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Antimicrobial Activity of Wheat Germ Oil: A Disk Diffusion Study

Buğday Ruşeymi Yağının Antimikrobiyal Aktivitesi: Bir Disk Difüzyon Çalışması

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ABSTRACT

Aim: Wheat germ oil possesses antimicrobial and antioxidant properties, which can be attributed to the presence of phytosterols and tocopherols in its composition. The relentless spread of antimicrobial resistance has prompted scientists to seek alternative antimicrobial agents. The aim of this study is to investigate the antimicrobial activity of commercially available wheat germ oil.

Method: The antimicrobial activity of wheat germ oil was evaluated using the disk diffusion method. The standard bacteria obtained from the American Type Culture Collection (ATCC) were utilized in this study. Four Gram-negative bacteria (*Acinetobacter baumannii* ATCC-17978, *Escherichia coli* ATCC-25922, *Klebsiella pneumoniae* ATCC-700603, and *Pseudomonas aeruginosa* ATCC-28539), two Gram-positive bacteria (*Staphylococcus aureus* ATCC-29213 and *Enterococcus faecalis* ATCC-29212), and one yeast fungus (*Candida albicans* ATCC-10231) were included in the study.

Results: The inhibition zone diameters of wheat germ oil against various microorganisms were measured as follows: *E. coli* (8.3 mm), *S. aureus* (10 mm), *K. pneumoniae* (8.7 mm), *A. baumannii* (8.7 mm), and *C. albicans* (11.67 mm). No antimicrobial activity was observed against *P. aeruginosa* and *E. faecalis*, as no inhibition zone was formed around the disks.

Conclusion: This study is pioneering in its investigation of the antimicrobial activity of wheat germ oil using the disk diffusion method. Given the increasing value placed on agents with antimicrobial properties, it is crucial to further explore the antimicrobial activity of wheat germ oil through additional research.

Keywords: Antimicrobial activity, antibacterial activity, antifungal activity, wheat germ oil, disk diffusion.

ÖZET

Amaç: Buğday ruşeymi yağı, bileşimindeki fitosteroller ve tokoferollerin varlığı sayesinde antimikrobiyal ve antioksidan özelliklere sahiptir. Antimikrobiyal direncin giderek yaygınlık göstermesi, bilim insanlarını alternatif antimikrobiyal ajanlar aramaya sevk etmiştir. Bu çalışmanın amacı, ticari olarak temin edilen buğday ruşeymi yağının antimikrobiyal aktivitesinin araştırılmasıdır.

Yöntem: Buğday ruşeymi yağının antimikrobiyal aktivitesi disk difüzyon yöntemi ile değerlendirilmiştir. Bu çalışmada Amerikan Type Culture Collection (ATCC)'den elde edilen standart bakteriler kullanılmıştır. Dört Gram-negatif bakteri (*Acinetobacter baumannii* ATCC-17978, *Escherichia coli* ATCC-25922, *Klebsiella pneumoniae* ATCC-700603 ve *Pseudomonas aeruginosa* ATCC-28539), iki Gram-pozitif bakteri (*Staphylococcus aureus* ATCC-29213 ve *Enterococcus faecalis* ATCC-29212) ve bir maya mantarı (*Candida albicans* ATCC-10231) çalışmaya dahil edilmiştir.

Bulgular: Buğday ruşeymi yağının çeşitli mikroorganizmalara karşı inhibisyon zon çapları şu şekildedir: *E. coli* (8.3 mm), *S. aureus* (10 mm), *K. pneumoniae* (8.7 mm), *A. baumannii* (8.7 mm) ve *C. albicans* (11.67 mm). Disklerin etrafında inhibisyon zonu oluşmadığı için *P. aeruginosa* ve *E. faecalis*'e karşı antimikrobiyal aktivite gözlenmemiştir.

Conclusion: Bu çalışma, disk difüzyon yöntemi kullanılarak buğday ruşeymi yağının antimikrobiyal aktivitesinin araştırılması açısından öncü niteliğindedir. Antimikrobiyal özelliklere sahip maddelere verilen değerin arttığı göz önünde bulundurulduğunda, buğday ruşeymi yağının antimikrobiyal aktivitesinin ilerleyen araştırmalarla desteklenmesi ve genişletilmesi büyük önem taşımaktadır.

Anahtar Kelimeler: Antimikrobiyal aktivite, antibakteriyel aktivite, antifungal aktivite, buğday ruşeymi yağı, disk difüzyon.

