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Research Article

Antibacterial and antioxidant activity of Black, Red, and Chinese cultivars of *Raphanus sativus*

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ABSTRACT

Our study sought to ascertain the biological activities of three distinct radish cultivars: Black, Red, and Chinese. The materials were subjected to lyophilisation for 72 hours, after which ethanolic extracts were obtained via the Soxhlet method. The antioxidant activity of the ethanolic extracts was assessed using the DPPH scavenging assay and the ferric reducing assay. At the same time, the total phenolic content was quantified by the Folin-Ciocalteu method. The antibacterial efficacy of the extracts against the test bacteria was assessed via the disc diffusion method. The findings revealed that Chinese radish exhibited the highest phenolic concentration, quantified at 18.1 mg GAE/g. The DPPH scavenging assay results indicated that, concerning the total phenolic content, the maximum scavenging activity was seen in Chinese radish at 72%. Subsequently, black radish exhibited a DPPH scavenging rate of 59%, while red radish demonstrated a rate of 39%. The ferric reducing power results corroborated the DPPH scavenging assay, indicating the activity hierarchy as Chinese radish, black radish, and red radish, respectively. Antibacterial activity of the ethanolic extracts obtained was also examined; according to the results, only the black radish extract showed antibacterial action against *Enterococcus faecalis* (13.5 mm) and *Staphylococcus aureus* (11 mm). This study revealed the effects of different radish cultivars on antioxidant and antibacterial activity.

Keywords: Antibacterial activity, DPPH scavenging, Food, Radish

Introduction

People have been using a variety of plants and their derivatives for medical purposes, such as treating infectious diseases, since ancient times. (Gorlenko et al., 2020). Numerous phytochemicals that are crucial to human health are present in them (Kaymak et al., 2018). Cures derived from plants are used in various traditional healing practices to treat many diseases, including bacterial infections. In addition, several medicines currently on the market were first developed in their raw form in folk medicine (Bilal et al., 2017; Li et al., 2020).

The most widely used root vegetable in the Brassicaceae family, radishes are cultivated and eaten all around the world. While other radish types vary in size, colour, and cultivation requirements, the popular red cultivars are the most commonly grown (Shin et al., 2015).

Different components of the radish are used for different therapeutic purposes in traditional medicine. For example, the juice from the leaves is used as a laxative and diuretic, and the roots are used to treat syphilis and relieve bladder pain (Faiyaz Ahmad et al., 2012). Numerous important characteristics and roles of *R. sativus* leaves and roots have been highlighted by different studies. These include their antibacterial qualities, antioxidant advantages, ability to prevent mutations, and ability to combat cancer (Noman et al., 2021). Numerous phytoconstituent types, such as flavonoids, phenols, and alkaloids, are thought to be responsible for the biological activity of radish (Shin et al., 2015).

The utilisation of radish in traditional medicine for treating many viral disorders has generated considerable curiosity in investigating its antibacterial characteristics (Faiyaz Ahmad et al., 2012; Jadoun et al., 2016). Studies have demonstrated that extracts derived from various components of the radish plant can successfully impede the growth of a wide range of bacterial strains, even those that are resistant to pharmaceutical medications. Radish roots and seeds have been found to inhibit the growth of various bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Pseudomonas pyocyaneus, Salmonella typhi, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Salmonella Enteritidis, Cronobacter sakazakii, Bacillus cereus* and *Enterococcus faecalis* (Faiyaz Ahmad et al., 2012; Jadoun et al., 2016; Lim et al., 2019). Phytochemicals such as phenolics, flavonoids, alkaloids, tannins, terpenes, and terpenoids can serve as antimicrobial and antioxidant (Muthusamy and Shanmugam, 2020).

Antioxidants are becoming more and more popular because of their ability to shield the body from diseases linked to oxidative stress and to stop oxidative deterioration in food and pharmaceutical items (Gulcin, 2020). Antioxidants are distinguished by their capacity to block singlet oxygen, donate hydrogen, serve as reducing agents, form complexes with metals, and neutralise free radicals. Antioxidants derived from plants help counteract the impacts of free radicals, which helps shield the body from several illnesses (Narayanaswamy and Balakrishnan 2013).

