The European Research Journal 2023;9(1):87-96 Original Article

DOI: 10.18621/eurj.1056629 *Nutrition and Dietetics*

# Investigation of antioxidant and antimicrobial activities of walnut (*Juglans regia* L.) kernel septum

**Elif Azize Özşahin Delibaş[1], Esin Kıray[2]**

*[1]Department of Nutrition and Dietetics, Gaziosmanpaşa University, Faculty of Health Sciences, Tokat, Turkey; [2]Department of Medical Services and Techniques, Ahi Evran University, Health Services Vocational School, Kırşehir, Turkey*

## ABSTRACT

**Objectives:** Walnut (*Juglans regia* L.) kernel septum (or septa) (WKS), a traditional nutraceutical material in China, has not been explored in detail. In this study, antimicrobial activity, total phenolic content (TPC) and antioxidant-oxidant status of WKS was investigated in case it may be clinically important in the management of various complications.

**Methods:** The WKS was extracted with ethanol in a Soxhlet device. TPC of WKS was analysed by using Folin-Ciocalteu's method. Antioxidant activity was obtained by using Rel Assay Diagnostics kits. The antimicrobial activity of WKS was evaluated against two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*), one Gram-negative bacteria (*Escherichia coli*) and one fungus (*Candida albicans*) strains using the agar diffusion method.

**Results:** The TPC of WKS was found to be 119.42 ± 2.39 mg GAE/gDW. It was determined that total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) values were 7.542 ± 0.389 mmol/L, 3.718 ± 0.287 µmol/L and 0.049 ± 0.001, respectively. WKS selectively inhibited the growth of Gram-positive bacteria and fungus, while *S. aureus* was the most susceptible one with 16 mm of inhibition zone. Gram-negative bacteria was resistant to the extract.

**Conclusions:** As far as we know, this paper is the first work that demonstrates the antioxidant-oxidant status of WKS by using the method described above, and moreover there are no scientific reports which have examined WKS in such a multidisciplinary experimental design. This study strongly supports the reported traditional use of WKS. Results indicated that WKS can be used as a pharmacological natural agent due to its high antioxidant and antimicrobial activities.

**Keywords:** *Juglans regia* L, walnut kernel septum, antioxidant, total phenolic content, antimicrobial activity

Herbal drugs are the oldest type of health service known by humankind. Medicinal plants have been used by all cultures throughout the history [1]. It is demonstrated in several studies that aromatic and medicinal plants are the source of many molecules with antioxidant and antimicrobial characteristics which can protect the body against both cellular oxidation reactions and pathogens. Thus, characterization of different parts of different types of plants is important in order to show their antioxidant and antimicrobial potentials [2].

In cases where reactive oxygen species (RES) are excessively produced or antioxidant mechanisms are insufficient, the failure of the balance between oxi-

***Received:*** *January 12, 2022;* ***Accepted:*** *July 31, 2022;* ***Published Online:*** *October 7, 2022*

***How to cite this article:*** *Özşahin Delibaş EA, Kıray E. Investigation of antioxidant and antimicrobial activities of walnut (Juglans regia L.) kernel septum. Eur Res J 2023;9(1):87-96. DOI: 10.18621/eurj.1056629*

e-ISSN: 2149-3189

***Address for correspondence:*** *Elif Azize Özşahin Delibaş, PhD., Gaziosmanpaşa University, Faculty of Health Sciences, Department of Nutrition and Dietetics, 60250, Tokat, Turkey. E-mail: elif.delibas@gop.edu.tr, Phone: +90 356 252 16 16 ext. 3956*

dant-antioxidant systems is defined as oxidative stress, and this condition has a significant role in the etiopathogenesis of many diseases [3]. Triggering factors caused by the modern lifestyle such as processed food, exposure to several chemicals, lack of exercise makes oxidative stress inevitable [4]. Weakening of antioxidant protection mechanisms that play an active role in decreasing the oxidative stress effects is a condition which increases tendency of body cells and tissues to develop diseases. Therefore, to continue having sufficient antioxidant levels without having excess dose are necessary to prevent many numbers of diseases and to take them under control [5].

It is known that phytochemicals such as phenolic compounds in plants decrease the risk of degenerative diseases by inhibiting the oxidation of macromolecules or decreasing the oxidative stress [6]. Bioactive materials including phenolic compounds have a large number of chemical functions and structures. It has been reported that bioactive components have a potential effect on health and particularly they have beneficial effects on prevention or delay of chronical diseases. However, complex, controlled, and long-term studies which would demonstrate these effects are still required [7].

On the other hand, increasing antibiotic resistance in bacteria is one of the main reasons of failure in fight with infectious diseases. Especially for an important number of immunosuppressed patients, infections are the most important reason of mortality and morbidity [8]. The rapid emergence of multidrug-resistant bacteria results in a constant requirement for novel antimicrobial components [9].

