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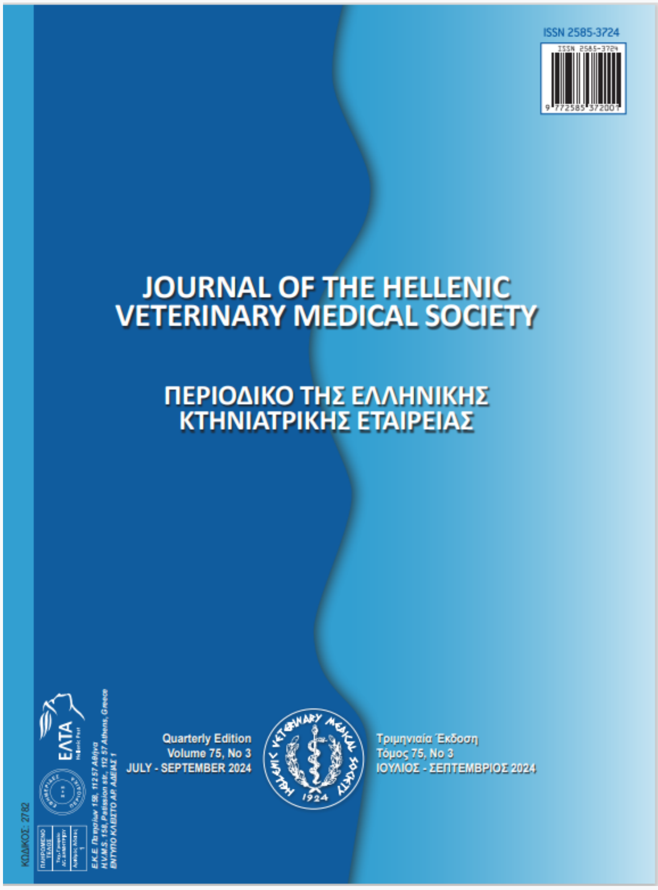

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Research article
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Antioxidant and antiproliferative effects of different *Raphanus Sativus* L. extracts

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ABSTRACT: This study aimed to investigate the phenolic and flavonoid levels, antioxidant activities and antiproliferative effects of Japanese, Chinese, Black, and Kohlrabi radish species on HEK293 immortalized human embryonic kidney cells incell culture medium. DPPH radical scavenging activity, total polyphenol, and flavonoid content of the extracts were measured spectrophotometrically. The highest total flavonoid content in sap of radish extraction and methanolic extraction was 0.12±0.02 mg/g RE and 0.10±0.00 mg/g RE in Chinese radish; the highest total polyphenol content was 0.32±0.03 mg/g GAE and 0.23±0.03 mg/g GAE in Black radish; the highest DPPH radical scavenging activity is approved 39.68±2.43 µg/g GAE in Japanese radish in sap of radish extraction and 84.59±3.50 µg/g GAE in Black radish in methanolic extraction respectively. DPPH radical scavenging activity was higher in the methanolic extraction of all radishes. The antiproliferative effects of different extracts in HEK293 cells were investigated by the MTT method. The IC50 concentration was found at lower doses in the methanolic extraction of whole radishes compared to the sap of radish extractions. The lowest dose of antiproliferative effect was observed in red radish (160 mg/ml) in methanolic extraction, and the antiproliferative effect increased dose-dependent. As a result, both antioxidant content and antiproliferative effect may vary depending on the type of radish, soil, and geographical conditions of the region where it is grown.

***Keywords*:** Radish; natural product; antioxidant; MTT; HEK293

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INTRODUCTION

Natural products play a role in correcting disorders caused by various diseases, especially cancer, diabetes, and neurodegenerative diseases (Riberio and Kim, 2023). Antioxidants found in foods play a role in strengthening the body's endogenous defenses against oxidative stress, which is responsible for chronic degenerative changes in most diseases (Contini et al., 2012). Polyphenols, which are secondary metabolites of plants, are natural compounds found in foods such as grains, vegetables, and fruits (Karamati et al., 2019).Most researchers accept that the antioxidant activity of fruits and vegetables is mainly due to the phenolic substances they contain, especially flavonoids (Orak, 2007).

