Araştırma Makalesi / *Research Article* **Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 9(3): 1460-1466, 2019**
Kimya / Chemistry ***Journal of the Institute of Science and Technology,* 9(3): 1460-1466, 2019**

DOI: 10.21597/jist.532824 **ISSN: 2146-0574, eISSN: 2536-4618**

Karpuzun (Citrullus Lanatus) Dış ve İç Kabuğundaki Antioksidanların Koruyucu Etkisi

Mesut IŞIK[1*]

ÖZET: İnsan sağlığına faydalı olduğu bilinen karpuz (Citrullus lanatus) yaygın olarak tüketilmektedir. Çalışma, *C. lanatus*'un dış ve iç kabuklarının fitokimyasal bileşenleri, serbest radikal temizleme aktiviteleri ve besleyici içeriğini değerlendirmek amacıyla yapılmıştır. Bu amaçla, çalışmada karpuzun dış ve iç kabuklarından elde edilen etanol ekstraktında toplam flavonoid / fenolik içeriği ve antioksidan aktivitesi belirlendi. Antioksidan aktivite, CUPRAC, DPPH, FRAP ve potasyum tiyosiyanat yöntemi gibi *in vitro* deneysel yöntemle belirlenmiştir. *C. lanatus* dış ve iç kabuğundaki toplam fenolik ve flavonoid içeriği sırasıyla 40.5 ve 32.4 mg GAE g^{-1} ve 12.6 and 5.8 mg QE g^{-1} olarak bulunmuştur. *C. lanatus* dış ve iç kabuğundan elde edilen etanol ektrakt konsantrasyonu (20 µg/ml), aynı konsantrasyondaki a-tokoferolle (% 85.3) karşılaştırıldığında maksimum lipit peraksidasyonunda % 73.2 ve % 72.0 oranında inhibisyon sağladı. Sonuç olarak, dış ve iç kabuklar metal indirgeme kapasitesi ve orta derecede serbest radikal giderme aktivitesine sahip olup ve aynı zamanda lipid peroksidasyon sürecini inhibe eder. Bu nedenle, *C. Lanatus*'un özellikle dış kabuğu, radikal süpürücüler gibi davranan, oksidasyonu inhibe eden veya geciktiren ve lipid peroksidasyonunu geciktirerek besin maddelerinin raf ömrünü uzatan doğal bir bileşene sahip olabilir.

Anahtar Kelimeler: Radical giderme aktivitesi, oksidatif stres, *citrullus lanatus*, antioksidan

Effect of Antioxidants as Preservatives in the Outer and Inner Shells of Watermelon (Citrullus Lanatus)

ABSTRACT: Watermelons (Citrullus lanatus) that are known to be beneficial to human health are widely consumed. The study was performed out to investigate the phytochemical components, free radical scavenging activities and nutritive contents of the outer and inner shells of *C. lanatus*. For this purpose, the study was determined the total flavonoid/phenolic content and antioxidant activity of ethanol extract prepared from outer and inner shells. The antioxidant activity was determined by in vitro experimental method, such as CUPRAC, DPPH, FRAP and potassium thiocyanate method. Total phenolic and flavonoid contents in outer and inner shell of *C. lanatus* were found to be 40.5 and 32.4 mg GAE g^{-1} and 12.6 and 5.8 mg QE g^{-1}, respectively. The concentration of ethanol extract obtained from *C. Lanatus* outer and inner shell (20 µg/mL) has inhibition of maximum lipid peroxidation in rate % 73.2 and % 72.0 respectively compared to that of α-Tokoferol (% 85.3) at the same concentration. As a result, the outer and inner shells have metal reduction capacity and moderate free radical removal activity and also inhibited the lipid peroxidation process. Therefore, *C. Lanatus*'s especially the outer shell may have a natural ingredient that acts as radical scavengers, inhibits or delays the oxidation and prolongs the shelf life of nutrients by delaying the lipid peroxidation.