To date, walnut is accepted as a natural functional food due to its nutritious content and medical benefits [10]. Many previous pharmacological studies have shown that different parts of *Juglans regia* L. have nutritious, cardiovascular, antidepressant, antisedative, antimicrobial, antioxidant, anticancerous, antidiabetic, antiinflammatory, antiparasitic, immunological, analgesic, gastrointestinal, endocrinal, and several other pharmacological effects [1]. Use of walnuts and walnut trees has a long history. Therefore, green husk, kernel, shell, or other parts such as flowers or leaves of walnut have always drawn attention and not only found place in conventional medicinal systems but they are also used in pharmaceutical and beauty industries [11-13].

The woody tissue, that divides the walnut fruit into two parts from inside called as 'Walnut Kernel Septum' (WKS) is a by-product which has been included in traditional medical literature and has been used in diseases such as diabetes, insomnia, diarrhea, renal diseases and reproductive disorders. However, it has not gained the reputation it deserves as there are very few numbers of scientific studies about it [14].

This study focuses on this part of the walnut which is treated as a waste and has only limited usage area. The aim of this study is to evaluate the total phenolic content, antioxidant, and antimicrobial capacity of ethanolic WKS extract with a multidisciplinary experimental design.

## METHODS

### Chemicals

All chemicals/reagents used in this study were purchased from Sigma (Sigma Aldrich Chemie GmbH, Schnelldorf, Germany) and Merck (Darmstadt, Germany). All reagents and solvents used were of analytical grade. Water used in all analyses was ultra-pure produced by a Milli-Q system (Millipore, Bedford, MA, USA). Total oxidant status (TOS) and total antioxidant status (TAS levels) were determined by using Rel Assay Diagnostic kit RL009 and RL010, respectively.

### Plant Material and Extraction Procedure

Walnuts (*Juglans regia* L.), which is naturally grown in Kaman district of Kırşehir province, were supplied commercially. The WKS of dried walnuts which separates the two kernel pieces were removed. The shadow-dried plant materials were chopped, just prior to pulverization with the help of a blender (Waring 8011 EB). WKSs (10 g) were extracted by a Soxhlet device (BUCHI Extraction System Model B811) with 100 mL ethanol (EtOH-%95, v/v, Merck) at 60°C for 8 hours. The extracts were filtered using Whatman filter paper (No:1) and then concentrated under vacuum at 40°C using a Rotary evaporator (Heidolph Hei-Vap Rotary Evaporator). Obtained ethanolic WKS extract was stored in a freezer at -20°C for further tests.

### Biological Materials

Based on their pathogenic importance, the following test micro-organisms were selected for the antimicrobial activity assay; S*taphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633 as gram-positive bacteria; *Escherichia coli* ATCC 25922 as gram-negative bacteria and *Candida albicans* ATCC 90028 as fungus. All standard bacterial and fungal strains were obtained from Department of Medical Microbiology, Ahi Evran University, Kırşehir.

## Quantitative Determination of TPC

Total phenolic content (TPC) of ethanolic WKS extract was determined by using the Folin-Ciocalteu (FC) method with minor modifications [15]. The principle of the method is based on the separation of a phenolic proton in alkaline medium and formation of a phenolate anion capable of reducing the FC reagent.

The prepared ethanolic WKS extract was diluted 100 times with Milli-Q water. 1 mL of the diluted sample was mixed with 1 mL of five times diluted FC reagent, prepared freshly before use. The reaction medium was kept at room temperature for 3 min. The mixture was then mixed with 3 mL of sodium carbonate ($Na_2CO_3$) solution (12.5%) and 15 mL of Milli-Q water. The solution was vortexed for 1 min and kept at 25 °C for 90 min. The absorbance value was obtained at 760 nm by using a spectrophotometer (Thermo Scienteilfic-Evolution 60S UV-Visible). Calibration curve used for quantification was obtained by using different concentrations of gallic acid. Gallic acid was used to obtain the standard curve (linear range 10-70 µg/mL with $R^2 = 0.9991$). The concentrations of phenolic compounds in all samples were expressed as micrograms of gallic acid equivalents per gram dry weight [µg of GAE/gDW].

## Measurements of TOS

The measurement is based on the principle that the oxidants in the sample oxidize the ferrous ion-orthodianicidine complex to ferric ion and the ferric ion forms a blue-green complex with the chromogen in an acidic environment [16]. The colour intensity of the complex, which is proportional to the total amount of oxidant present in the sample, is measured spectrophotometrically at 530 nm wavelength. Hydrogen peroxide ($H_2O_2$) solution is used as a standard in the calibration of the method and the results are expressed as µmol $H_2O_2$ equiv/L. The assay was carried out as follows: 500 µl of Reagent 1 was mixed with 75 µl ethanolic WKE and OD1 (optical density) values were obtained at 530 nm 25 µL Reagent 2 was added to the mixture. OD2 values were obtained at 530 nm after 5 min incubation at 37°C. The TOS value was calculated by using the formula given in the kit prospectus.

