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RESEARCH ARTICLE

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Evaluation of antibacterial activity of *Triticum monococcum* seeds, *Castanea sativa* seeds and *Begonia maculata* leaves against several bacterial strains

Triticum monococcum tohumu, *Castanea sativa* tohumu ve *Begonia maculata* yapraklarının antibakteriyel aktivitesinin çeşitli bakteri türlerine karşı değerlendirilmesi

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ABSTRACT

There are serious research projects to prevent the bacteria to antibiotics. Thus, scientists are looking for new antibiotic derivatives using plants. Einkorn wheat seeds (*Triticum monococcum*), Chestnut seed (*Castanea sativa*) and *Begonia* leaves were used for antibacterial tests on bacterial strain. The study of antibacterial effects of Einkorn wheat seed and begonia leaves has not been seen in the literature. The antimicrobial effects of these plant leaves and seeds were investigated in Muller-Hilton media at 37 C° for 48 hours. After incubation, all plates were observed for zones of growth inhibition. The parameters of these zones were measured in millimeters. All tests were performed under sterile conditions and repeated three times. It was observed that the wheat and chestnut seeds had no antibacterial effect against *Staphylococcus aureus* bacteria. However, they have been found to have a significant antibacterial effect against *Escherichia coli* bacteria. Furthermore, antimicrobial effects of *Begonia maculata* leaves against *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeroginosa* bacteria were investigated. *E. coli* bacteria were affected by the leaves more than the other bacteria. *K. pneumoniae* bacteria were affected less while *P. aeruginosa* bacteria were affected the least.

ÖZ

Bakterilerin antibiyotiklere direncini önlemek için ciddi araştırmalar var ve bilim insanları, bitkileri ve doğayı kullanarak yeni antibiyotik türevleri aramaktadırlar. Siyez buğdayı tohumları (*Triticum monococcum*), kestane meyvesi (*Castanea sativa*) ve begonya yaprakları bazı bakteri türlerine karşı antibakteriyel test için kullanılmışlardır. Siyez buğday tohumu ve Begonya yapraklarının antibakteriyel etkileriyle ilgili çalışma literatürde görülememiştir. Bu bitki yaprak ve tohumlarının antimikrobiyal etkisi Muller-Hilton besiyerinde 37 C° sıcaklıkta 48 saat süreyle incelenmiştir. İnkübasyondan sonra, tüm plakalar büyüme inhibisyon bölgeleri için gözlendi. Bu bölgelerin parametreleri milimetre cinsinden ölçüldü. Tüm testler, steril koşullar altında gerçekleştirildi ve üç kez tekrarlandı. Bu çalışmada, buğday ve kestane tohumlarının *Staphylococcus aureus* bakterisine karşı antibakteriyel etkisi olmadığı görülmüştür. Ancak siyez buğdayı ve kestane tohumlarının, *Escherichia coli* baktesine karşı önemli bir antibakteriyel etkiye sahip olduğu bulunmuştur. Buna ilaveten; *Begonia maculata* bitki yapraklarının *E. coli, Klebsiella pneumoniae* ve *Pseudomonas aeroginosa* bakterilerine karşı antimikrobiyal etkileri incelendi. Özellikle *E. coli* bakterileri her iki yaprak çeşidinden diğer bakterilere göre daha fazla etkilemiştir.

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1. INTRODUCTION

Bacteria can improve resistance to antibiotics that are used in treatment of disease. New antimicrobial compounds are needed to be used against diseases and bacterial infections. Natural antimicrobial agents have been developed to prevent bacterial diseases and to control bacterial resistance to antibiotics (Shetty & Lin, 2005). WHO stated that antibiotic resistance is the biggest threat to global health and food safety and can affect everyone (WHO, 2018). In addition to this, CDC (*Centers for Disease Control and Prevention*) estimates that antibiotic-resistant bacteria in the United States (CDC, 2015) cause at least two million diseases and

23,000 deaths each year. Plants containing numerous components are valuable sources with antimicrobial properties that are important for drug design against diseases (Das et al., 2010; Bhattacharjee & Islam, 2015).