Radish (Raphanus sativus L.), a member of the Brassicaceae family, has high nutritional and bioactive components (Yang et al., 2022)and is a root vegetable widely grown in tropical and temperate regions of the world (Baloch et al., 2014). Radish should not only be seen as a vegetable, it is also rich in medicinal compounds (Curtis, 2003).Radish roots are also used for appetizing, strengthening, urinary, and expectorant purposes (Kaya, 2013). The first records of radish are found in the ''*Tang Materia Medica"*, a Chinese pharmacopeia, and it is thought to have been used as a traditional Chinese herbal medicine for more than 1400 years (Kim et al., 2014).It is also known that horseradish, which is frequently used in upper respiratory tract diseases, has a good expectorant effect (Sabuncu, 2019) and white radish juice is added to most cough syrups (Akan et al., 2013).

The pharmaceutical potential of radish is reportedly due to its beneficial secondary metabolites such as glucosinolates, isothiocyanates, and polyphenols (Manivannan et al., 2019). Glycosinolates, which are highly present in the structure of cruciferous vegetables, are hydrolyzed by myrosinase enzyme, and important degradation products are released (Knez et al., 2022). Sulforaphane is one of these breakdown products. Studies have reported that sulforaphane is effective in preventing cancer occurrence (Lugasi et al., 2005). Radish roots are reported to be rich in peroxidase, an oxidoreductase involved in the scavenging of harmful free radicals (Curtis, 2003).Radish is not only rich in protein and vitamins A & C (Baloch et al., 2014) but also in folic acid, ascorbic acid, vitamin B6, riboflavin, calcium, potassium, and magnesium (Akan et al., 2013).

This study aimed to investigate the phenolic and flavonoid levels, antioxidant activities and antiproliferative effects of two different extracts of Japanese, Chinese, Black, and Kohlrabi radish species on HEK293 immortalized human embryonic kidney cells in a cell culture medium.

MATERIAL AND METHODS

Material

In the study, sap of radish extractions and methanolic extractions of Japanese, Chinese,Black, and Kohlrabi radishes were supplied from the market as herbal material.

Extraction

The sap of radish extraction was performed by modifying the method of Jakmatakul et al. (2009). For sap of radish extraction, radishes were pressed with the help of a juicer. Methanolic extraction was performed by modifying the method of Vitali et al. (2009).

The extracts were concentrated by rotary evaporator for cell culture applications. The sap of radish extractions was diluted with cell culture medium (DMEM) and methanolic extracts were dissolved with DMSO and diluted with DMEM and used in cell culture studies.

Antioxidant Activities

To determine the antioxidant activities of the extracts, DPPH radical scavenging (Shirazi et al., 2014) activity measurement analysis and to determine the antioxidant components, total polyphenol (Shirazi et al., 2014), and total flavonoid content (Boateng et al., 2008) analyses were determined spectrophotometrically. Three separate extractions were performed for each radish and all analyses were carried out in three separate extractions.

Antiproliferative Effects

IC50 values were determined as a result of MTTassays (Meral, 2018) performed on HEK293 immortalized human embryonic kidney cells in cell culture.

Statistical Analysis

Descriptive statistics for the data were calculated and presented as "Arithmetic Mean ± Standard Error". GLM analysis was used to examine the effect of Radish and Extraction factors on DPPH, Flavonoid, and Phenolic contents. The SPSS package program was used for the analysis (Field, 2013).