Our work sought to assess the antioxidant activity (employing two distinct methods: DPPH from three radish varieties (black, red, and Chinese), utilising the Soxhlet method. The antibacterial efficacy of the produced extracts was evaluated against *Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus,* and *Salmonella Typhimurium.*

Materials and Methods

Materials

Different varieties of radishes (with red exteriors, red interiors, and black radishes) were obtained from local marketplaces in Sakarya and subsequently processed according to the forthcoming processes for extract preparation. The bacterial strains *Staphylococcus epidermidis* ATCC 12228*, Bacillus subtilis* ATCC 6633*, Escherichia coli* ATCC 8739*, Enterococcus faecalis* ATCC 29212*, Staphylococcus aureus* ATCC 29213, *and Salmonella Typhimurium A*TCC 14028 were obtained from the Microbiology Laboratory at Sakarya University's Institute of Science, Department of Biology.

Plant Material and Preparation of Plant Extracts

The washed and cleaned samples were chopped and freeze-dried for 72 hours using a lyophilizer. 10 grams of sample ground into powder in an electric grinder were extracted in a Soxhlet apparatus (Semerci et al., 2024). 70% ethanol was used as a solvent. The obtained extracts were filtered and then freed from solvents using a rotary evaporator at 55 °C for approximately 15 min. The obtained extracts were then dissolved in their solvents and prepared as stock solutions at a concentration of 1 mg/mL.

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu protocol (Singleton & Rossi, 1965) was employed to quantify the total phenolic content. The experimental protocol involved the combination of 100 μL of an ethanolic extract (1000 µg/mL) with 200 µL of a 50% Folin-Ciocalteu reagent, followed by a 2-minute exposure period. Next, 1 mL of a 2% Na_2CO_3 solution was introduced, and the mixture was vigorously agitated. The mixture was thereafter kept in the dark for 1 hour. Absorbance was quantified at 760 nm using a spectrophotometer (Shimadzu UV mini-1240). The total phenolic content was measured by cross-referencing a calibration curve generated from a sequence of gallic acid standards with concentrations of 5, 10, 20, 30, and 40 mg/L. The results were quantified in mg of GAE per 100 g. The experiment was performed in three replicates.

Antioxidant Activity

DPPH Assay

The antioxidant activity was evaluated by a modified iteration of the Blois experimental procedure (Blois, 1958). This procedure involved combining 1 mL of a 0.004% DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical solution in ethanol with 1 mL of the extract solution, which was likewise in ethanol. After 30 minutes of storage in the absence of light, the mixes were subjected to optical density measurement at 517 nm using a spectrophotometer. Ethanol served as a reference substance throughout the testing process. The percentage of inhibition shown by the samples was determined using the following formula.

$$\%\text{Inhibition} = \frac{Acontrol - Asample}{Acontrol} \times 100$$

Reducing Power

The reduction potential of plant extracts derived from ethanol was assessed using the methodology described by Semerci et al. (2024). A total of 1200 µL of a 0.2 M phosphate buffer with a pH of 6.6, together with 1% potassium ferricyanide $[K_3Fe(CN)_6]$, were added to each 500 µL extract. The quantities of these substances varied between 20 and 100 micrograms per unit volume. The combination was afterwards maintained at a standard temperature of 50°C for 20 minutes. Subsequently, 1250 µL of a 10% trichloroacetic acid solution was introduced, and the mixture was vortexed at 2500 revolutions per minute for 10 minutes. Following the extraction of 500 microliters of the supernatant from the combination, 1250 µL of distilled water and 250 µL of a 1% (w/v) $FeCl_3.6H_2O$ solution were added. The absorbance of the final solution was measured at a wavelength of 700 nm using a UV-Vis spectrophotometer, with pure water serving as the reference source.