Wheat (*Triticum* spp.), a member of the Poaceae family, is an ancient grain whose origin is South-West Asia. It is also one of the world's leading cereal products (Fao, 2019). Moreover, it is known for its highly effective medicinal values and various therapeutic drugs that provide protection against a variety of diseases such as obesity, diabetes, heart disease, colon diseases, and appendicitis (Hadjivassiliou et al., 2003). Whole wheat contains phytochemicals such as phenolic, carotenoids, lignans and vitamin E, which have beneficial effects on health (Berger et al., 2005).

Chestnut seed is one of the traditional foods of the Turks. It is a seed that has been consumed lovingly since ancient times. All type of chestnut is native to the northern hemisphere. They are members of Fagaceae. Chestnut seed is an annual plant. There are four main economic species of chestnut: *Castanea dentata* (Marshall) Borkh., *C. mollissima* Blume (Chinese), *C. crenata* Siebold & Zucc. (Japanese), and *C. sativa* Mill. (European) (Ertürk et al., 2006; Burnham, 1988). The bark, leaves, and flowers of chestnut tree was first discovered in Ancient Roman times for a high potential of drug production, not just for producing seed. The chestnut contains different amounts of Ca, P, K, Mg, S, Fe, Cu, Zn and Mn (Pereira-Lorenzo et al., 2006; Borges et al., 2008; Er et al, 2013). Besides, it has been shown to include compounds such as PUFA, tocopherols, L-ascorbic acid, carotenoids, polyphenols, and vitamin E with beneficial health effects (Simopoulos, 1991; Barreira et al., 2009; Desmaison & Adrian, 1986; Neri et al., 2010; Ribeiro et al., 2007).

The genus *Begoniae* has approximately 1400 species distributed primarily in the tropical and subtropical areas of the world (Clement et al., 2004). Begonia is now considered one of the five largest species of vascular plants. It is widely spread in the rainforest, in damp areas and near the waterways (Hoover et al., 2004). The plants exhibit a wide range of colors, shapes and patterns that are rarely seen in other plants (Sheue et al., 2012). Nowadays, many varieties of this ornamental plant are grown, however, summer and autumn blooming tuber species are more popular (Hessayon, 1980). Many begonias are cultivated as a popular ornamental plant and more than 10000 begonia hybrid species were reported (Awal et al., 2008). There are also studies on the use of this genus in the treatment of diabetes. *Begonia malabarica* Lam. (Begoniaceae) was used as a diabetes medicine by the Malasar clan in the Indian (Pandikumar et al., 2007) and Pandikumar et al. (2009) confirm the traditional use of *Begonia malabarica* by the Malasar tribe in the treatment of diabetes.

Bacteria can increase the resistance to antibiotics used in the treatment of the disease. Therefore, new antimicrobial compounds are needed to prevent bacterial infections. Natural antimicrobial agents have been developed to treat bacterial diseases and to control bacterial resistance to antibiotics (Shetty and Lin, 2005). Therefore, alternative antibiotic sources are sought from different plant parts. The research on bacterial resistance should seek continuation as bacteria improve the protection. This study was conducted to investigate the cheaper and more natural antibiotic sources of bacteria to prevent resistance against antibiotics.

2. MATERIAL AND METHOD

2.1. Seeds and leaves extraction

Einkorn wheat seeds (*Triticum monococcum* L.) (Figure 1) were collected from the city of Ihsangazi Kastamonu and Chestnut seed (*Castanea sativa* Mill.) (Figure 1) were collected from the city of Sinop. The seeds were extracted in the wet state. The seeds are first powdered with the blender and 10 g of the sample are extracted with 50 ml of chloroform with shaking at 150 rpm for 3 days at room temperature. Then specimens passed through 0.45 µm teflon filters. The resulting liquid was stored at + 4 ° C until ading Muller Hilton medium.

Begonia maculata Raddi plant leaves (Figure 1) were used in this study and the plant obtained from Istanbul. The leaves were extracted in the wet state. 1 gram of *B. maculata* leaves extracted for 1 hour with 50 ml methanol and then filtered through Whatman No.1 filter paper. The filtrate was evaporated. The extract was diluted with 10 ml of dimethylsulfoxide and then passed through a 0.45 µm sterile filter. The resulting liquid was stored at -25 °C until use.

(a) **(b)** **(c)**

Figure 1: Photograph shows the Einkorn wheat seeds (a), Chestnut seeds (b), and *Begonia maculata* leaves (c).