RESULTS

Total flavonoid content (Figure 2) was found as 0.12±0.02 mg/g RE, 0.06±0.00 mg/g RE, 0.02±0.00 mg/g RE, and 0.05±0.00 mg/g RE in the sap of radish extraction of Chinese radish, Black radish, Kohlrabi radish, and Japanese radish, respectively; while 0.10±0.01 mg/g RE, 0.04±0.00 mg/g RE, 0.02±0.01 mg/g RE and 0.04±0.01±0.01 mg/g RE were found in methanolic extraction. The total flavonoid content of the extracts was higher in all radishes except Kohlrabi radish. The highest flavonoid content was found in Chinese radish in both sap of radish extraction and methanolic extraction. The lowest flavonoid content was found in both the sap of extraction and methanolic extraction of Kohlrabi radish. Radish * Extraction interaction for the flavonoid content is statistically insignificant ($P>0.05$) (Table 1).

Total polyphenol content (Figure 3) was found as0.21±0.04 mg/g GAE, 0.32±0.03 mg/g GAE, 0.08±0.00 mg/g GAE and 0.27±0.08 mg/g GAE in the sap of radish extraction of Chinese radish, Black radish, Kohlrabi radish, and Japanese radish, respectively;while0.21±0.02 mg/g GAE, 0.23±0.03 mg/g GAE, 0.14±0.01 mg/g GAE and 0.21±0.06 mg/g GAE were found in methanolic extraction. The totalpolyphenol content of the extracts was higher in all radishes exceptKohlrabi radish.The highest total polyphenol content was found in black radish in both sap of radish extraction and methanolic extraction.The lowest total polyphenol content was found in both sap of extraction and methanolic extraction of Kohlrabi radish.Extraction and Radish * Extraction effects for the phenolic content are statistically insignificant ($P>0.05$) (Table 1).

DPPH radical scavenging activity (Figure 4) was found as 30.02±3.70 µg/g GAE, 26.37±2.27 µg/g GAE, 19.18±1.57 µg/g GAE and 39.68±2.43 µg/g GAE in the sap of radish extraction of Chinese radish, Black radish, Kohlrabi radish, and Japanese radish, respectively; while 56.48±1.14 µg/g GAE, 84.59±3.50 µg/g GAE, 68.06±3.19 µg/g GAE and 80.94±7.36 µg/g GAE were found in methanolic extraction.DPPH radical scavenging activity of methanolic extracts was higher than that of the sap of radish extracts in all radishes.The highest DPPH radical scavenging activity was detected in the methanolic extraction of Black radish. The lowest DPPH radical scavenging activity was detected in the sap of radish extraction of Kohlrabi radish. For the DPPH variable, the effects of Radish, Extraction, and Radish* Extraction are statistically significant ($P<0.05$).The difference in DPPH radical scavenging activity between sap of radish extraction and methanolic extraction of Chinese radish, Black radish, Kohlrabi radish, and Japanese radish was statistically significant.The difference in DPPH radical scavenging activity between Kohlrabi radish and Japanese radish was statistically significant In the sap of radish extraction.The difference in DPPH radical scavenging activity between Black radish and Chinese radish was statistically significant in methanolic extraction (Table 1).

The IC50 (mg/ml) concentrations (Figure 5) of Chinese radish, Black radish, Kohlrabi radish, and Japanese radish were found to be 4950 mg/ml, 3900 mg/ml, 3250 mg/ml, and 980 mg/ml in the sap of radish extraction, respectively, while 160 mg/ml, 240 mg/ml, 240 mg/ml and 200 mg/ml in methanolic extraction.Methanolic extracts showed a higher antiproliferative effect.In methanolic extraction, the highest antiproliferative effect was observed in Chinese radish (IC50=160 mg/ml). In radish juice, the highest antiproliferative effect was observed in Japanese radish (IC50=980 mg/ml).

