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Antimicrobial and Antifungal Activities of Koruk (Unripe Grape) Juice Against Bacterial and Phytopathogenic Fungal Strains

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ABSTRACT

This study evaluated the antimicrobial and antifungal effects of verjuice obtained from unripe fruits of *Vitis vinifera L.* against selected bacterial and fungal microorganisms. The findings revealed that verjuice exhibited a marked inhibitory effect on all tested bacteria and inhibited the growth of *Verticillium dahliae* and *Fusarium oxysporum*, while showing no effect against *Aspergillus niger*. To date, no scientific study has been reported on the antifungal effects of verjuice against the phytopathogenic molds tested in this study. This study, therefore, makes a unique contribution by exploring the antimicrobial potential of koruk juice against both bacterial and fungal pathogens. The results indicate that verjuice could be considered a natural preservative in food safety applications and a potential agent for the biological control of phytopathogenic molds.

Keywords: Antibacterial activity, Antifungal effect, Fungal Strains, Koruk juice, *Vitis vinifera L.*

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INTRODUCTION

The antimicrobial properties of grape juice, particularly from unripe grapes (known as "koruk"), have become a significant area of focus in food science and technology, as researchers seek natural alternatives to synthetic food preservatives. Unripe grapes, which are widely used as an acidifying and flavouring agent in Turkish cuisine, possess a unique chemical composition that contributes to their potent antimicrobial and antioxidant effects [1]. The effectiveness of these products stems primarily from their high levels of organic acids and phenolic compounds, which have been shown to inhibit the growth of various foodborne pathogens [2]. This has led to a growing body of research exploring the application of koruk products in food safety.

Several studies have investigated the efficacy of koruk juice as a natural antimicrobial agent. For example, researchers have demonstrated its inhibitory effect against *Salmonella typhimurium* on salad vegetables [3]. In their study investigating the antimicrobial effect of unripe grape juice, Gargi and Şengün (2021) marinated meat samples with unripe grape juice and reported that, following marination, the populations of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella Typhimurium* were significantly reduced [4]. In this study, koruk juice significantly reduced the population of *S. typhimurium* strains, with the antimicrobial effect being dependent on the specific bacterial strain and the method of juice preparation. Another study further confirmed the broad-spectrum antibacterial activity of koruk juice, noting its effectiveness against *Bacillus cereus*, with a Minimum Inhibition Concentration (MIC) ranging from 31.3 to 500.0 µg/mL [5]. The bactericidal effect was also observed on all tested cultures at concentrations between 250.0 and 500.0 µg/mL. The high acidity and phenolic content are identified as the primary reasons for this inhibition, making unripe grape products a promising natural preservative.

The application of koruk has been extended beyond vegetables to include meat products, particularly as a marination liquid. Research has shown that koruk products can effectively act against foodborne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella Typhimurium* on poultry meat. These studies found that marination with koruk juice could reduce pathogen counts and improve the overall microbiological safety of the meat. In one experiment, marination with 50% koruk juice for 48 hours was the most effective treatment for reducing *E. coli* O157:H7 counts [6]. Similarly, a study on meat inoculated with these same pathogens showed that marination with koruk juice and dried koruk pomace significantly reduced bacterial counts, with the inactivation effect being more pronounced when a low inoculum dose was used [7]. The use of dried koruk pomace in "kofte" (meatballs) also showed a significant reduction in pathogen counts, indicating its potential in various meat product formulations [8].

In addition to its use in meat and vegetables, koruk juice has been tested on seafood. One study compared the antibacterial effects of unripe grape, lemon, and pomegranate juice against *Vibrio parahaemolyticus* in mussels. The results indicated that unripe grape juice had the highest antibacterial effect among the three, suggesting its potential for ensuring food safety in seafood products as well [9]. The high antibacterial efficacy of koruk juice, compared to other acidic fruit juices, highlights its unique composition and potential as a versatile food safety agent.

The antimicrobial action of koruk products is intrinsically linked to their rich phytochemical profile. Unripe grapes have been found to contain a high concentration of total phenolic and flavonoid compounds [10]. For instance, the total phenolic content in koruk juices can range from 152.11 to 317.71 mg GAE/L, with a high variability depending on the grape variety. This phenolic richness is directly correlated with high antioxidant activities, which further contribute to the overall preservative effect [1]. The low pH and high acidity of koruk juice, with pH values typically between 2.44 and 2.78, create an unfavourable environment for many spoilage and pathogenic microorganisms, thus extending the shelf life and safety of foods to which it is applied [3]. This synergy between low pH, high acidity, and bioactive compounds makes unripe grape products an excellent natural alternative to chemical preservatives. The findings from these collective studies underscore the potential for koruk products to be utilised as a natural, effective, and consumer-friendly means of improving food safety across various food industries.