2.2. Bacterial strains and antibiotics

Bacterial strains: *Escherichia coli* ATCC 25922*, Klebsiella pneumoniae, Pseudomonas aeroginosa,* and *Staphylococcus aureus* were used in this study. Antibiotics: ampicillin 10µg/µl (AMP10), gentamicin 10µg/µl (CN10), ciprofloxacin 5 µg/µl (CIP5), erythromycin 15 µg/µl (E15), amikacin 30 µg/µl (AK30), Cefoxitin 30 µg/µl (FOX30), Sulfamethoxazole with trimethoprim 25 µg/µl (SXT25).

Table 1. Bacterial strains and antibiotics used in this study.

Antibiotics	Bacteria strain
Ampicillin 10µg (AMP10)	*Escherichia coli* ATCC 25922
Gentamicin 10µg (CN10)	*Staphylococcus aureus*
Ciprofloxacin 5 µg (CIP5)	*Klebsiella pneumoniae*
Erythromycin 15 µg (E15)	*Pseudomonas aeroginosa*
Amikacin 30 µg (AK30)	
Cefoxitin 30 µg (FOX30)	
Sulfamethoxazole with trimethoprim 25 µg (SXT25)	

2.3. Agar well diffusion method

The medium was prepared as follows. The medium is melting by heating to 34 g/L. It is autoclaved at 121°C for 15 min. The medium is poured to 4mm thickness. The discs are allowed to come to room temperature before starting the treatment. Distilled water is placed in 2-2.5 ml of glass test tubes. Colony is taken from the petri using a sowing rod with McFarland between 0.5-0.6. Single colonies should be preferred when colonization is taken. The colonies are homogenously mixed and vortexed with the distilled water in the tubes. McFarland of the mixture which has been adjusted is spread over the colony where it falls evenly (Spread by rotating 3 times). Discs are placed on the surface of the agar with a sterile forceps so that they are 2 to 2.5 cm apart and 1.5 cm away from the edge of the plaque. The discs are pressed on the discs with a slight pressure on the agar. The resulting zone diameters are evaluated (Collins & Lyne, 1987; Bauer et al., 1966).

3. RESULTS

Einkorn wheat seeds (*Triticum monococcum*) and Chestnut seed (*Castanea sativa*) were used for antibacterial test against to *Escherichia coli* and *Staphylococcus aureus*. *B. maculata* plant leaves were used for antibacterial test against to *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeroginosa* bacteria.

The antimicrobial effects of plant leaves and seeds were investigated in Muller-Hilton media at 37 C° for 48 hours. After incubation, all plates were observed for zones of growth inhibition and the parameters of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times. ampicillin 10µg/µl (AMP10), gentamicin 10µg/µl (CN10), ciprofloxacin 5 µg/µl (CIP5), erythromycin 15 µg/µl (E15), amikacin 30 µg/µl (AK30), Cefoxitin 30 µg/µl (FOX30), Sulfamethoxazole with trimethoprim 25 µg/µl (SXT25), were used as positive controls.

It was observed that there was antibacterial no effect found in chestnut and Einkorn wheat seed extract (cloroform) against any *Staphylococcus aureus* strain. Einkorn wheat seed extract (cloroform) showed the

highest diameter of inhibition zone (34 mm), followed by 24 mm, and 23 mm at the dose of 5 µg/µl against *E. coli.*

Chestnut seeds against *E. coli* bacterium antibacterial effect was slightly less than wheat seeds. Chestnut seed extract showed a highest zone of inhibition of 33 mm, followed by 25 mm and 24 mm, and 21 mm against *E. coli* (Figure 2; Table 2, 3).

B. maculata leaves examined for antimicrobial effect on three bacteria and antimicrobial effect was observed in only two bacteria. *B. maculata* leaves extract was not effective *on P. aeroginosa*. *E. coli* bacteria had more effected leaves varieties than other bacteria, followed by *K. pneumoniae* and *P. aeroginosa* (Figure 3; Table 2, 3).