Keywords: Radical cavenging activity, oxidative stress, *citrullus lanatus*, antioxidant

[1] Mesut IŞIK (**Orcid ID:** 0000-0002-4677-8104), Department of Pharmacy Services, Health Services Vocational School, Harran University, Şanlıurfa, Turkey

* Sorumlu Yazar/Corresponding Author: Mesut IŞIK, e-mail: mesutisik16@gmail.com, misik@atauni.edu.tr

Geliş tarihi / *Received:*26.02.2019
Kabul tarihi/*Accepted:*21.03.2019

INTRODUCTION

Many studies have shown that the most important causes of food degradation during storage and processing is lipid peroxidation. It is known that radicals cause the lipid peroxidation has a serious effect in the progression the many pathological diseases such as brain dysfunction, cancer promotion, neurodegenerative, atherosclerosis, immune system decline and heart (Czinner et al., 2001; Işık et al., 2015). When added as an additive to food products, antioxidants play a role as radical scavengers. They have important functions such as prolonging shelf life by delaying the lipid peroxidation process, inhibit or retard the oxidation process and preventing radical chain reactions of oxidation (Young and Woodside, 2001). The synthetic antioxidants that generally used in foods to retard or prevent lipid oxidation is butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). However, it is thought that there are limitations due to some side effects and carcinogenicity in the use of the compounds (Velioglu et al., 1998; Işık et al 2017). Therefore, consumers have become more conscious about the safety of foodstuffs and the nutritional value.

The natural food, which is believed to be healthier, safer and less exposed to hazards, is more preferred than synthetic (Farag et al., 1986). Therefore, the preservation of natural products and their antioxidant effect has been the focus of attention in recent years. The use of herbal products with antioxidant content in processed foods is becoming more important in the food industry than in the use of unnatural antioxidants (Madsen and Bertelsen, 1995; Albayrak et al., 2010).

Cucurbitaceae family, which has important economic importance, contains approximately 122 genera and 900 species, many of them used as folk medicines and/or food. *Citrullus lanatus* (Watermelon), which grows in many countries, represents a large part of the Mediterranean diet, which dates back nearly 3000 years (Vaughan and Geissler, 2009; Simpson, 2010).

Watermelon (*C. lanatus*, family Cucurbitaceae), which can adapt to different environmental conditions is an important vegetable product grown in many countries (Greenway and Pratt, 2001). The antioxidant activities of these products, anti-inflammatory effects and some basic nutrients, have been emphasized in many studies (Sun and Wang, 2010; Abdelwahab et al., 2011). *C. Lanatus* consumption is known to reduce the frequency of many diseases because watermelon contains a rich A and C vitamin (Greenway and Pratt, 2001). *C. lanatus* fruit has abundantly a content of vitamins C and A. Many other compounds previously isolated from watermelon, for example, previous studies have been found in compounds such as cis-3 nonenal, trans,trans,cis-2,4,6-nonatrienal, cis-6-nonenal and cis-2-nonenal isolated from watermelon (Beaulieu, 2006). In many studies, it has been reported that *C. lanatus* fruit has therapeutic effects and it is known that these effects are due to antioxidant and some phytochemical compounds (Yadav and Agarwala, 2011). For example, beta carotene and lycopene have an important role in the treatment of many diseases such as atherosclerosis and cancer (Charoensiri et al., 2009; Adetutu et al., 2015).

Although the benefits of *C. Lanatus* fruit on health in terms of content are known, no studies have been done on the species grown in Osmaniye region of Turkey in terms of nutrient content and antioxidant benefits. Thus, in the study, we aimed to determine antioxidant potential and to identify phytochemical components in *C. lanatus*.

MATERIALS AND METHODS

Chemicals

Gallic acid and $CuCl_2$, Folin-Ciocalteu reagent (Ferrozine®), Na-K phosphate buffer, the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), Trolox, α-tocopherol, 2,9-dimethyl-1,10-phenanthroline (neocuproine) ethanol and other chemicals used were obtained from Sigma-Aldrich and Merck.

Preparation of Sample Extracts

C. lanatus fruits was obtained from Osmaniye in 2017. The obtained parts were dried at 30 °C in the shade and left to mix with 100 ml ethyl alcohol for extraction. The Sample was extracted using a rotary evaporator (Stone Staffordshire England) at 50 °C to remove ethanol after filtering with Whatman No 1 Paper. The extracts were stored in a dark at -20°C until experimental studies (Işık et al., 2015).

