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Antibacterial and antioxidant activity of some seeds used as food

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ABSTRACT

In this study, antioxidant and antibacterial activities of *Brassica nigra*, *Linum usitatissimum*, *Salvia hispanica*, *Nigella sativa*, *Chenopodium quinoa* seeds prepared with ethanol were determined. Antioxidant capacity has been measure by evaluating DPPH radical scavenging activity level and antibacterial activities were investigated by using the disc diffusion method. Highest antibacterial activity level has been detected for the seed of *N. sativa*. As a result of this study there exist no any antibacterial effect in the seeds of *B. nigra* and *L. usitatissimum*. The extract of *N. sativa* seed has shown an inhibition zone of 14.5 mm on *Staphylococcus aureus*, whereas this value has been detected to be 10.5 mm for both on the seeds of *Enterococcus faecalis* and *Staphylococcus epidermidis*. IC_{50} value showing the 50% scavenging value of DPPH radical has been determined to be 51 µg/mL for the seed extrafct of *B. nigra* as the highest one between the all seed samples searched. All seeds used in the current study which are known to be functional food have shown certain level of antioxidant activity.

Keywords: Antioxidant, Antibacterial, Seed

Introduction

Today, about 40000 plant species are known, some of which are collected from the nature, while others are cultured and produced. A significant part of the plants used for health treatments are collected from the nature and these plants are named as medicinal plants. It is well known for the medicinal plants that they have been used for many similar purposes such as food, medicine, cosmetics and spices since the beginning of human history (Acıbuca and Bostan Budak, 2018; Semerci et. al., 2020).

Medicinal plants and some spices contain highly useful phytochemical and antioxidant properties due to various chemicals contained in them (Virendra et. al., 2013). Mustard with antioxidant properties makes the skin look brighter and more vivid. The oil created from the seed is used to relieve the pain in the various parts of the human body. The oil obtained from mustard seeds cannot be used for nutritional purposes due to its fatty acids, especially due to its high amount of erucic acid, but it can be used for different purposes in the pharmaceutical and cosmetic industries (Gıdık, 2016).

L. usitatissimum is often called "functional food", "bioactive food" and "endocrine active food" and its nutrient value together with its protective properties originate from its distinct ingredients (İşleroğlu et.al., 2005). Flax or flax seed are among the oldest crops grown for oil and fiber (Jhala and Hall, 2010). Flax plant contains essential fatty acids, omega-3 and 6 fatty acids, linoleic acid, alpha linolenic acid (ALA) necessary for health protection. It is also known as a good source of antioxidants (Üstü and Keskin, 2019).

S. hispanica is classified as functional food due to essential nutrients in its structure. It has a protective effect against cardiovascular diseases, nervous system disorders, inflammatory and diabetes (Ergene and Bingöl, 2019). As a natural source of chia seeds n-3 fatty acids, it is effective in lowering blood triglycerides and regulating blood cholesterol levels thanks to its β-sterol content.

N. sativa has properties such as repairing cell damages that may occur in the human body. It has also immune stimulating, anti-inflammatory, anticancer, antioxidant, antiastmatic, hypoglycemic, antimicrobial and antiparasitic characteristics (Al Ali et.al., 2008).

C. quinoa seeds are rich in nutrients. It is a good source of minerals such as protein, calcium, iron and vitamins E and B. All 8 essential amino acids necessary for tissue development in humans are found in the seed of this plant (Tan and Yöndem, 2013).

In this study, it is aimed to clarify the antibacterial and antioxidant activities of *B. nigra*, *L. usitatissimum*, *S. hispanica*, *N. sativa*, *C. quinoa* seeds.

Materials and Methods

Seeds of *B. nigra* L., *L. usitatissimum* L., *S. hispanica* L., *N. sativa* L., *C. quinoa* Wild. used in the research were taken from the herbalist in Sakarya province. Plant seeds taken from the herbalist were crushed in an electric grinder and powdered.

Preparation of Herbal Extracts

Extracts were prepared by weighing the ground seed samples to 10 g and adding them separately into 100 mL ethanol (Tunç et.al., 2019). These prepared extracts are kept in a cool and dark environment for 3 days and mixed in a magnetic mixer at regular intervals. Extracts were prepared according to the maceration method. At the end of the process, the extracts were filtered through filter paper and the solvents in the extract were removed with a rotary evaporator. After these processes, the extracts were prepared at the determined concentration (6400 µg/disc) by adding solvents that used in the extraction process. Of the raw extracts obtained, empty sterile discs with the radius of 6 mm were absorbed 10 µL and kept in a dark sterile environment for 24 hours.