## Measurements of TAS

The principle of the measurement is based on the reduction of dark blue-green 2,2'-azinobis 3-ethyl benzothiazoline-6-sulphonate radical to colourless ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) form by the antioxidants in the sample [17]. The degree of ABTS reduction, which depends on the amount and capacity of antioxidants, is determined by the change in absorbance caused by the colour difference at 660 nm wavelength in the spectrophotometer. The method is calibrated with a stable antioxidant standard solution called "Trolox", which is widely used as a traditional standard for TAS measurement assays. The assay results are expressed in mmol Trolox equiv/L.

The assay was carried out as follows: 500 µl of Reagent 1 was mixed with 30 µl ethanolic WKE and OD1 values were obtained at 660 nm 75 µL Reagent 2 was added to the mixture. $OD_2$ values were obtained at 660 nm after 5 min incubation at 37°C. The TAS value was calculated using the formula given in the kit prospectus.

## Measurements of OSI

The percentage ratio of TOS to TAS gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress. In order to perform the calculation, the unit of TAS, mmol Trolox equivalent/L, was changed to µmol Trolox equivalent/L. The OSI value was calculated according to the following formula [17]:

OSI = [(TOS, µmol $H_2O_2$ equivalent/L) / (TAS, µmol Trolox equivalent/L) × 100]

## Determination of Antimicrobial Activity

Antimicrobial effects of ethanolic WKS extract were tested using the agar well method [18]. Bacteria was grown in Nutrient-Broth Agar (NBA) medium which was kept at 37°C overnight. Suspensions were prepared from these bacteria the next day according to 0.5 McFarland (a final inoculum of $1.5 \times 10^8$

CFU/mL) turbidity value. 100 µl of 0.5 McFarland bacterial suspension prepared in turbidity was added into 5 ml soft agar (0.5% agar) and poured onto the Müller-Hinton Agar (MHA) medium prepared in petri dishes. Wells were opened with the help of a 6 mm diameter glass pipette on the media that were left to dry for a while. Two wells were drilled in NBA medium where microorganisms were spread. 25 µl of ethanolic WKS extract was pipetted into one, while ethanol, the solvent of the extraction process, was pipetted into the other as a negative control. Ethanolic WKS extract and pure ethanol were sterilized by passing them through membrane filters with 0.22 µm pore diameter before use. On the other hand, Tetracycline (30 mg), Penicillin G (10 U), Sulbactam (10 mcg) + Ampicillin (10 mcg), Gentamicin (10 mcg), Rifampin (5 mcg), Teicoplanin (30 mcg), Ciprofloxacin (5 mcg), Chloromphenical Sterile discs containing (30 mg) were also carefully placed in MHA media and used as positive control. Antimicrobial activity was determined by measuring the zone diameters formed after 24 hours of incubation at 35 oC. After the incubation process, the inhibition zones were measured. The results were given as mean value of three independent measurements. The antibacterial activity was measured in terms of the diameter (mm) of clear zone of growth inhibition. The sensitivity was evaluated according to the National Committee for Clinical Laboratory Standards (NCCLS) [NCCLS, 1998] and the antimicrobial activity of the WKS was evaluated.

## Statistical Analysis

All samples were analysed in triplicate (n = 3) and the results were expressed as mean ± SD (Standard Deviation).

## RESULTS

### *TPC*

In this study, phenolic concentration in ethanolic extract of WKS was measured and TPC of WKS was found as 119.42 ± 2.39 mg (GAE)/gDW. Experiments are made in 3 parallels.

### *Antioxidant Activity*

In this study, antioxidant, and oxidant potentials of WKS has been determined and the oxidative stress index, which demonstrates how much the available antioxidant compounds can suppress the oxidant compounds, has been calculated. It was found that endogen antioxidant capacity produced by WKS was 7.542 ± 0.389 mmol/L, while oxidant compound level that is obtained due to environmental effects and metabolic activities was determined to be 3.718 ± 0.287 µmol/L. It was determined that the OSI, which shows the percentage of oxidant compounds that are tolerated by endogen antioxidant compounds was 0.049 ± 0.001 (Table 1). In the antibiogram test *E. coli* and *C. albicans* have been found to be resistant to the antibiotics; Ampicillin/Sulbactam, Rifampin, and Penicillin, while most effected by Ciprofloxacin and less effected by Chloramphenicol. *S. aereus*, has been effected by all antibiotics except Ampicillin/Sulbactam and has provided large inhibition diameters. *B. subtilis*; has shown resistance to the antibiotics; Rifampin, Penicillin and Teicoplanin, while most effected by Ciprofloxacin and least effected by Ampicillin/Sulbactam (Fig. 1). So, it has been demonstrated by the inhibition zone diameters formed that the antibiotics are efficient against many of the selected microorganisms.