Disc Diffusion Method

30 µL of extracts (1 mg/mL), each prepared at varying concentrations, were applied to 6 mm diameter sterile discs using a micropipette. Bacterial suspensions with a 0.5 McFarland density were made from fresh 24-hour cultures of bacteria (*Staphylococcus epidermidis ATCC 12228, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 8739, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 29213, and Salmonella Typhimurium ATCC 14028*). These suspensions were inoculated onto Mueller-Hinton agar plates with sterile swabs, and the discs soaked in the extracts were placed on the agar. The Petri dishes containing the agar and discs were incubated at 37°C for 24 hours (Baran et al., 2024). After incubation, the inhibition zones were measured using a digital calliper.

Results and Discussion

Numerous studies have investigated the health effects of eating cruciferous vegetables in recent years. Because these veggies contain large amounts of phenolic compounds, such as tocopherols, carotenoids, and ascorbic acid, research indicates that they are a valuable natural source of antioxidants (Goyeneche et al.,2015). Most significantly, their capacity to scavenge free radicals or stop low-density lipoproteins from oxidising is what gives them their antioxidant properties. Due to their ability to prevent disease and promote health, polyphenolic substances have gained attention in the fields of nutrition and medicine today (Beevi et al., 2011). The phenolic content of three distinct radish cultivars was assessed in our study; the findings are shown in Figure 1. Chinese radish had the highest phenolic content with 18.1 mg GAE/g. Following it were the Black radish (12.3 mg GAE/g) and the Red radish (14.6 mg GAE/g).

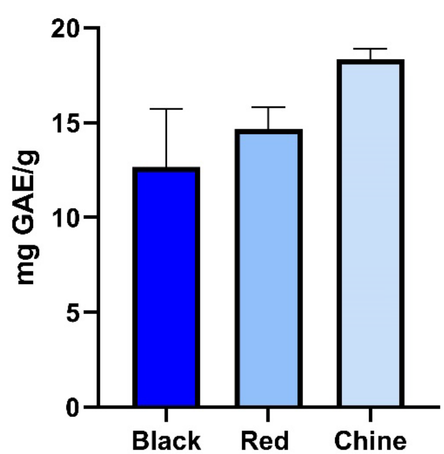

Figure 1. Total Phenolic Content of Radish Samples

According to a Romanian study on the total phenolic content of various radish cultivars, the black radish has 13.7 mg GAE/g of total phenolics, whereas the red radish has 12.9 mg GAE/g (Bors et al., 2015). Another investigation found a correlation between the solvent's polarity and the extraction yield (Semerci et al., 2024). Different extraction solvents produced varying amounts of phenolic content; water extraction produced the highest yield, while hexane extraction produced the lowest. It has been demonstrated in the literature that several variables, including extraction temperature, solvent type, and solvent concentration, influence phenolic extraction (Razali et al., 2008; Beevi et al., 2011). Polar solvents were used in comparable investigations to extract the most common polyphenols (Ghaffar & Perveen, 2024; Semerci et al., 2024). In our study, it was found that the phenolic content of the extracts obtained with aqueous ethanol solvent was higher than the values reported in the literature.

Numerous bioactive substances with potent antioxidant properties can be found in plants. The value of various plant species as sources of novel antioxidant chemicals might be highlighted by studies that try to ascertain their antioxidant activity (Chaves et al., 2020; Eruygur et al., 2024). In our study, the antioxidant activity results evaluated using two different extraction methods (DPPH scavenging test and iron reduction) are presented in Figure 2 and Figure 3. The highest DPPH scavenging rates were observed in the Chinese radish (72% at 1000 µg/mL), followed by the Black radish (59% at 1000 µg/mL) and the Red radish (39% at 1000 µg/mL). In a study evaluating the antioxidant activity of four different radish species, the DPPH radical scavenging activity in the radish extracts was found to be 30.02 ±3.70 µg/g GAE for Chinese radish, 26.37 ±2.27 µg/g GAE for Black radish, 19.18 ±1.57 µg/g GAE for Kohlrabi radish, and 39.68 ±2.43 µg/g GAE for Japanese radish (Tiraş et al., 2024). A further investigation indicated that the Valentine ethanol extract at 800 µg/mL exhibited 18.71 ±0.58% DPPH activity, whereas Cherry Belle had 15.43 ±1.25% DPPH activity (Kim et al., 2016). The existence of multiple methodologies for assessing antioxidant activity complicates comparisons, resulting in disparate findings among research in the literature. The dry matter and other constituents in radishes can fluctuate based on factors like cultivation settings, climatic factors, maturation circumstances, harvest timing, and genotype (Solmaz, 2017).

