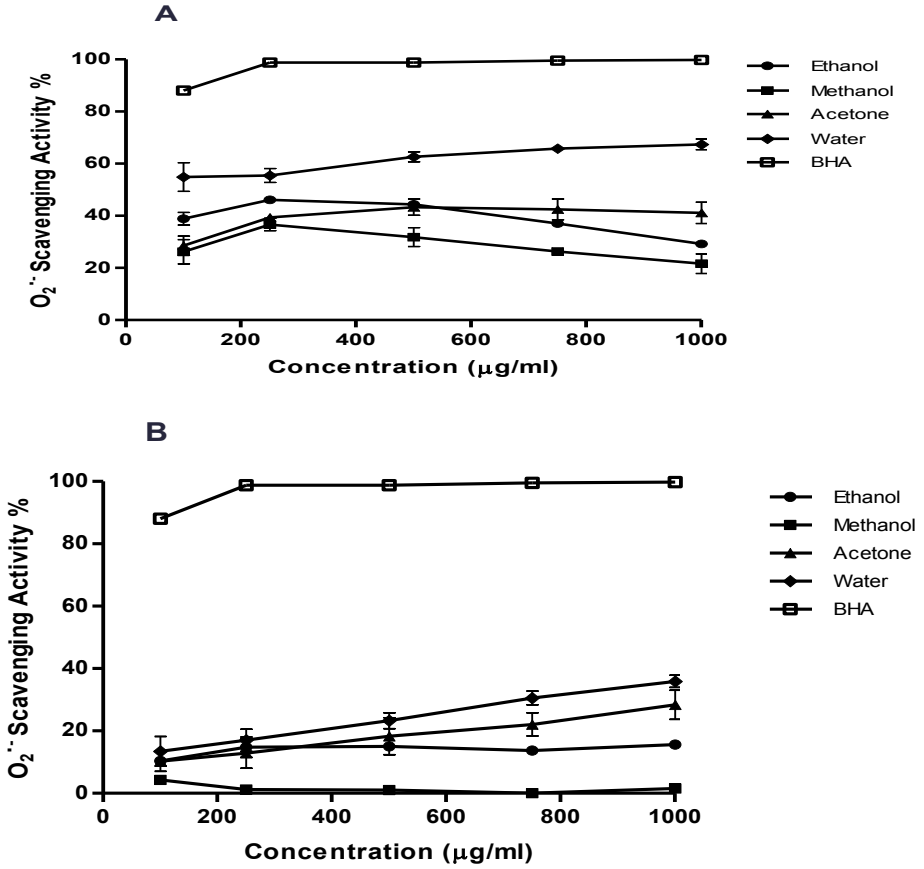

Figure 5: Superoxide radical scavenging activity of kohlrabi leaf (A) and tuber (B)

Water extract of the leaf showed a high superoxide radical scavenging activity. Activity of leaf water extract was about 70 % whereas that of tuber water extract was about 40 %. Besides methanol, ethanol and acetone extracts of leaf showed an inhibition in the range of 20 % and 45 %. For acetone and ethanol extracts of kohlrabi tuber, superoxide radical scavenging activity was maximum 25 % while methanol extract didn't show any activity. Our results show similarity with Koksal and Gulcin (2008), who demonstrated that water and ethanol extracts of cauliflower had effective results in superoxide radical scavenging activity experiment.

3.6 Test for Reducing Power.

The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potential antioxidant activity (Ferreiraw *et al.* 2007). The reducing power capacities of the kohlrabi leaf and tuber extracts are shown in Figure 6.

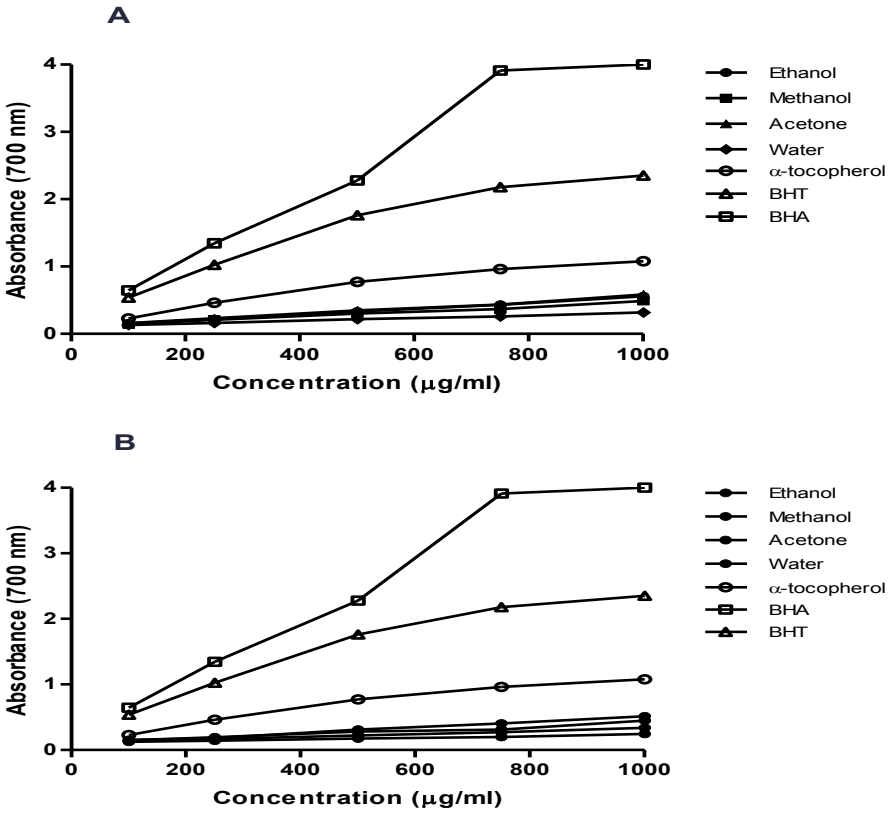

Figure 6: Reducing power capacity of kohlrabi leaf (A) and tuber (B)

As seen as in Fig 6, reducing power of leaf and tuber extracts of kohlrabi was low when compared to standards. Kohlrabi leaf and tuber extracts had similar reducing power capacity. Guo et al. (2008), Köksal and Gulcin (2008) reported that methanol and water extracts of broccoli and cauliflower had effective reducing power while Syhamala et al. (2005) reported that ethanol extract of dried white cabbage leaf had low reducing power similar to our results.

4. Conclusion

In the present study, we compared some of the antioxidant properties in different parts of white kohlrabi started to be grown recently in the Turkey. Results exhibited that antioxidant activity of fresh kohlrabi leaf was better than that of tuber. When antioxidant activity of kohlrabi was compared to other members of *Brassicaceae* family, it was determined that kohlrabi leaves and tuber had higher antioxidant activity than cauliflower but lower than kale. Antioxidant activity of leaf extracts was close to that of some kinds of broccoli. This research stated that kohlrabi extracts have a potent antioxidant capacity and kohlrabi leaf extracts are more effective than tuber extracts as a health supplements and nutraceuticals. When fresh kohlrabi leaves are eaten in salads, it may help our antioxidant defense system in its struggle with free radicals.

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