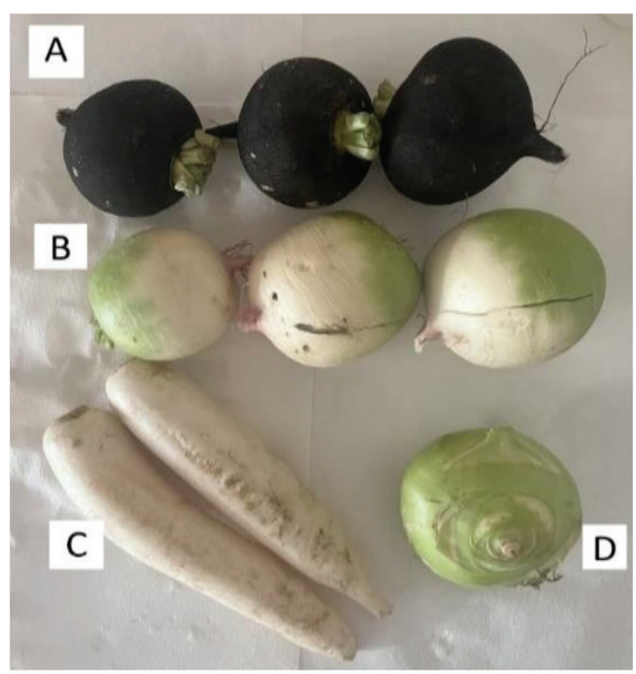

Fig 1. Types of radish used; A: Black radish, B: Chinese radish, C: Japanese radish, D: Kohlrabi radish