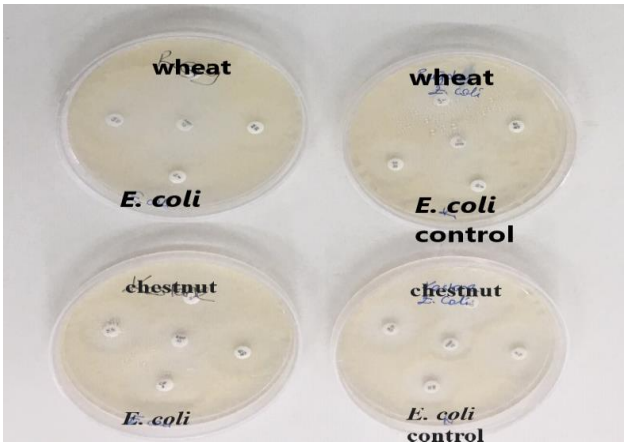
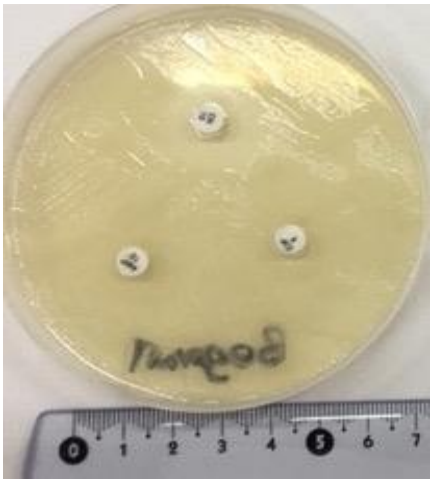


Figure 2: Photograph shows the antibacterial activity of wheat seed extract (upper part) and Chestnut seed extract (lower part) against the strain of *Escherechia coli.*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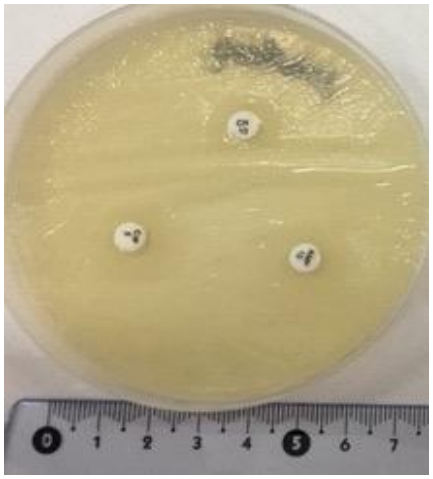
b)

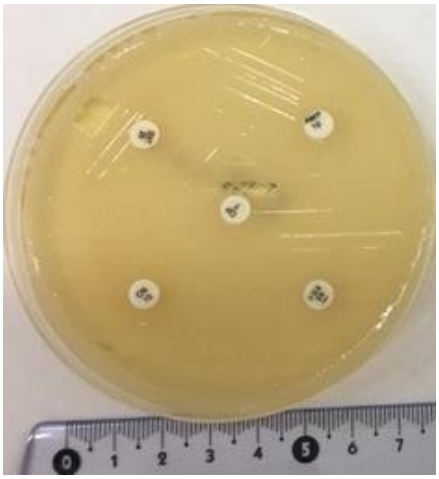
c)

Figure 3. Photograph shows the antibacterial activity of *B. maculata* leaves extract against the strains of *E. coli* and*, K. pneumoniae;* a) *B. maculata* leaves and *E. coli b) B. maculata* leaves and *K. pneumoniae* c) *B. maculata* leaves and *E. coli* control.

Table 2. Comparison of inhibition zones of Einkorn wheat, chestnut seeds, *B. maculata* against *E. coli, K. pneumoniae, S. aureus, P. aeruginosa*. (inhibition zone mm)

Bacteria	Chestnut 1	C2	C3	C4	C5	Wheat 1	W2	W3	W4	W5	*B. maculata* Raddi 1	BmR 2	BmR 3	BmR 4	BmR 5
E. coli	24	21	25	25	33	23	23	24	34	34	2	-	14	-	2
K. pneumoniae											8	-	7	-	8
S. aureus	-	-	-	-	-	-	-	-	-	-					
P. aeroginosa											-	-	-	-	-

*Zone of inhibition, including the diameter of the disc.

Chestnut 1 AMP10, Chestnut 2 (C2) AK30, Chestnut 3 (C3) CN10, Chestnut 4 (C4) E15, and Chestnut 5 (C5) CIP5. (-) indicates that there is no significant difference.