FRAP and CUPRAC Method as Metal Reduction Potential

The Ferric Cyanide (Fe3+) Reducing Antioxidant Power Assay (FRAP) assay was performed with the modified method of the previously described Oyaizu (1986) method. When the method is reduced to the ferrous (Fe^{2+}) ion to ferric tripyridyl triazene (Fe^{3+}-TPTZ) complex at 700 nm, the complex is formed. It is based on the spectrophotometric measurement of the complex. (Oyaizu 1986; Bursal and Köksal 2011; Işık et al 2015). The reduction capacity for cupric ions (Cu^{2+}) was determined by Cupric Ions Reducing Assay (CUPRAC) assay as previously described (Bursal and Köksal, 2011; Işık et al 2015). A volume of 0.25 ml neocuproine (7.5 mM) in ethanol, 0.25 mL NH_4Ac (1 M) and 0.25 mL $CuCl_2$ (0.01 M) was mixed with sample at different amounts (10, 20, 30μg/mL) and standards.

DPPH Scavenging Activity

DPPH scavenging activities of *C. lanatus* outer and inner shell was determined according to the method described by Blois (1958). In the method, the stable DPPH radical is removed with free radical scavenging activity of the sample. Briefly, The ethanol extract (10, 20, and 30μg/mL) from the sample were prepared and then volume adjusted to 3 mL with ethanol. then the prepared DPPH solution (1 mL, 0.1M) was added, followed by incubation in the dark for 30 minutes. DPPH elimination activity of the sample after incubation was measured spectrophotometrically (Blois, 1958; Işık et al., 2017).

Determination of Total Phenolic and Flavonoids

As described previously, total phenolic analysis was performed using Folin-Ciocalteu reagent. Folin- Ciocalteu phenol reagent (0.5 mL) were added to 23 ml sample water mixture and 3 min later, 2 % Na_2CO_3 (1.5 mL). The mixture was vortexed and then kept in the room temperature for 30 min. Then, the samples were measured at 760 nm. The phenolic content outer and inner shell of *C. lanatus* was determined by using a standard curve of gallic acid (Bursal and Köksal 2011; Bursal et al 2010). The total flavonoid content determined by colorimetric method was performed according to the procedure described in our previous study. One mg of the sample is added to 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL CH_3COOK (1M). The mix was was incubated for 40 minutes at room temperature and then measured at 415 nm (Ross et al., 2002; Işık et al., 2017).

Determination with Ferric Thiocyanate Method of TOS

Total antioxidant activity (TOS) of outer and inner shell in *C. lanatus* was determined with thiocyanate method as previously described. In this method, peroxidase formed as a result of linoleic acid oxidize Fe^{2+} to Fe^{3+} and former ion reacts with thiocyanate to form a complex which give absorbance at 500 nm. The TOS amount was calculated according to the

thiocyanate method we have done in previous studies (Işık et al., 2017: Gulcin et al., 2010). The inhibition of linoleic acid oxidation was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = 100 - \left(\frac{A_S}{A_C} \text{ x } 100 \right)$$

RESULT AND DISCUSSION

Using the standard curve (gallic acid and quercetin), total flavonoid and phenolic content in ethanol extract obtained from outer and inner shell of *Citrullus lanatus* showed in Table 1.

Table 1. Total flavonoids and phenolic contents in outer and inner shell of *Citrullus lanatus*

	Total phenolic compound (µg GAE mg^{-1} extract)	**Total amount of flavonoid (µg QE mg^{-1} extract)**
Citrullus lanatus outer shell	40.5	12.6
Citrullus lanatus inner shell	32.4	5.8

The scavenging effect the sample is shown in Figure 1A. For the comparison purpose, standard antioxidants (trolox, α-tocopherol and BHT) have been used as reference. The result showed that ethanol extract of the sample is able to scavenge DPPH• radical even at low concentration (10 µg/L). However increases in the concentration did not lead a further reducing power. The reducing power of α-tocopherol, trolox and *C. lanatus* extract, are presented in Figure 1B,1C.

Compared to synthetic antioxidants (trolox and a-tocopherol), the polar fractions of all extracts showed radical scavenging activity close to synthetic antioxidants. Inhibition rates of the oxidized linoleic acid in outer and inner shell of *C. lanatus* were very close to each other. The result showed that ethanolic extract in outer and inner shell (20 µg/mL) of the *C. lanatus* caused a reduction inhibited lipid peroxidation up to 48 hours (Figure 1D) The inhibition rate of the substance were 85.3%, 83.1% and 72.05-73.2 % for α-tocopherol, trolox and *C. lanatus* outer and inner shell, respectively. These results clearly show that ethanolic extract obtained from the outer and inner shell of *C. lanatus* is a remarkable potent antioxidant activity.