Supply of Bacterial Strains

The microorganisms used in the study were obtained from the strain collection of Sakarya University, Faculty of Arts and Sciences, Department of Biology, Microbiology Research Laboratory. *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228 bacteria were used in the study.

Disc Diffusion Method

Antibacterial activity of extracts was determined using the disc diffusion method. The concentration of the previously activated strains was adjusted to an average of 0.5 McFarland overnight and Müeller Hinton Agar was cultivated with a sterile swab. Extracts impregnated discs with the pliers were placed in Müeller Hinton Agar in aseptic conditions where bacteria were cultivated. They were incubated at 37 ° C for 24 hours. If there is an inhibition zone against that pathogen around the disc as a result of the incubation, the zone diameters (mm) have been measured from the back of the petri by using a digital caliper.

All studies were carried out in triplicate. Gentamicin was used as the positive control and ethanol as the negative one.

Determination of Antioxidant Activity

For example, antioxidant activity was investigated by modified DPPH free radical scavenging method (Blois, 1958). After taking 1 mL of standart solution and ectracts prepared in various concentrations we have added a DPPH solution of 1 mL with 0.04% concentration. After vortexing, they were left in a dark place for 30 mins and their absorbance have been measere at 517 nm. The results have been attained by evaluating the DPPH% radical scavenging activity and IC_{50} value. During the experiments DPPH and ethanol together with ascorbic acid as standart have been used:

DPPH% scavenging activity= 100 x (Absorbance of control-Aborbance of sample)/Absorbance of control

The regression value of the extracts prepared at different concentrations was calculated with the results obtained from the% DPPH scavennging activity. IC_{50} ratios were determined from this equation.

Results and Discussion

To our knowledge this is the first study demonstrating both the antibacterial and antioxidant activities at the same time for five functional foods, namely *B. nigra*, *L. usitatissimum*, *S. hispanica*, *N. Sativa*, *C. quinoa*.

Inhibition zone diameter measurements of extracts prepared from *B. nigra*, *L. usitatissimum, S. hispanica*, *N. Sativa*, *C. quinoa* plants against test microorganisms are given in Table 1.

While *B. nigra* and *L. usitatissimum* seed extracts do not form inhibition zone diameters, *S. hispanica* seed extract has been determined to form an 8 mm inhibition zone diameter on *S. epidermidis* and 9 mm on *S. aureus*.

N. sativa seed extract has shown an inhibition zone diameter of 10.5 mm on *E. faecalis* and *S. epidermidis*, and 14.5 mm on *S. aureus. C. quinoa* seed extract produced 9.25 mm inhibition zone diameter only on *S. aureus.*

In a study, it was reported that black seed extract prepared with diethylether was observed on gram positive bacteria (*S. aureus*) while there was no antibacterial activity on gram negative bacteria (*E. coli*) (Mohammed and Mohammed, 2000).

Gerige et. al. (2009) examined the antimicrobial activity of the oils obtained from the seeds of black seed and determined that this oil formed a 18 mm *B. subtilis* 10 mm inhibition zone diameter on *S. aureus*.

In this study, while *N. sativa* seed extract was determined to have 14.5 mm inhibition zone diameter on *S. aureus*, no antibacterial activity was observed on *B. subtilis*. This is thought to be due to differences in extract preparation methods or differences in growing site.

In the *S. hispanica* study, many bacteria were studied and the effect of Chia seed on them was investigated.

Table 1. Inhibition zone diameters of seed extracts on test bacteria

Plant extracts (6400 µg/disc)	TEST MICROORGANISMS Inhibition Zone Diameter (mm)				
	E. coli	*B. subtilis*	*E. faecalis*	*S.epidermidis*	*S. aureus*
B. nigra	0	0	0	0	0
L. usitatissimum	0	0	0	0	0
S. hispanica	0	0	0	8 ±0	9 ±0
N. sativa	0	0	10.5 ±0.7	10.5±0.7	14.5 ±3.3
C. quinoa	0	0	0	0	9.25 ±0.3
Gentamicin	19	17	20	21	20