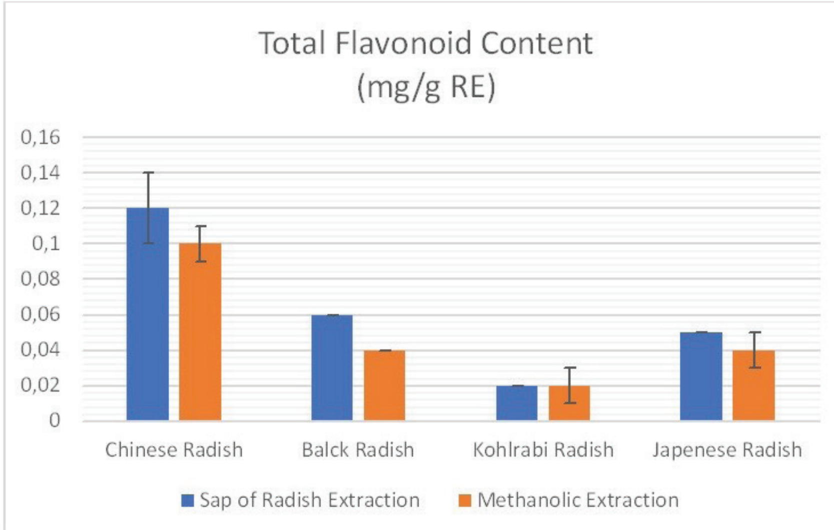

Fig 2. Total flavonoid content results of radishes

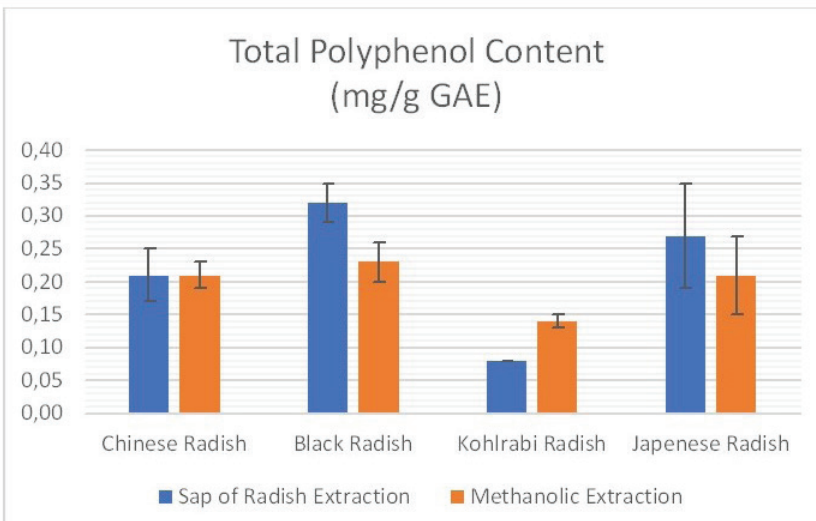

Fig 3. Total polyphenol content results of radishes

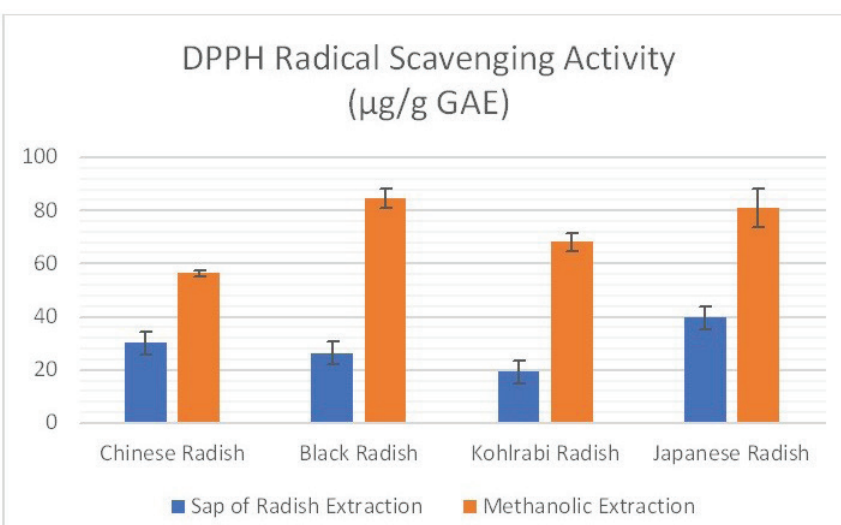

Fig 4. DPPH radical scavenging activity results of radishes

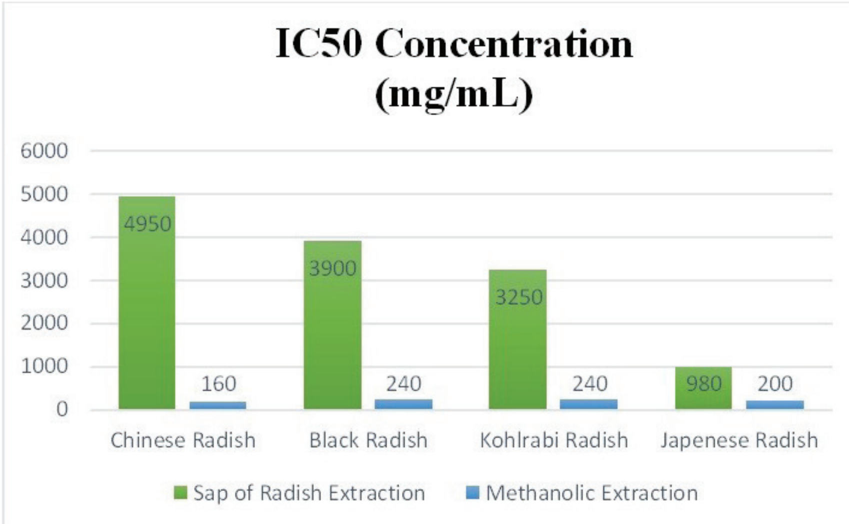

Fig 5. IC50 concentration results of radishes

Table 1. Results of statistical analysis

		Sap of Radish Extraction	Methanolic Extraction	p
DPPH Radical Scavenging Activity	Chinese Radish	30.02±3.70^{AB,b}	56.48±1.14^{C,a}	P<0.05
	Black Radish	26.37±2.27^{AB,b}	84.59±3.50^{A,a}	
	Kohlrabi Radish	19.18±1.57^{B,b}	68.06±3.19^{BC,a}	
	Japanese Radish	39.68±2.43^{A,b}	80.94±7.36^{AB,a}	
Total Flavonoid Content	Chinese Radish	0.12±0.02	0.10±0.01	P>0.05
	Black Radish	0.06±0.00	0.04±0.00	
	Kohlrabi Radish	0.02±0.00	0.02±0.01	
	Japanese Radish	0.05±0.00	0.04±0.01	
Total Polyphenol Content	Chinese Radish	0.21±0.04	0.21±0.02	P>0.05
	Black Radish	0.32±0.03	0.23±0.03	
	Kohlrabi Radish	0.08±0.00	0.14±0.01	
	Japanese Radish	0.27±0.08	0.21±0.06	