A number of recent studies emphasize that reactive oxygen species (ROS) are produced continuously in cells and that oxidative stress occurs if the antioxidant defense system is insufficient. The oxidative stresses play a role in the pathogenesis of many diseases and cause severe damage to tissues and biomolecules in biological systems. There are endogenous and exogenous antioxidant defense systems in the cells against these harmful effects of ROS. Many fruits and plants consumed are known to be rich in antioxidant content (Eze, 2006; Catala, 2009). It has been shown by many studies that medicinal plants and fruits are becoming extremely popular as antioxidants all over the world and herbal formulations and some dietary which have the potential to free radical scavenging, can be effective in the treatment of many disease such as chronic diseases (Tiwari and Tripathi, 2007).

A group of investigators said that phytochemicals were able to remove free radicals because of their antioxidant content, and they also stated that they reduced the inflammatory process (Charoensiri et al., 2009; Abdelwahab et al., 2011; Adetutu et al., 2015). Flavonoids, carotenoids, isoflavones, ellagic acid, indoles and lignans are one of the best examples of phytochemicals. Research have been reported that phenolic compounds such as condensed tannins, gallotannins and flavonoids

have bioactivities such as anti-allergic, anticancer and anti-inflammatory properties. Moreover, the some molecular targets of pro-inflammatory mediators also have been known to inhibit by the compounds mentioned above. (Raza et al., 2007; Okwu, 2004; Gülçin, 2012). According to a study done by Okwu, the flavonoids, which prevent oxidative cell damage, have water soluble form and anticancer activity. It is well known that the use and consumption of such additives, which are in water soluble form, are important for foodstuffs. The flavonoids in *C. lanatus* fruit can provide protection against tumor formation, microbes, free radicals and inflammation (Okwu, 2004).