### *Antimicrobial Activity*

In this study, the antimicrobial potential of WKS has been investigated on two Gram-positive (*S. aureus, B. subtilis*), one Gram-negative (*E. coli*) bacteria, and *C. albicans*, an opportunistic yeast that can become pathogenic when our immune system is compromised. Ethanol, the extract solvent, was used as negative control, while Tetracycline (30 mg), Penicillin G (10 U), Sulbactam (10 mcg) + Ampicillin (10 mcg), Gentamicin (10 mcg), Rifampin (5 mcg), Teicoplanin (30 mcg), Ciprofloxacin (5 mcg), Chloram-

**Table 1. TAS, TOS and OSI values**

| | TAS (mmol/L) | TOS (µmol/L) | OSI |
|---|---|---|---|
| **Walnut kernel septum** | 8.407 ± 0.399 | 6.533 ± 0.366 | 0.078 ± 0.008 |

Values are presented as mean ± SD. Experiments are made in 3 parallels. TAS = Total antioxidant status, TOS = Total oxidant status, OSI = Oxidative stress index

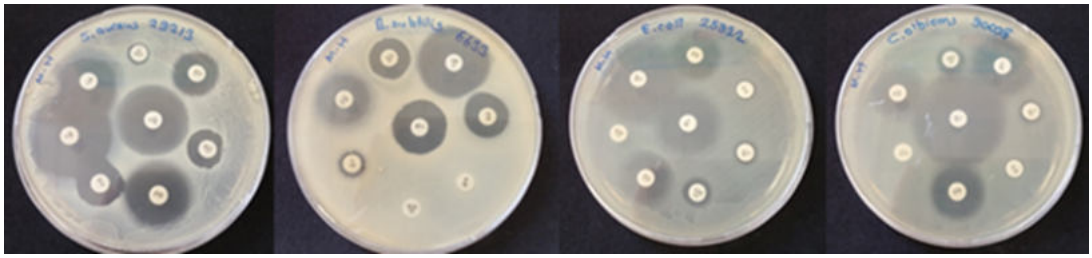

**Fig. 1.** **Antibiogram test.**

phenicol (30 mg) were used as positive control (Fig. 1).

In the antibiogram test *E. coli* and *C. albicans* have been found to be resistant to the antibiotics; Ampicillin/Sulbactam, Rifampin, and Penicillin, while most effected by Ciprofloxacin and less effected by Chloramphenicol. *S. aereus*, has been effected by all antibiotics except Ampicillin/Sulbactam and has provided large inhibition diameters. *B. subtilis*; has shown resistance to the antibiotics; Rifampin, Penicillin and Teicoplanin, while most effected by Ciprofloxacin and least effected by Ampicillin/Sulbactam (Fig. 1). So, it has been demonstrated by the inhibition zone diameters formed that the antibiotics are efficient against many of the selected microorganisms.

According to our findings, the results indicate that WKS ethanolic extract had antimicrobial effects against S. aereus (16 mm), *B. subtilis* (15 mm), and *C. albicans* (14 mm) with varying extent, but did not have any inhibitory effect on *E. coli* (12 mm) in terms of antimicrobial activity. The diameter of inhibition zone is shown in Table 2.

## DISCUSSION

Due to their strong antioxidant characteristics and significant effects on preventing several diseases related with oxidative stress, herbal polyphenols are increasingly drawing attention. In the last few years, identification of phenolic compounds has been an important field in the researches about health and medicine. Phenolic compounds are the most found secondary metabolites of plants and have a wide distribution among plant tissues. Therefore, there are several studies in which different parts of plants are examined [19].

In a recent study by Hu *et al*. [20] in which phenolic composition and nutritious characteristics of WKS was investigated, TPC of WKS has been reported as 73.66 ± 0.73 g/100 gDW. In another study conducted by Liu *et al*. [21], polyphenol profile of WKS was aimed to be identified and it was determined that there were 75 individual phenolic compounds. Also, TPC of WKS has been expressed in terms of 122.78 ± 2.55 mg GAE/gDW. In an experimental study conducted by Ghiravani *et al*. [22] in 2016 on diabetic rats, polyphenol content of WKS ethanol extract has been found as 21.64 ± 1.44 mg GAE/gDW.

Also, Regueiro *et al*. [23] has reported mean total polyphenol content of different walnut extracts as 2.464 ± 22 mg GAE/100 g and highlighted that the results have varied between 1.576 and 2.499 mg GAE/100 g and this was consistent with other studies [24, 25]. In a comprehensive review article by Jahanban-Esfahalan *et al*. [26] , methanolic extract of walnut leaves were compared with aqueous extract (27.92 ± 1.40 mg); and it was stated that methanolic extract has demonstrated the maximum polyphenol content (94.39 ± 5.63 mg GAE/g extract). In another study conducted by Akbari *et al*. [27], phenolic compounds of different parts such as walnut hull, shell, pellicle (brown skin), and kernel have been investigated. TPC for hull, shell, skin and kernel were reported as follows, respectively: 24.68 ± 4.28mg GAE/g, 18.04 ± 4.20 mg GAE/g, 52.05 ± 1.27 mg GAE/g and 1.45 ± 0.12 mg GAE/g. In another study conducted by Popovici [28], ethanol extracts of walnut leaves, shell and WKS were investigated in respect of their total phenol content. In that article, total phenol content of WKS has been found significantly more than that of both leaves and shell extracts. Solvent extractions are the most convenient procedures to prepare extracts of plant materials due to their ease of use, efficiency, and large applicability. However, many methods such as