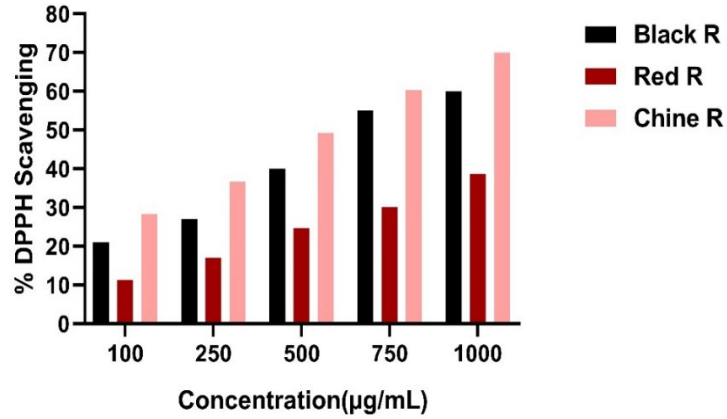

Figure 2. DPPH scavenging activity of radish extracts at different concentrations

Research indicates that radish roots possess chemicals including vanillin, pyrogallol, gallic acid, coumaric acid, caffeic acid, and trans-ferulic acid. Moreover, it has been indicated that the free hydroxyl groups in phenolic substances are chiefly accountable for their antioxidant efficacy (Zhang et al., 2014). The pronounced DPPH radical scavenging activity demonstrated by *R. sativus* extracts can be attributed to the presence of polyphenols, which have been documented to possess radical scavenging capabilities in several model systems (Beevi et al, 2011; Zrouri et al., 2021).

The DPPH scavenging activity showed similar characteristics to the reducing power of the plant extract. An increase in iron reduction rates was observed in all three cultivars with the increase in extract concentration (Figure 3). The results obtained from this study suggest that the significant reducing ability of different radish varieties is a result of their antioxidant activities. Therefore, it can be assumed that the polyphenols in radishes may act as reductants by donating electrons to free radicals and terminating free radical-mediated chain reactions.

In a study, radish leaves and stems were reported to have iron reducing power in the range of 1.68-2.83 mM/g of water, methanol and acetone (Beevi et al., 2010). In another study, it was observed that root extracts at a concentration of 250 µg/mL showed a significant ability to reduce ferric ions and the reducing power of root extracts varied between 0.417 nm and 0.651 nm (Beevi, et al., 2011) *R. sativus* species have been shown in our study and in literature studies to be able to chelate iron ions in a concentration-dependent manner.

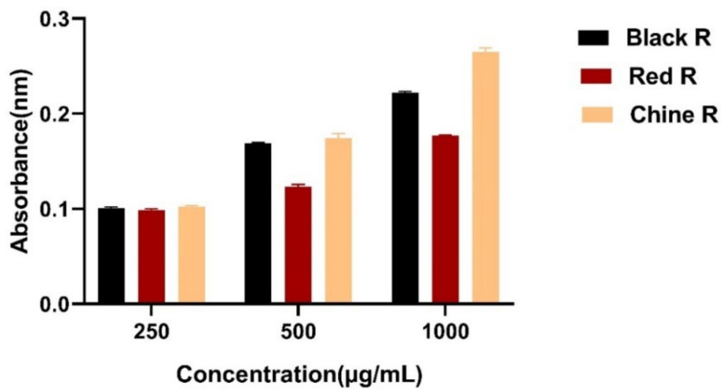

Figure 3. The iron reduction rate of radish extracts: Results are means ± standard deviation of three parallel measurements