A, B, C: Indicates the difference between radishes
a, b: Indicates differences between extraction methods

DISCUSSION

Nutrition with diets rich in fruits and vegetables, especially cruciferous vegetables, has been reported to play a role in reducing the risk of many chronic diseases, including cancer (Donaldson, 2004; Hanlon et al., 2007). The dry matter and other compounds contained in radishes may vary depending on some conditions. These conditions can be sorted by various factors such as growing conditions, climatic conditions, ripening conditions, harvest time, and genotype (Solmaz, 2017).

Goyeneche et al. (2015)investigated the chemical characterization and antioxidant capacity of red radish (*Raphanus sativus* L.) leaves and roots and reported that the total flavonoid content of red radish roots was 267.47±6.38 mg quercetin (QE)/100 g.Noman et al. (2021)investigated the antioxidant and anticancer activities of white radish (*Raphanus sativus* L.) leaf and root extracts grown in Saudi Arabia and found the total flavonoid content of the ethanolic extract of white radish roots as 24.4 mg QE/g.Keyata et al. (2021) investigated the phytochemical contents, antioxidant activities, and functional properties of *Raphanus sativus L*, *Eruca sativa L.,* and *Hibiscus sabdariffa L.* species grown in Ethiopia and found that the total flavonoid content of radish roots was 5.28±0.70 mg catechin equivalent (CE)/g.In this study, total flavonoid content was found to be lower than in the studies conducted by Goyeneche et al. (2015), Noman et al. (2021), and Keyata et al. (2021). It is thought that this difference may be due to the difference in extraction methods used in the study and the difference in radish varieties.

Solmaz (2017) prepared extraction using 3 different solvents and analyzed the total polyphenol content in his thesis study with Black radish. The total polyphenol content of water, methanol, and ethanolic extractions was reported as 37.23±1.98 mg GAE/100g, 17.79±2.15 mg GAE/100g, and 31.33±1.27 mg GAE/100g, respectively.The total polyphenol content of water, methanol, and ethanolic extractions was reported as 37.23±1.98 mg GAE/100g, 17.79±2.15 mg GAE/100g, and 31.33±1.27 mg GAE/100g, respectively.Sabuncu (2019) reported the total polyphenol content as 318.11 mg GAE/100g; 508.94 mg GAE/100g; 381.85 mg GAE/100g; 710.04 mg GAE/100g; 288.35 mg GAE/100g and 291.31 mg GAE/100g, respectively, in the thesis study investigating the antioxidant capacity of methanolic extracts of 6 different radishes including Chestnut, Horseradish, White, Chinese, Japanese and Hazelnut radish.While the total polyphenol content detected in this study was compatible with the results of Solmaz (2017), it was lower than the results of Sabuncu (2019). It is thought that this difference may be due to the soil and geographical conditions of the region where radish is grown.

Beevi et al. (2010) investigated the effects of hexane extract of radish root on the viability of HeLa, A549, MCF-7 and PC-3 cells in India and found 24 h MTT results (IC50) as 8.78±0.43 µg/ml; 10.24±0.65 µg/ml; 8.36±0.21 µg/ml; 20.87±0.77 µg/ml, respectively. Pocasap & Weerapreeyakul (2016), Thailand, reported the result of a 24-hour MTTassay (IC50) >50 µg/ml on HCT116 colon cancer cell line with dichloromethane extract of Chinese radish root. Noman et al. (2021) found the DPPH radical scavenging activity of ethanolic extract of white radish roots to be 40.6±2 µg/ml. Noman et al. (2021)using the ethanolic extract of white radish roots on A549, HepG2, MCF-7, and MDA-MB-231 cell lines, found the MTT assay results (IC50) as 250.6±3.1 µg/ml, 444.6±1.6 µg/ml, 306.3±2.2 µg/ml and 470±2.6 µg/ml, respectively.In this study, the IC50 values determined by the MTT assay were found to be higher than in other studies. It is thought that this difference may be due to the type of radish, changes in the extraction method, and differences in the growing conditions of radish (such as soil and geographical conditions).