The purpose of this study is to evaluate the antimicrobial and antifungal effects of grape juice derived from "koruk," the unripe form of the grape fruit, on various bacteria and mold species. The inhibitory effects of different concentrations of koruk juice were investigated against clinically and environmentally significant bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus aureus* using the agar well diffusion method. This study also represents the first time that the effect of koruk juice on the growth of phytopathogenic molds, such as *Fusarium oxysporum*, *Verticillium dahliae* and *Aspergillus niger* was evaluated using the poisoned food technique. The first-time use of these phytopathogenic molds to determine the antifungal efficacy of grape juice enhances the originality of this research and its contribution to the literature, emphasizing its scientific importance for the development of naturally sourced biocontrol strategies. This study aims to reveal the potential uses of koruk juice as a microbial growth inhibitor in light of the increasing interest in naturally sourced antimicrobial agents.

METHODS

Unripe Grape

The unripe grape (*Vitis vinifera L.*) used in this study was sourced from the Balıkesir/Ayvalık region of Turkey between June and July 2024.

Test Microorganisms

To determine the antibacterial activity of koruk juice, three Gram-positive bacteria (*Bacillus cereus* ATCC 7064, *Staphylococcus aureus* ATC 6538P and *Enterococcus faecalis*), two Gram-negative bacteria (*Escherichia coli* ATCC 11230 and *Pseudomonas aeruginosa*) and three fungal strains (*Fusarium oxysporum*, *Verticillium dahlia*) were used. The microorganism strains used in the study were obtained from the Department of Art and Science Faculty and the Agriculture Department of Plant Protection of Van Yüzüncü Yıl University.

Preparation of Koruk Juice from Unripe Grape

Unripe grapes were subjected to a pressing process and subsequently filtered through filter paper (Filter-Lab, 40 × 40 cm) to obtain the extract. The pH of the resulting extract used in the study was measured with a Jenway 3310 standard digital pH meter. Six different concentrations (10%, 15%, 20%, 25%, 50%, and 100%) were prepared and stored at +4 °C for the experiments.

Cultivation of Target Microorganisms

The target bacteria were inoculated into tubes containing 7 ml of Nutrient Broth (NB) and incubated at 37±0.1°C for 24 hours to promote growth. Molds were inoculated onto Petri plates containing Malt Extract Agar (MEA) and incubated for 7 days at 28°C for growth.

Agar Well Diffusion Method

For the test bacteria, Petri plates containing Mueller-Hinton Agar and the agar well diffusion method were used. A corkborer was used to create 6 mm diameter wells in the agar. Bacteria (100 µl) were dropped onto the plates and spread over the surface of the medium using a sterile glass rod (Drigalski spatula). 100 µl of each of the koruk juice concentrations was added to each well. Additionally, antibiotic discs were used for positive control. Imipenem (10 mg) was used for *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterococcus faecalis*, and *Escherichia coli*, while Chloramphenicol (30 µg) was used for *Staphylococcus aureus*. The resulting inhibition zones were measured in millimetres after 48 hours of incubation. The antibiotics used are detailed in Table 1. The inhibition zones formed after 48 hours of incubation were measured in millimetres.

Poisoned food technique

The poisoned food technique was used for the molds. Molds were first grown on Petri plates containing MEA by incubating them at 28°C for 7 days. At the end of the 7th day, 5% of the medium volume in each sterile Petri dish was replaced with concentrations of 25%, 50%, and 100%. MEA cooled to 50°C was then added, homogenized, and a 15 mm diameter section of each mold culture, cut with a cork borer, was placed in the center of the prepared plates. Cycloheximide was used for the positive control, and only MEA was used for the negative control. The plates were incubated at 28°C for 8 days. The growth of the molds was measured in millimeters and recorded every 2 days.

RESULTS

The pH of this solution was measured at 2.25.