The application of radish in traditional medicine for the treatment of numerous infectious disorders has generated considerable interest in investigating its antibacterial properties. Numerous studies have shown that extracts from various plant components can prevent the growth of a diverse array of bacterial strains, including those resistant to drugs (Janjua et al., 2013; Jadoun et al., 2016). The antibacterial efficacy of ethanolic extracts from three distinct radish cultivars examined in this study against test bacteria is illustrated in Table 1—only the black radish showed antibacterial action. In comparison to the positive control, the antibiotic gentamicin, black radish had moderate antibacterial activity against *E. faecalis* and *S. aureus*. Methanol extracts from the taproots of white and black radishes have been reported to exhibit the ability to inhibit a variety of foodborne pathogens such as *Arthrobacter atrocyaneus, Corynebacterium ammoniagenes, Enterobacter hormaechei, Kocuria rosea, Neisseria subflava, Pantoea agglomerans, Proteus vulgaris, Psychrobacter immobilis, Shigella dysenteriae, Bacillus sphaericus*, and *Corynebacterium flavescens* (Umamaheswari et al., 2012; Lim et al., 2019). Jamuna et al. (2015) evaluated the antioxidant and antibacterial properties of radish root by applying different extraction methods. In their studies, they found that cold extraction of fresh radish root was better than dried cold extraction or Soxhlet in biological activity studies. It was observed that the extraction method and the solvent used may cause differences in antibacterial activity.

Alcoholic extract of radish leaves has been reported to effectively inhibit a variety of bacteria, including *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli,* as well as the yeast *Candida albicans* (Jaafar et al., 2020). In another study, it was reported that radish ethanolic extract created an inhibition zone diameter of 23 mm in *E. coli*, 22 mm in *S. aureus* and 22 mm in *S. epidermidis* (Mohamed et al., 2024). Our findings regarding antibacterial activity are partially aligned with the existing literature. Black radish extracts exhibited modest antibacterial efficacy against *S. aureu*s and *E. faecalis*, corroborating observations that methanol extracts of radish roots inhibit many foodborne pathogens. Our investigation did not detect significant antibacterial activity in red or Chinese radish extracts, indicating potential variations in their phytochemical make-up.

Table 1. shows the measurement of the inhibitory effect of solutions on different types of bacteria.

Extracts	Test bacteria (Inhibition Zones (mm))					
	Ec	Sa	Se	St	Ef	Bc
Red	0	0	0	0	0	0
Black	0	11 ±0.3	0	0	13.5 ±0.7	0
Chine	0	0	0	0	0	0
Gentamicin	18 ±0.1	21 ±0.1	21 ±0.1	22 ±0.1	19 ±0.1	21 ±0.1
N.control	0	0	0	0	0	0

Ec-*Escherichia coli*, Sa-*Staphylococcus aureus*, Se-*Staphylococcus epidermidis*, St-*Salmonella Typhimurium*, Ef-*Enterecoccus faecalis*, Bs-*Bacillus subtilis*

Other authors have confirmed that radish species prevent the growth of different bacteria. The antibacterial mechanism of plant extracts or specific phytochemicals, like those from *R. sativus* L., can be complex. These chemicals may engage with the microbial cell membrane, enhancing its permeability and altering cellular metabolism. Moreover, they can inhibit the intake and assimilation of vital nutrients necessary for the appropriate reproduction and growth of bacteria. Injury to the microbial cell membrane can alter permeability, causing disruption of multiple processes within bacterial cells (Jaafar et al., 2021; Ziemlewska et al., 2024).

Conclusion

In our study, the antioxidant activity of three different radish cultivars was evaluated; the iron reducing power results confirmed the DPPH scavenging experiment. Antioxidant activity was determined in both analyses as Chinese radish, black radish and red radish, respectively. Among the prepared ethanolic radish extracts, only black radish extract showed antibacterial activity against *Enterococcus faecalis* and *Staphylococcus aureus*. The results obtained in this study revealed the potent antioxidant properties of Chinese radish and the antibacterial activity of black radish. It shows that radishes can be used as a promising source of bioactive natural ingredients in the food sector.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare that they have no actual, potential, or perceived conflicts of interest related to this article.

Ethics committee approval: The authors declare that this study does not involve experiments with human or animal subjects, and therefore, ethics committee approval is not required.

Data availability: Data will be made available at the request of the author(s).

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Disclosure: -

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