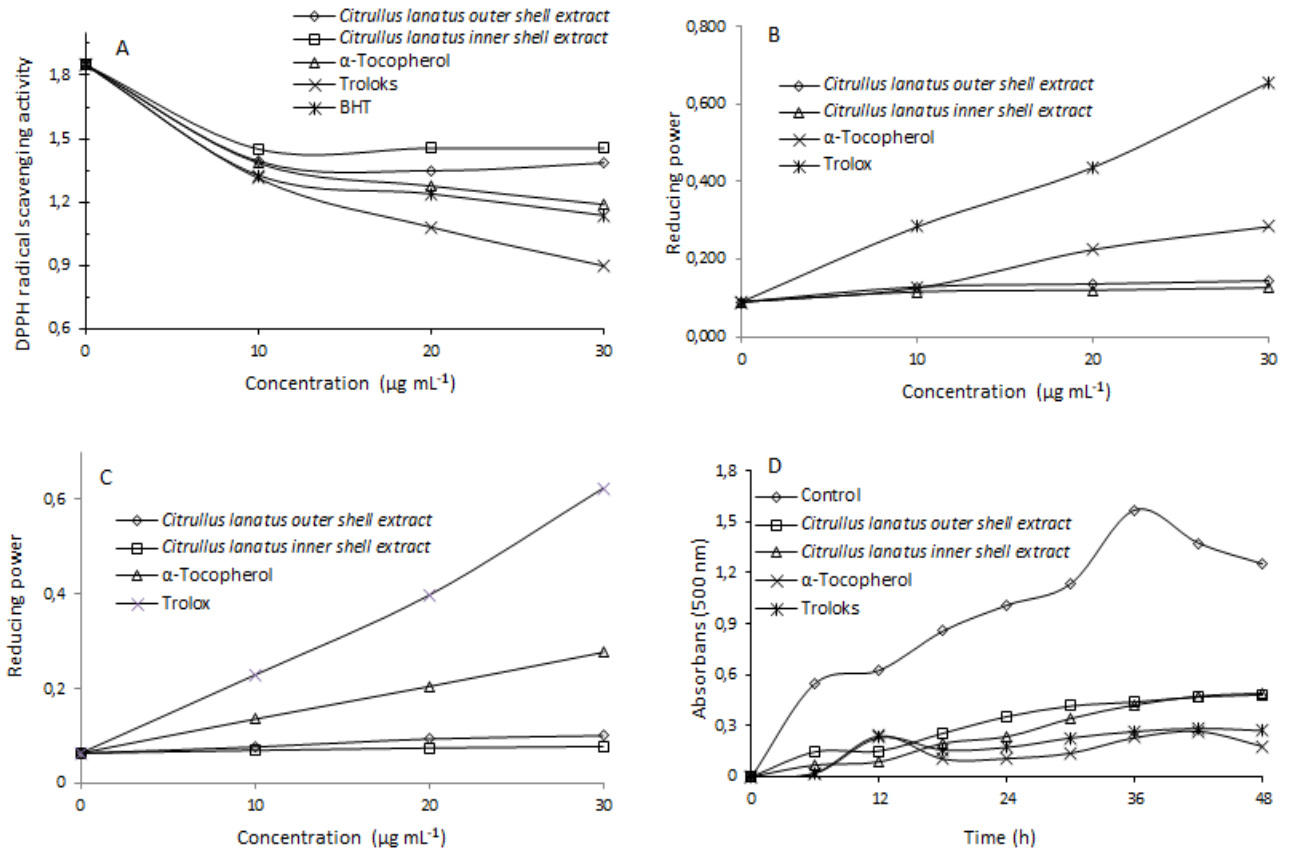

Figure 1. Reducing power and free radical-scavenging capacities in different concentrations (10-30 µg/mL) of Phlomis pungens and reference antioxidants determined by (A) DPPH (B) FRAP and (C) CUPRAC method. (D) Inhibition rate of linoleic acid oxidation

According to a study conducted by Abdelwahab et al, phytochemical analyzes of *C. lanatus* seed and other parts were made and it was stated that phytochemicals were present in significant amounts. In the studies conducted, phytochemicals emphasized many benefits to health. Phytochemicals in *C. lanatus* fruit extract were determined, and reported that they had basic nutritional values and free radical scavenging activity. It is also emphasized that this fruit may not be used as a specific lipid, carbohydrate and protein source according to its basic nutritional values. In studies conducted, it emphasizes the health benefits of phytochemicals. Phytochemical analyzes in seed and other parts of *C. lanatus* have been made and it is stated that phytochemicals are found in considerable amounts (Abdelwahab et al., 2011).

CONCLUSION

In conclusion, in this study, the outer and inner shell of *C. lanatus* showed DPPH radical removal and metal reduction activity and inhibited lipid peroxidation. The ethanol extract including the outer and inner shell of *C. lanatus* has demonstrated radical scavenging activities. As a result, it is thought that the important source of natural antioxidant may be due to the flavonoids content of beta-carotene and lycopene. *C. lanatus* can be a important source of antioxidants to prevent or treat the oxidative stress-related diseases due to this feature in the outer and inner shell. As we know, the most recent deterioration of the watermelon under the outer shell shows the protective effect of the substances found in the outer shell. Nowadays, synthetic compounds used as antioxidants are known to be replaced by natural products due to side effects, both in food processing and in pharmaceutical fields. Therefore, the results presented in this study are important for the emergence of plant-derived products which may be useful for such industries.

REFERENCES

Abdelwahab SI, Hassan LEA, Sirat HM, 2011. Anti-Inflammatory Activities of Cucurbitacin E Isolated from *Citrullus lanatus* var. Citroides: Role of Reactive Nitrogen Species and Cyclooxygenase Enzyme Inhibition. Fitoterapia, 82: 1190-1197.

Adetutu A, Olorunnisola OS, Owoade OA, 2015. Nutritive values and antioxidant activity of *Citrullus lanatus* fruit extract. Food and Nutrition Sciences, 6(11): 1056.

Albayrak S, Aksoy A, Sagdic O, Hamzaoglu E, 2010. Compositions, antioxidant and antimicrobial activities of Helichrysum (Asteraceae) species collected from Turkey. Food Chemistry: 119(1); 114-122.

Beaulieu JC, 2006. Effect of Cutting and Storage on Acetate and Non-Acetate Esters in Convenient, Ready to Eat Fresh-Cut Melons and Apples. HortScience, 41: 65-73.