**Table 2. Antibacterial and antifungal activity of WKS**

| Mean inhibition zone (mm)* | Microorganisms | Positive control | | | | | | | | Negative control | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SAM | C | RIF | PCN | TET | TEC | GM | CPFX | Ethanol | WKS |
| | ***B. subtilis 6633*** | 11 | 22 | R | R | 18 | R | 16 | 34 | 12 | 15 |
| | ***S. aereus 29213*** | R | 23 | 27 | 15 | 20 | 14 | 17 | 25 | 12 | 16 |
| | ***E. coli 25922*** | R | 19 | R | R | 17 | 15 | 17 | 26 | 12 | 12 |
| | ***C. albicans 90028*** | R | 22 | R | R | 18 | R | 19 | 28 | 12 | 14 |

*Inhibition zone including well diameter (5 mm). SAM = Ampicillin/sulbactam, C = Chloramphenicol, RIF = Rifampin, PCN = Penicillin, TET = Tetracycline, TEC = Teicoplanin, GM = Gentamicin, CPFX = Ciprofloxacin R = Resistant

microwave, ultrasound supported extractions have been developed in recent years. Rusu *et al*. [29] have found total phenol content of WKS as 67.03 ± 9.76 mg GAE/gDW by Ultra-Turrax extraction method and 31.27 ± 5.24 mg GAE/gDW by maceration method. Taken together, different extraction methods, different types and parts of plants, use of different solvents have made it more difficult to compare the results of these studies; however, it can be easily distinguished that WKS is a rich source of phenolic compounds and yet, it has more phenolic content than other different parts of walnut.

It is reported that oxidative stress is related with development of many metabolic and chronical disorders. Within this context, antioxidants are referred as valuable molecules as they prevent the damage due to oxidative stress in defense mechanisms [30]. It is also reported that in case of antioxidant insufficiency, exogen antioxidant supplement (food/preparation) for the organism can also prevent formation of oxidative stress [31]. Therefore, it is especially important to evaluate antioxidants present in biological materials and food in respect of quantity and activity. Kusano and Ferrari [32] have not only highlighted that TAS measurement can be a reliable biomarker which can be used in diagnosis and prognosis of several pathophysiological conditions, but also have stated that to prevent initiation and development of diseases, to execute nutritional interventions including anti-aging strategies, it can also be used in determination of antioxidant-rich food.

The exploration of new antioxidant sources in nature can be possible by determining the antioxidant capacity of different parts of plants. Yet, these parts of plants might have significant medical potential even if they do not have nutritional features [33]. Currently, many total antioxidant capacity tests such as FRAP (Ferric Reducing-Antioxidant Power) [34], ORAC (Oxygen Radical Absorbance Capacity) [35], TEAC (Trolox Equivalent Antioxidant Capacity) [36] are widely used in the analysis of biological tissues and/or food materials [32]. In this study, a different automated method was used which directly measures the total antioxidant capacity colorimetrically, which is far less affected by the presence of uric acid, as reported by Erel *et al*. [17]. The most important advantage of this method is that it has not measured only a single compound's antioxidant capacity, but it has measured the total antioxidant capacity of all antioxidants in the biological sample [37]. So, known or unknown, any type of antioxidant interactions including possible additive effects have been evaluated as a whole.

No reference values could have been found as there were not found any study in literature which determines the antioxidant potential of WKS by the method we use. However, with the method reported by Erel, by different studies conducted on different plant types, TAS; TOS; OSI values of *Thymbra spicata* [38], *Gundelia tournefortii* [39], *Rumex crispus* [40] have been reported as 8.399, 6.831, 6.758; 6.530, 3.712, 5.802; 0.078, 0.054, and 0.086, respectively. When compared with these studies, TAS value of WKS was higher than that of *G. tournefortii* and *R. crispus*, and lower than that of *T. spicata*, which have been evaluated as having antioxidant properties. It is considered that these differences are due to the potential of the plants to produce compounds with antioxidant characteristics. In this regard, it can be said that WKS has a significant antioxidant potential.

In a review study where different extracts of dif-

ferent walnut parts such as kernel, skin, shell, husk have been examined comparatively in respect of antioxidant features, results obtained by DPPH (2.2.-Diphenyl-1-picrihydrazile), FRAP and ORAC methods have been reported [26]. In another study in which antioxidant activity of walnut male flowers was investigated, IC50 values were reported for DPPH and ABTS as 75.17 ± 4.43 µg/ml and 63.40 ± 5.73 µg/ml, respectively, while FRAP value was reported as 54.35 ± 3.12 µmol/L FeSO4/mg ethanolic extract [41]. It is understood that different parts of walnut might have different antioxidant potentials.