INTRODUCTION

Essential oils possess a variety of biological activities due to the bioactive compounds present in their composition. Because of these properties, they have been used for many years in both traditional and modern medicine [1]. Wheat germ oil is a natural product that possesses antimicrobial and antioxidant properties due to its phytosterols and tocopherols [2,3]. Antimicrobial resistance (AMR) represents one of the most significant health threats confronting modern medicine globally. The inappropriate use of antimicrobials, coupled with the rise in antimicrobial resistance, has intensified the search for alternative antimicrobial agents. In this context, antimicrobial agents derived from natural herbal products are critically important due to their potential as alternative therapies to combat antimicrobial-resistant microorganisms [4,5]. It has been reported that wheat germ oil disrupts the membrane permeability of microorganisms due to the presence of lipophilic compounds in its structure [6]. However, studies examining the antimicrobial activity of wheat germ oil are quite limited. The aim of this study is to investigate the antibacterial and antifungal activities of commercially available wheat germ oil. The findings of this study are expected to contribute to the existing literature by demonstrating the potential of natural antimicrobial agents.

MATERIALS and METHODS

The wheat germ oil used for *in vitro* antimicrobial activity was purchased from a commercial supplier.

Microbial Strains and Microbial Inoculum

The standard bacteria obtained from the American Type Culture Collection (ATCC) were utilized in this study. Four Gram-negative bacteria (*Acinetobacter baumannii* ATCC-17978, *Escherichia coli* ATCC-25922, *Klebsiella pneumoniae* ATCC-700603, and *Pseudomonas aeruginosa* ATCC-28539), two Gram-positive bacteria (*Staphylococcus aureus* ATCC-29213 and *Enterococcus faecalis* ATCC-29212), and one yeast fungus (*Candida albicans* ATCC-10231) were included in the study. Overnight cultures of the bacteria were grown on 5% sheep blood agar at 37°C, while the yeast fungus was cultured on Sabouraud Dextrose Agar (SDA) (Aklab, Türkiye) at 30°C for 48 hours. Microbial colonies from these young cultures were diluted in sterile saline solutions (0.9% NaCl) to adjust the McFarland standard of No. 0.5, which corresponds to approximately 10^8 colony-forming units (CFU)/mL for bacterial suspensions and approximately 10^7 CFU/mL for yeast suspensions.

Disk Diffusion Method

In order to evaluate the antimicrobial properties of wheat germ oil, the agar disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) guideline M02-A12, was employed with several modifications [7,8]. Wheat germ oil was applied at a volume of 15 µL to each of the sterile blank paper disks (Bioanalyse, Türkiye), which had a diameter of 6 mm, and were placed in a sterile petri dish (FıratMed, Türkiye). The disks were allowed to absorb the oil for 30 minutes at room temperature. At the same time, the prepared microorganism inoculum was applied to Mueller Hinton Agar (MHA) (Aklab, Türkiye) medium by evenly spreading it over the entire surface using a sterile swab. Then, wheat germ oil-impregnated the paper disks were placed on agar plates in triplicate. The conventional antibiotic and antifungal disks served as

positive controls, while distilled water-impregnated disks were used as negative controls. A distance of 24 mm is maintained between the paper disks. Before incubation, the prepared plates were stored at +4°C for one hour to facilitate the diffusion of oil from the disks into the medium while preventing microbial growth. Then, all prepared plates were incubated in an oven at 37°C for 24 hours. At the end of the incubation period, the zones of inhibition formed around the disks were measured in millimeters (mm) using a ruler. The diameter of the disks (6 mm) was included in the measurements. The average diameters of the inhibition zones are presented as results. Each test was conducted in triplicate.

Statistical Analysis

Data from this study were analyzed using GraphPad Prism software (version 10.0; Boston, USA). The results are reported as arithmetic means with standard deviations.

RESULTS

In this study, we investigated the antibacterial and antifungal activity of commercially available wheat germ oil *in vitro* against four Gram-negative bacteria (*A. baumannii* ATCC-17978, *E. coli* ATCC-25922, *K. pneumoniae* ATCC-700603, and *P. aeruginosa* ATCC-28539), two Gram-positive bacteria (*S. aureus* ATCC-29213 and *E. faecalis* ATCC-29212), and one yeast fungus (*C. albicans* ATCC-10231). The inhibition zone diameters of wheat germ oil against the microorganisms were measured as follows: *E. coli* (8.3 mm), *S. aureus* (10 mm), *K. pneumoniae* (8.7 mm), *A. baumannii* (8.7 mm), and *C. albicans* (11.67 mm). No antimicrobial activity was observed against *P. aeruginosa* and *E. faecalis*, as no inhibition zone was formed around the disks. The inhibition zone diameters are summarized in Table 1.