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

Bursal E, Köksal E, Gülçin İ, 2010. In vitro Antioxidant Properties and Phenolic Content of Ginger (Zingiber officinale Rosc.). Root, 2010.

Catala A, 2009. Lipid peroxidation of membrane phospholipids generates hydroxy-alkanals and oxidized Phospholipids active in physiological and/or pathological conditions. Chem.Phys.Lipids, 157 (1):1-11.

Charoensiri R, Kongkachuichai R, Suknicom S, Sungpuag P, 2009. Beta-Carotene, Lycopene and Alpha-Tocopherol Contents of Selected Thai Fruits. Food Chemistry, 113: 202-207.

Charoensiri R, Kongkachuichai R, Suknicom S, Sungpuag P, 2009. Beta-Carotene, Lycopene and Alpha-Tocopherol Contents of Selected Thai Fruits. Food Chemistry, 113: 202-207.

Czinner E, Hagymási K, Blázovics A, Kéry Á, Szoke, É, Lemberkovics É, 2001. The in vitro effect of Helichrysi flos on microsomal lipid peroxidation. Journal of Ethnopharmacology, 77: 31–35.

Eze MO, 2006.The oxygen paradox and the place of oxygen in our understanding of life,aging,and death. Ultim.Real Meaning (URAM):stud.Med.Health.29 (1):46-61.

Farag RS, Badei AZ, Heweij FM, El-Baroty GSA, 1986. Antioxidant activity of some spices essential oils on linoleic acid oxidation in aqueous media. Journal of the American Oil Chemists Society, 66. 792–799.

Greenway HT, Pratt SG, 2001. Fruit and Vegetable Micronutrients in Diseases of the Eye. In: Watson, R., Ed., Fruits, Vegetables and Herbs in Health Promotion. CRC Press, Boca Raton.

Gulcin I, 2012. Antioxidant activity of food constituents: an overview. Arch Toxicol, 86(3):345-91.

Gülçin İ, Huyut Z, Elmastaş M, Aboul-Enein HY, 2010. Radical scavenging and antioxidant activity of tannic acid. Arabian Journal of Chemistry, 3(1): 43-53.

Işık M, Dikici E, Tohma H, Köksal E, 2017. Antioxidant Activity and Total Phenolic/Flavonoid Contents of *Phlomis pungens* L. Hacettepe J. Biol. & Chem., 45 (3): 425–433.

Işık M, Korkmaz M, Bursal E, Gulcin I, Koksal E, Tohma H, 2015. Determination of antioxidant properties of Gypsophila bitlisensis bark. Int. J. Pharmacol, 11; 366-371.

Madsen HL, Bertelsen G, 1995, Spices as antioxidants. Trends in Food Science and Technology, 6: 271–277.

Okwu DE, 2004. Phytochemicals and Vitamin Content of Indigenous Species of South Eastern Nigeria. Journal of Sustainable Agriculture and the Environment, 6: 30-34.

Oyaizu M, 1986. Studies on products of browning reaction--antioxidative activities of products of browning reaction prepared from glucosamine. Eiyogaku zasshi Japanese journal of nutrition.

Raza F, Gholam AG, Mohammad, HHK, 2007. Antioxidant Activity of Various Extracts of Old Tea Leaves and Black Tea Wastes (Camellia sinensis L.). Food Chemistry, 100: 231-236.

Ross JA and Kasum CM, 2002. Dietary flavonoids: Bioavailability, metabolic effects, and safety. Annual Review of Nutrition, 22: 19-34.

Sun H, Wang ZB, 2010. Effects on Exercise Endurance Capacity and Antioxidant Properties of Astragalus Membranaceus Polysaccharides (APS). Journal of Medicinal Plants Research, 4: 982-986.

Tiwari OP, Tripathi YB, 2007. Antioxidant properties of different fractions of Vitex negundo Linn. Food Chem., 100: 1170-1176.

Velioglu YS, Mazza G, Gao L, Oomah BD, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 46: 4113–4117.

Yadav RNS, Agarwala M, 2011. Phytochemical Analysis of Some Medicinal Plants. Journal of Phytology, 3: 10-14.

Young IS, Woodside JV, 2001. Antioxidants in health and disease. Journal of Clinical Pathology, 54: 176–186.