The only study that has been found in literature about the antioxidant activity of WKS has been the study of Rusu *et al*. in which ABTS, DPPH and FRAP methods were used. According to this study, antioxidant activity was reported as 174,28 ± 8,22 mg trolox equivalent (TE)/g DW WKS by ABTS method, while 255,89 and 400,97 mg TE/g WKS extract by DPPH and FRAP methods; WKS was evaluated as having good antiradical effects [29].

Wang *et al*. [42] have reported that WKS has formed anti-inflammatory activity by inhibiting nitric oxide production and claimed that this activity has been due to the presence of gallic acid, ethyl gallate and (+)-dehydrovomifoliol. Meng *et al*. [43] have also reported that semi-maximal inhibitor concentration of WKS extract (IC50) has been 1.06 mg/mL, and this was far less than that is for polysaccharide obtained from potato peel (11.57 mg/mL) and more than that of ascorbic acid (0.077 mg/mL).

Although it is not possible to compare these values as antioxidant activity is expressed in different ways, different methods are applied and results are given in different units, in general all of them has demonstrated that WKS has high antioxidant activity. It is claimed that highly phenolic compounds can be responsible from high antioxidant activity of WKS [44].

Pharmacological agents already in use modulate oxidative stress [45]. WKS is also considered to have high antioxidant potential and can be recommended as a natural antioxidant agent.

Nowadays, the emergence of antibiotic-resistant strains and the presence of various side effects are problems to be solved in the fight against infectious diseases. Limited options for preventing or treating bacterial/fungal infections have led medical science to nature, to discover new and different antimicrobial agents [46].

In this study the results indicate that fungal/bacterial species have different sensitivities towards WKS extract. It can be said that WKS is more effective on gram (+) bacteria compared to gram (-) bacteria. There are other studies which have reported that plant extracts are more effective on gram (+) bacteria [47]. It is claimed that this is caused by the lipopolysaccharide layer outside the cell wall of gram (-) bacteria which makes them more resistant [48].

Many numbers of herbal extracts with antimicrobial characteristics have been reported [49]. But, there are limited number of studies whose subject area is antimicrobial characteristics of WKS. Similar to our study, in a recent study, it was reported that WKS has shown varying degrees of antibacterial effects on Gram-positive bacteria (*S. aureus, Staphylococcus epidermidis, Enterecoccus faecalis* and *Enterecoccus faecium*) (with MIC (minimum inhibitory concentration) ranging from 8.59 to 275 µg/ml); while Gram-negative (*E. coli, Klepsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis*) strains have shown very low sensitivity (with MIC values of 275 µg/ml). In the same study in which antimicrobial activity of WKS has been determined by using Ciprofloxacin which is a wide-spectrum antibiotic, it was also reported that administration of WKS in highest doses (275 ug/ml) have affected the growth of Gram-positive and Gram-negative bacteria strains most of which are resistant to Ciprofloxacin [50]. In another study conducted by Rusu *et al*. [51], it was reported that WKS has shown MIC (minimum inhibition concentration) levels between 0.012-3.12 mg/mL; WKS antimicrobial activity was measured against Gram-positive (*S. aureus*) and Gram-negative (*E. coli, P. aeruginosa, Salmonella enteritidis*) bacteria and two fungi (*C. albicans* and *C. parapsilosis*), and the lowest effect was found against E. coli. Different than our study, in two experimental studies conducted by Meng *et al*. [43], the water soluble polysaccharide fraction isolated from WKS has been found to show significant antibacterial activity against two Gram-negative bacteria strains (*E. coli* and *P. aeruginosa*) and also against two Gram-positive strains (*S. aureus* and *Listeria monocytogenes*) depending on the dosage (0.2-1.2 mg/mL).

The results presented above clearly prove that this part of the plant is a promising source of new antimicrobial agents.

### Limitations

This study was carried out on crude WKS extract. The elucidation of metabolic pathways, metabolic regulations, or the biosynthesis and roles of macromolecules are still in obscurity. More in-depth studies are needed in the future to clarify the molecular mechanisms responsible for the antioxidant and antimicrobial effect of WKS.

## CONCLUSION

In our study, it was aimed to get more information about WKS which is a by-product with a limited use at present. In sum, the results from our study showed that WKS has a high phenolic content, and remarkable antioxidant and antimicrobial activity. Nevertheless, clinical studies are also required in order to investigate other possible pharmacological activities, safety, and efficacy of WKS.

*Authors' Contribution*

Study Conception: EAÖD, EK; Study Design: EAÖD, EK; Supervision: EAÖD, EK; Funding: EAÖD, EK; Materials: EAÖD, EK; Data Collection and/or Processing: EAÖD; Statistical Analysis and/or Data Interpretation: EAÖD; Literature Review: EAÖD; Manuscript Preparation: EAÖD and Critical Review: EAÖD, EK.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

*Financing*

The authors disclosed that they did not receive any grant during conduction or writing of this study.