Table 3. Control group of Comparison of inhibition zones of Einkorn wheat, chestnut seeds *B. maculata* against *E. coli, K. pneumoniae, S. aureus, P. aeruginosa*. (inhibition zone mm)

Bacteria	Chestnut 1	C2	C3	C4	C5	Wheat 1	W2	W3	W4	W5	*B. maculata* Raddi 1	BmR 2	BmR 3	BmR 4	BmR 5
E. coli	18	23	22	28	28	15	16	20	26	27	-	-	7	-	-
K. pneumoniae											6	-	3	-	6
S. aureus	-	-	-	-	-	-	-	-	-	-					
P. aeroginosa											-	-	-	-	-

Control Group (Bacteria + Antibiotic)

*Zone of inhibition, including the diameter of the disc.

Chestnut 1 AMP10, Chestnut 2 (C2) AK30, Chestnut 3 (C3) CN10, Chestnut 4 (C4) E15, and Chestnut 5 (C5) CIP5(-) indicates that there is no significant difference.

4. DISCUSSION

In this study, AMP, CN, AK, E and CIP (Table 1) antibiotics were used as the control group in order to investigate the antibacterial effects of chestnut and Einkorn wheat seeds on Gram positive bacterium *S. aureus* and Gram negative bacterium *E. coli*. Besides, *B. maculata* leaves examined for antimicrobial effect on *E. coli, P. aeruginosa* and *K. pneumoniae.*

There are no more studies in the literature about the antibacterial effect of chestnut seeds. Živković et al. investigated the antimicrobial activity determination of 30% (v/v) ethanol extracts of C. sativa seed in various test microorganisms (*Staphylococcus aureus, Sarcina lutea, Bacillus cereus, Proteus mirabilis, Lactococcus lactis* ssp. *lactis, Micrococcus pyrogenes* var. albus, *Salmonella typhimurium*) and these seeds showed antimicrobial activity only in *L. lactis* ssp. *lactis* bacteria (Živković et al. 2010).

In recent years, herbal sources have been seen as a good alternative to combating harmful microorganisms. This study was conducted to investigate the cheaper and more natural antibiotic sources of bacteria to prevent resistance against antibiotics. The methanolic extracts of *B. maculata* leaves were moderate antimicrobial effects against the test microorganisms (*E. coli* and *K. pneumoniae*), with inhibition zones at 2.0 to 14.0 mm, and 7.0 to 8.0 mm, respectively. Notably, *E. coli* is more susceptible to the extract of *B. maculata* leaves (inhibition zones is 14.0 mm) as compared to standard antibacterial antibiotics CN. Besides, the extracts of *B. maculata* showed reduced antibacterial activity against *K. pneumoniae* and *P. aeroginosa* than some of the standard antibiotics (Table 3). It was observed that there was antibacterial no effect found in *B. maculata* leaves extract against *P. aeroginosa* strain. When a literature review was made to compare this study, but, there is no study in the literature on the antibacterial effect of *B. maculata* leaves.

The aim of this study was to investigate the antibacterial effects of Einkorn wheat *(Triticum monococcum*) and chestnut (*Castanea sativa*) seeds against *E. coli* and *S. aureus*. In the literature, there is no study about the antibacterial effect of chestnut seeds and Einkorn wheat. Therefore, this situation shows the specificity of our study. Chestnut and Einkorn wheat germ did not show any antibacterial effect against *S. aureus* strain.

Saha and colleagues showed that seed extracts of wheat varieties (namely Pavon 76) provided a highest zone of inhibition against *S. aureus* and *E. coli* species **(**Saha et al., 2018**)**. Choie et al. investigated the antibacterial activity of two wheats (Woori and Winter wheat) seeds against bacteria *S. aureus* and *E. coli* that cause contamination in the cosmetic and food industry. They showed that Woori and Winter wheat have the potential to be used as natural antimicrobials against *S. aureus* and *E. coli* species **(**Choi et al., 2014**)**. In this original study, Einkorn wheat seeds were observed to have antibacterial effect on *E. coli* bacteria.

Scientists are looking for new antibiotic derivatives that use plants and nature, as serious research has been done to prevent bacterial resistance to antibiotics. This study showed that Einkorn wheat, *Begonia* leaves and chestnut seeds were examined for natural antibiotic effect. Based

on this study, it is aimed to study a broader range of natural antibiotics that include more plant varieties.

Conflict of Interest: The author have no financial conflicts of interest to declare.

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