CONCLUSIONS

In this study, the highest flavonoid and polyphenol substance content in both sap of radish extraction and methanolic extraction and DPPH* radical scavenging activity in methanolic extraction were determined in Chinese radish. At the same time, the highest antiproliferative effect was found in the methanolic extract of Chinese radish. In this direction, it has been concluded that radish consumption can be a natural product that can be evaluated in terms of health. As a result, both the antioxidant content and the antiproliferative effect may vary depending on the type of radish, the soil, and the geographical conditions of the region where it is grown. In addition, it was determined that the antiproliferative effect of radish extract increased in a dose-dependent manner. It is thought that new studies on the effects of radish, which is a natural product, on metabolic pathways in different diseases will be beneficial.

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CONFLICT OF INTEREST

None declared

REFERENCES

Akan S, Veziroğlu S, Özgün Ö & Ellialtıoğlu Ş (2013) Turp (Raphanus sativus L.) sebzesinin fonksiyonel gıda olarak değerlendirilmesi. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *23*(3), 289-295.

Baloch PA, Uddin R, Nizamani FK, Solangi AH & Siddiqui AA (2014) Effect of nitrogen, phosphorus, and potassium fertilizers on growth and yield characteristics of radish (Raphinus sativus L.). *American-Eurasian Journal of Agricultural & Environmental Sciences*, *14*(6), 565-569.

Beevi SS, Mangamoori LN, Subathra M & Edula JR (2010)Hexane extract of Raphanus sativus L. roots inhibits cell proliferation and induces apoptosis in human cancer cells by modulating genes related to apoptotic pathway. *Plant foods for human nutrition*, *65*, 200-209.

Boateng J, Verghese M, Walker LT & Ogutu S (2008) Effect of processing on antioxidant contents in selected dry beans (Phaseolus spp. L.). *LWT-Food Science and Technology*, *41*(9), 1541-1547.

Contini M, Baccelloni S, Frangipane MT, Merendino N &Massantini R (2012) Increasing espresso coffee brew antioxidant capacity using phen olic extract recovered from hazelnut skin waste. *Journal of Functional Foods*, *4*(1), 137-146.

Curtis IS (2003)The noble radish: past, present and future. *Trends in Plant Science*, *8*(7), 305-307.

Donaldson MS (2004)Nutrition and cancer: a review of the evidence for an anti-cancer diet. *Nutrition Journal*, *3*(1), 1-21.

Field A (2013) Discovering Statistics Using Ibm Spss Statistics. 4th Ed., Sage Publication Ltd.

Goyeneche R, Roura S, Ponce A, Vega-Gálvez A, Quispe-Fuentes I, Uribe E & Di Scala K (2015)Chemical characterization and antioxidant capacity of red radish (Raphanus sativus L.) leaves and roots. *Journal of Functional Foods*, *16*, 256-264.

Hanlon PR, Webber DM & Barnes DM (2007)Aqueous extract from Spanish black radish (Raphanus sativus L. Var. niger) induces detoxification enzymes in the HepG2 human hepatoma cell line. *Journal of agricultural and food chemistry*, *55*(16), 6439-6446.

Jakmatakul R, Suttisri R & Tengamnuay P (2009) Evaluation of antityrosinase and antioxidant activities of Raphanus sativus root: comparison between freeze-dried juice and methanolic extract. *The Thai Journal of Pharmaceutical Sciences*, *33*(1), 22-30.

Karamati Jabehdar S, Mirzaei Aghjehgheshlagh F, Navidshad B, Mahdavi A, Staji H (2019)In vitro antimicrobial affect of phenolic extracts and resistant starch on Escherichia coli, Streptococcus spp., Bifidobacterium and Lactobacillus spp.. Kafkas Univ Vet Fak Derg, 25 (2): 137-146.