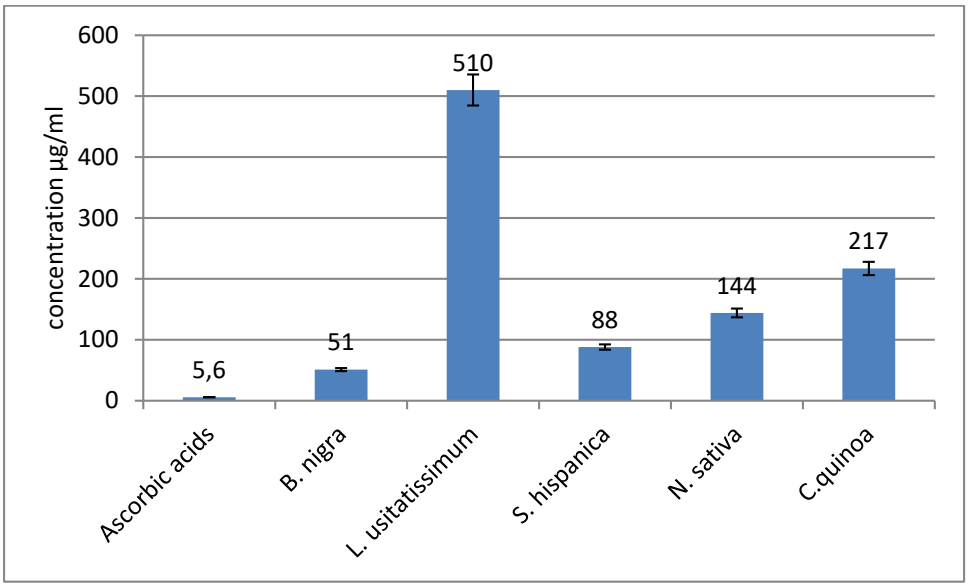

Figure 1. IC_{50} values measured for the seed extracts and ascorbic acid.

Thus, the ability to scavenge free radicals is an important antioxidant property in minimizing oxidative cellular damage. In a series of *in vitro* tests the essential oils from spices and medicinal plants have exhibited remarkable antioxidant activity (El-Baroty et al., 2010).

IC_{50} value is expressed as the amount of exract inhibiting the radical concentration to a degree of 50%. The lower value of IC_{50} shows that the antioxidant level of the plant is higher. The highest DPPH antioxidant activity level for the current study has been measured for the *B. nigra* seed extract, whereas the lowest one detected for the *L. usitatissimum* seed extract. IC_{50} values for the seed extract are shown in Figure 1.

In a work done on the seed of *N. sativa* collected from the Koycegiz (Muğla), its seed oil constituents and anitoxidant activity level have been evaluated and IC_{50} value has been detected to be 52.61 µg/mL using scavenging activity of DPPH radical (Sıcak and Erdoğan, 2019).

In another work done for the comparison of the antioxidant and antimicrobial activities of *C. quinoa* seed grown in Korea, America and Peru, it has been found that the IC_{50} values determined by using the DPPH radical scavenging activity was 250 µg/mL in Korea, whereas it was detected to be 260 µg/mL and 470 µg/mL in America and Peru, respectively (Park et.al., 2017). The results of the current study are in good accordance with that study, even though the results may show slight changes owing to the collection area of the samples.

IC_{50} value of DPPH radical scavenging activity for the seed of *B. nigra* has been detected to be 63.09 µg/mL (Alam et.al., 2011). *B. nigra* seed extract prepared with ethanol has shown IC_{50} value of 71.59 µg/mL in work done by (Krishnan-Radha et.al., 2015). In the current study we have measured IC_{50} value to be 51 µg/mL, which are found to be compare well with the other work done by several researchers.

Anwar and Przybylski have studied the antioxidant activity of *L. usitatissimum* and found that for the extracts prepared in different solvent and concentration levels there existed some differences in the activity level. For instance, for the 100% methanol concentration level it has been detected to be 83.6, whereas it is 81.3 for 80% for the methanol and 42.2, 100% for the ethanol concentrations The IC_{50} value for *L. usitatissimum* has been obtained to be 510 µg/mL for the current study.

Conclusions

As a result, the highest microbial activity prepared out of *B. nigra*, *L. usitatissimum, S. hispanica*, *N. sativa*, *C. quinoa* seed extracts has been found in *N. sativa.* Antibacterial activity level order is as follows *N. sativa*, *S. hispanica*, *C. quinoa*. The highest antioxidant activity level has been detected for the seed of *B. nigra*. The antioxidant activity level order is as follows: *B. nigra*, *S. hispanica*, *N. Sativa*, *C. quinoa*, *L. usitatissimum.*

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

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Disclosure: -

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