*Acknowledgements*

I am grateful to Selma Çetinçift Doğan for providing checking/correction services by examining this document with great care in terms of word choice, style and consistency, as well as grammar, spelling and punctuation.

## REFERENCES

1. Al-Snafi AE. Chemical constituents, nutritional, pharmcological and therapeutic importance of Juglans regia-A review. IOSR J Pharm 2018;8:1-21.
2. Wojdyło A, Oszmıańskı J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem 2007;105:940-9.
3. Amira AM. Oxidative stress and disease: an updated review. Res J Immunol 2010;3:129-45.
4. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front Physiol 2020;11:694.
5. Kusano C, Ferrari B. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J Cell Mol Biol 2008;7:1-15.
6. Silva BM, Andrade PB, Valentao P, Ferreres F, Seabra RM, Ferreira MA. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. J Agric Food Chem 2004;52:4705-12.
7. Barut Uyar B, Sürücüoğlu MS. [Biologically active components in foods]. Bes Diy Derg 2010;38:69-76. [Article in Turkish]
8. Taşova Y. İmmunosupresif hastalarda gelişen infeksiyonlar: sık rastlanan gram-pozitif kok enfeksiyonları. Güneş Kitabevi. Türkiye: Ankara; 2003: pp. 27-43..
9. Deshpande RR, Kale AA, Ruikar AD, Panvalkar RS, Kulkarni AA, Deshpande NR et al. Antimicrobial activity of different extract of Juglans regia L. against oral microflora. Int J Pharm Pharm Sci 2011;3:200-1.
10. Bouabdallah I, Bouali I, Martínez-Force E, Albouchi A, Perez Camino MDC, Boukhchina S. Composition of fatty acids, triacylglycerols and polar compounds of different walnut varieties (Juglans regia L.) from Tunisia. Nat Prod Res 2014;28:1826-33.
11. Beiki T, Najafpour GD, Hosseini M. Evaluation of antimicrobial and dyeing properties of walnut (Juglans regia L.) green husk extract for cosmetics. Color Technol 2018;134:71-81.
12. Ebrahimi S, Jamei R, Nojoomi F, Zamanian Z. Persian walnut composition and its ımportance in human. Health Int J Enteric Pathog 2017;6:3-9.
13. Pereira JA, Oliveira I, Sousa A, Valentão P, Andrade PB, Ferreira ICFR et al. Walnut (Juglans regia L.) leaves: phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food Chem Toxicol 2007;45:2287-95.
14. RBN Prasad. Encyclopaedia of food sciences and nutrition. In: Walnuts and pecans. 2nd ed. Academic Press, USA: London; 2003: pp. 6071-9.
15. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.
16. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103-11.
17. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277-85.
18. Holder IA, Boyce ST. Agar well diffusion assay testing of

bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns 1994;20:426-9.
19. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010;1:7313-52.
20. Hu Q, Liu J, Li J, Liu H, Dong N, Geng YY, et al. Phenolic composition and nutritional attributes of diaphragma juglandis fructus and shell of walnut (Juglans regia L.). Food Sci Biotechnol 2020;29:187-96.
21. Liu P, Li L, Song L, Sun X, Yan S, Huang W. Characterisation of phenolics in fruit septum of Juglans regia Linn. by ultra performance liquid chromatography coupled with Orbitrap mass spectrometer. Food Chem 2019;286:669-77.
22. Ghiravani Z, Hosseini M, Mehdi M, Taheri H. Evaluation of hypoglycemic and hypolipidemic effects of internal septum of walnut fruit in alloxan-induced diabetic rats. Afr J Tradit Complement Altern Med 2016;13:94-100.
23. Regueiro J, Sánchez-González C, Vallverdú-Queralt A, Simal-Gándara J, Lamuela-Raventós R, Izquierdo-Pulido M. Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap-Orbitrap mass spectrometry. Food Chem 2014;152:340-8.
24. Abe LT, Lajolo FM, Genovese MI. Comparison of phenol content and antioxidant capacity of nuts. Cienc Tecnol Aliment 2010;30:254-25.
25. Vinson JA, Cai Y. Nuts, especially walnuts, have both antioxidant quantity and efficacy and exhibit significant potential health benefits. Food Funct 2012;3:134-40.
26. Jahanban-Esfahlan A, Ostadrahimi A, Tabibiazar M, Amarowicz R. A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (Juglans regia L.) fruit and tree. Molecules 2019;24:2133.
27. Akbari V, Jamei R, Heidari R, Esfahlan AJ. Antiradical activity of different parts of Walnut (Juglans regia L.) fruit as a function of genotype. Food Chem 2012;135: 2404-10.
28. Popovici C. Soxhlet extraction and characterisation of natural compounds from walnut (Juglans regia L.) by-products. Ukr Food J 2013;2:328-36.
29. Rusu ME, Gheldiu AM, Mocan A, Moldovan C, Popa DS, Tomuta I, et al. Process optimization for improved phenolic compounds recovery from walnut (Juglans regia L.) Septum: phytochemical profile and biological activities. Molecules 2018;23:2814.
30. Aminjan HH, Abtahi SR, Hazrati E, Chamanara M, Jalili M, Paknejad B. Targeting of oxidative stress and inflammation through ROS/NF-kappaB pathway in phosphine-induced hepatotoxicity mitigation. Life Sci 2019;232:116607.
31. Prior RL, Cao G. In vivo total antioxidant capacity: comparison of different analytical methods. Free Radic Biol Med 1999;27:1173-81.
32. Kusano C, Ferrari B. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J Cell Mol Biol 2008;7:1-15.
33. Chibane LB, Degraeve P, Ferhout H, Bouajila J, Oulahal N. Plant antimicrobial polyphenols as potential natural food preservatives. J Sci Food Agric 2019;99:1457-74.
34. Ustundag Y, Huysal K, Kahvecioglu S, Demirci H, Yavuz S, Sambel M, et al. Establishing reference values and evaluation of an in-house ferric reducing antioxidant power (FRAP) colorimetric assay in microplates. Eur Res J 2016;2:126-31.
35. Cao G, Booth SL, Sadowski JA, Prior RL. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am J Clin Nutr 1998;68:1081-7.
36. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci 1993;84:407-12.
37. MacDonald-Wıcks LK, Wood LG, Garg ML. Methodology for the determination of biological antioxidant capacity in vitro: a review. J Sci Food Agric 2006;86:2046-56.
38. Mohammed FS, Şabik AE, Sevindik E, Pehlivan M, Sevindik M. Determination of antioxidant and oxidant potentials of thymbra spicata collected from Duhok-Iraq. Turkish J Agric Food Sci Technol 2020;8:1171-3.
39. Saraç H, Demirbaş A, Durna S, Ata M. [Evaluation of nutrients and biological activities of kenger (Gundellia tournefortii L.) seeds cultivated in Sivas province]. Turkish J Agric Food Sci Technol 2019;7:52-8. [Article in Turkish]
40. Durna Daştan S, Durukan H, Demirbaş A, Dönmez E. [Bioactivity and therapeutic properties of evelik (Rumex crispus), a naturally growing and edible plant in Sivas province]. Turkish J Agric Food Sci Technol 2019;7:67-71. [Article in Turkish]
41. Muzaffer U, Paul VI. Phytochemical analysis, in vitro antioxidant and antimicrobial activities of male flower of Juglans regia L. Int J Food Prop 2018;21:345-56.
42. Wang D, Mu Y, Dong H, Yan H, Hao C, Wang X, et al. Chemical constituents of the ethyl acetate extract from diaphragma juglandis fructus and their inhibitory activity on nitric oxide production in vitro. Molecules 2017;23:72.
43. Meng Q, Li Y, Xiao T, Zhang L, Xu D. Antioxidant and antibacterial activities of polysaccharides isolated and purified from Diaphragma juglandis fructus. Int J Biol Macromol 2017;105:431-7.
44. Ghiravani Z, Hassanzadeh-Taheri M, Hassanzadeh-Taheri M, Hosseini M. Internal septum of walnut kernel: a neglected functional food. Res J Pharmacogn 2020;7:81-92.
45. Li H, Horke S, Förstermann U. Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci 2013;34:313-9.
46. Okunade AL, Elvin-Lewis MPF, Lewis WH. Natural antimycobacterial metabolites: current status. Phytochemistry 2004;65:1017-32.
47. Zoral FB, Turgay Ö. [A research on total phenolic content, antioxidant activity and antimicrobial effects of various food wastes]. KSÜ Doğa Bilim Derg 2014;17:24-33. [Article in Turkish]
48. Kavak DD, Altiok E, Bayraktar O, Ülkü S. Pistacia terebinthus extract: as a potential antioxidant, antimicrobial and possible β-glucuronidase inhibitor. J Mol Catal B Enzym 2010;64:167-71.
49. Acquaviva R, D'Angeli F, Malfa GA, Ronsisvalle S, Garozzo A, Stivala A, et al. Antibacterial and anti-biofilm activities of walnut pellicle extract (Juglans regia L.) against coagulase-negative staphylococci. Nat Prod Res 2021;35:2076-81.

50. Genovese C, Cambria MT, D'Angeli F, Addamo AP, Malfa GA, Siracusa L, et al. The double effect of walnut septum extract (Juglans regia L.) counteracts A172 glioblastoma cell survival and bacterial growth. Int J Oncol 2020;57:1129-44.

51. Rusu ME, Fizesan I, Pop A, Mocan A, Gheldiu AM, Babota M, et al. Walnut (Juglans regia L.) septum: assessment of bioactive molecules and in vitro biological effects. Molecules 2020;25:2187.