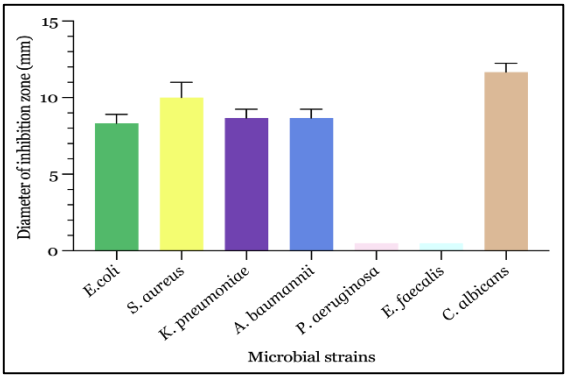
Table 1. Inhibition zone diameters of wheat germ oil against microorganisms

Microorganisms	Diameter of Inhibition Zone* (mm)			
	I-Z_1	I-Z_2	I-Z_3	I-$Z_{\bar{x}}$ (Mean ±SEM, mm)
Escherichia coli	8	9	8	8.3±0.58
Staphylococcus aureus	9	10	11	10±1
Klebsiella pneumoniae	8	9	9	8.7±0.58
Acinetobacter baumannii	9	9	8	8.7±0.58
Pseudomonas aeruginosa	-	-	-	-
Enterococcus faecalis	-	-	-	-
Candida albicans	12	12	11	11.67±0.58

I-Z: Inhibition zone, $Z_{\bar{x}}$: Arithmetic mean of zones, (Mean ±SEM, mm), *Diameter of inhibition zones including the diameter of the disk (6 mm). -: No inhibition zone; 6 mm, diameter of blank paper disk.

When the results of the study were analyzed, it was determined that wheat germ oil exhibited the most potent antimicrobial effect against *C. albicans* (11.67 mm). Consequently, it is evident that the antifungal effect of wheat germ oil surpasses its antibacterial effect. Among the tested bacteria, the most significant antibacterial effect was observed against *S. aureus* (10 mm). The antimicrobial activity of wheat germ oil is summarized in Figure 1.

Figure 1. Antimicrobial Activities of wheat germ oil. (The values represent the means ± standard deviation of data independent experiments. No inhibition zone was observed in *P. aeruginosa* and *E. faecalis*. The diameter of the paper disk is 6 mm).



DISCUSSION

Antimicrobial resistance continues to spread unabated worldwide. It is increasingly common for conventional antibiotics to be ineffective against resistant microorganisms. To address this challenge, scientists are actively seeking new alternative agents with antimicrobial properties [9]. In this context, plants exhibiting antimicrobial activity, along with their extracts and oils, are of critical importance. The literature has documented the antimicrobial properties of various oils, reporting their effectiveness at different rates [10,11]. However, research on the antimicrobial activity of wheat germ oil remains relatively scarce.

In this study, the antimicrobial activity of wheat germ oil was found to be moderate, nearly resistant. The antifungal activity was observed to be higher than the antibacterial activity. In a separate study examining the antimicrobial properties of extracts from various parts of the wheat plant, such as the root and germ, the average antibacterial activity measured 12 mm against *S. aureus* and 9 mm against *E. coli*. Antibacterial activity was tested at different concentrations, and no antibacterial effects were observed at lower doses [11]. In another study, the antibacterial effects of wheat germ extract were investigated using a bacterial growth method. The results indicated that *S. aureus* was the most sensitive bacterium. Additionally, it was reported that Gram-positive bacteria exhibited greater sensitivity compared to Gram-negative bacteria. It has been suggested that the increased resistance of Gram-negative bacteria may be associated with multidrug resistance pumps situated in the outer membrane of the bacterial cell wall [12]. In another study, the antimicrobial effects of wheat bran were evaluated using a broth microdilution test. The most susceptible bacteria identified were *Listeria monocytogenes*, followed by *S. aureus* and *E. coli* [13].

CONCLUSION

According to the results obtained from this study, wheat germ oil exhibits moderate antimicrobial activity, with antifungal effects being stronger than antibacterial effects. However, no antimicrobial activity was observed against certain bacterial species. The existing literature indicates that the number of studies investigating the antimicrobial activity of wheat germ extracts and oils is quite limited. Additionally, since the studies employed the liquid microdilution method rather than the disk diffusion method, there is insufficient data for comparison. Our study is pioneering in investigating the antimicrobial activity of wheat germ oil using the disk diffusion method. However, the antimicrobial properties of oils can be attributed to various biocomponents within their structure. Consequently, the inability to identify which specific biocomponent in wheat germ oil exhibits antimicrobial activity represents a limitation of our research. Given that agents with antimicrobial properties are increasingly valued, it is crucial to further investigate the antimicrobial activity of wheat germ oil through additional research.

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Ethics Committee Approval

This study does not require approval from an Ethics Committee.

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