Kaya S (2013)*Tıbbi botanik ve tıbbi bitkiler*. Ankara: Medisan Yayınevi.

Keyata EO, Tola YB, Bultosa G & Forsido S F (2021)Phytochemical contents, antioxidant activity and functional properties of Raphanus sativus L, Eruca sativa L. and Hibiscus sabdariffa L. growing in Ethiopia. *Heliyon*, *7*(1).

Kim KH, Moon E, Kim SY, Choi SU, Lee JH & Lee KR (2014)4-Methylthio-butanyl derivatives from the seeds of Raphanus sativus and their biological evaluation on anti-inflammatory and antitumor activities. *Journal of Ethnopharmacology*, *151*(1), 503-508.

Knez E, Kadac-Czapska K, Dmochowska-Ślęzak K & Grembecka M (2022)Root Vegetables—Composition, Health Effects, and Contaminants. *International Journal of Environmental Research and Public Health*, *19*(23), 15531.

Lugasi A, Blázovics A, Hagymási K, Kocsis I & Kéry Á (2005): Antioxidant effect of squeezed juice from black radish (Raphanus sativus L. var niger) in alimentary hyperlipidemia in rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(7), 587-591.

Manivannan A, Kim J-H, Kim D-S, Lee E-S & Lee H-E (2019)Deciphering the nutraceutical potential of Raphanus sativus—A comprehensive overview. *Nutrients*, *11*(2), 402.

Meral S Ç (2018)*Akciğer kanseri hücrelerinde alfa lipoik asit ve piperlonguminin hücre canlılığına etkileri.* Yayımlanmış yüksek lisans tezi, Ankara Üniversitesi, Ankara.

Noman OM, Nasr FA, Alqahtani AS, Al-Zharani M, Cordero MAW, Alotaibi AA, Bepari A, Alarifi S & Daoud A (2021)Comparative study of antioxidant and anticancer activities and HPTLC quantification of rutin in white radish (Raphanus sativus L.) leaves and root extracts grown in Saudi Arabia. *Open Chemistry*, *19*(1), 408-416.

Orak HH (2007) Total antioxidant activities, phenolics, anthocyanins, polyphenol oxidase activities of selected red grape cultivars and their correlations. *Scientia Horticulturae*, *111*(3), 235-241.

Pocasap P & Weerapreeyakul N (2016)Sulforaphane and sulforaphane in commonly consumed cruciferous plants contributed to antiproliferation in HCT116 colon cancer cells. *Asian Pacific Journal of Tropical Biomedicine*, *6*(2), 119-124.

Ribeiro RIMA & Kim B (2023)Anti-cancer effects of natural products against reproductive cancers. *Frontiers in Pharmacology*, *14*, 1132795.

Sabuncu M (2019)*Farklı Turp (Raphanus sativus* L.*) Tiplerinin Antioksidan Kapasite ve Biyoalınabilirliklerinin Belirlenmesi.* Yayımlanmış yüksek lisans tezi, Bursa Uludağ Üniversitesi, Bursa.
Solmaz M (2017)*Kara Turpun (Raphanus sativus* L. *niger) Bazı Biyoaktif Bileşenlerinin Ekstarksiyonu*. Yayımlanmış yüksek lisans tezi, Ondokuz Mayıs Üniversitesi, Samsun.
Shirazi, O. U., Khattak, M. M. A. K., & Shukri, N. A. M. (2014). Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. Journal of pharmacognosy and phytochemistry, 3(3), 104-108.
Vitali D, Dragojević IV & Šebečić B (2009) Effects of incorporation of integral raw materials and dietary fiber on the selected nutritional and functional properties of biscuits. *Food Chemistry*, *114*(4), 1462-1469.
Yang M, Hou CY, Hsu HY, Hazeena SH, Santoso SP, Yu CC, ... & Hsieh CW (2022) Enhancing Bioactive Saponin Content of Raphanus sativus Extract by Thermal Processing at Various Conditions. *Molecules*, *27*(23), 8125.