Table 1. Commercial antibiotic discs used for positive control

Antibiotic Disc Name	Disc Content	Bacteria
İmipenem	10 µg	*Pseudomonas aeruginosa*
		Bacillus cereus
		Enterococcus faecalis
		Escherichia coli
Kloramfenikol	30 µg	*Staphylococcus aureus*

Table 2. Effect of extract concentrations on test bacteria (given in mm)

Bacteria	P.C^{p}	100%	50%	25%	20%	15%	10%
Pseudomonas aeruginosa	24	18	14	10	-	-	-
Bacillus cereus	24	22	14	11	-	-	-
Staphylococcus aureus	18	18	14	-	-	-	-
Enterococcus faecalis	22	14	12	9	8	-	-
Escherichia coli	26	18	14	10	-	-	-

- : No Zone, **P.C^{p}**: Positive Control

Table 3. Effect of extract concentrations on Verticillium dahliae (given in mm)

Time (Day)	P.C^{p}	N.C^{n}	100%	50%	25%
2	-	20	20	26	26
4	-	41	26	34	34
6	-	50	34	45	42
8	-	69	36	51	49

-: No Growth, **P.C^{p}**: Positive Control, **N.C^{n}**: Negative Control

Table 4. Effect of extract concentrations on Fusarium oxysporum (given in mm)

Time (Day)	P.C^{p}	N.C^{n}	100%	50%	25%
2	-	22	29	31	37
4	-	48	34	44	50
6	-	64	46	48	60
8	-	85	57	53	74

-: No Growth, **P.C^{p}**: Positive Control, **N.C^{n}**: Negative Control

Table 5. Effect of extract concentrations on Aspergillus niger (given in mm)

Time (Day)	P.C^{p}	N.C^{n}	100%	50%	25%
2	-	90	90	90	90
4	-	90	90	90	90
6	-	90	90	90	90
8	-	90	90	90	90

-: No Growth, **P.C^{p}**: Positive Control, **N.C^{n}**: Negative Control

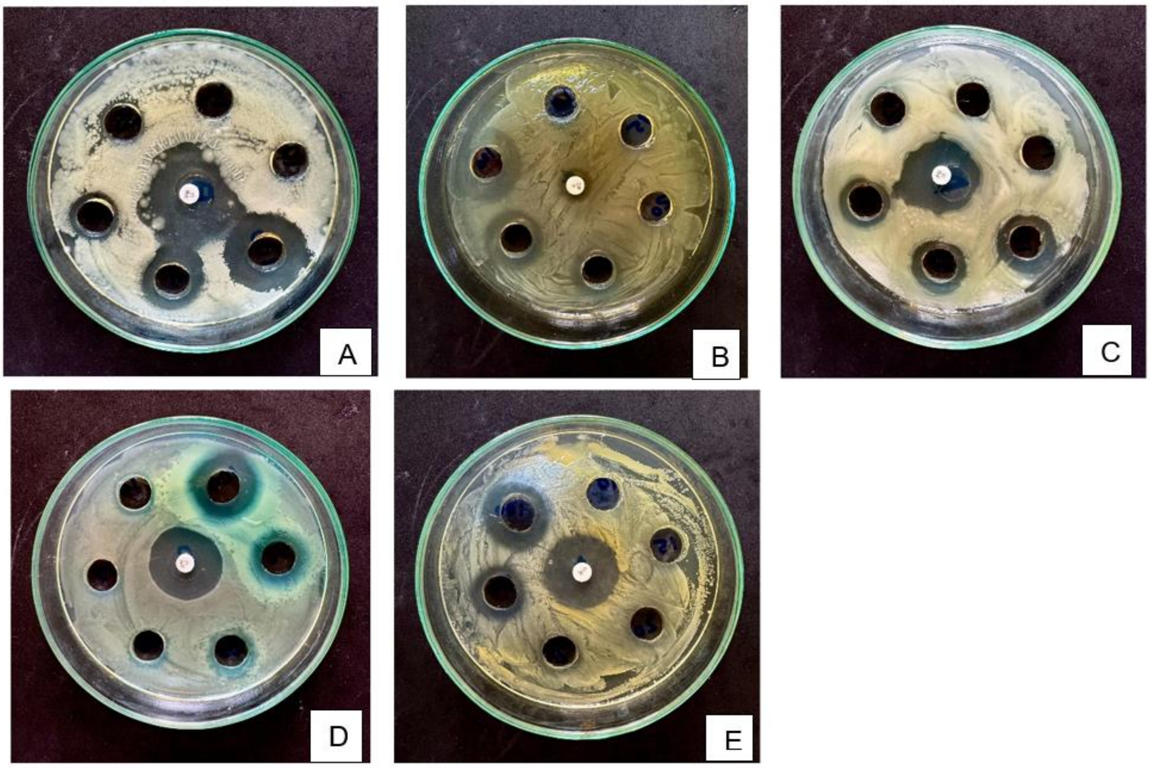

Figure 1. *Antimicrobial effect of the extract on tested bacterial strains (A: Bacillus cereus, B: Escherichia coli, C: Enterococcus faecalis, D: Pseudomonas aeruginosa, E: Staphylococcus aureus).*

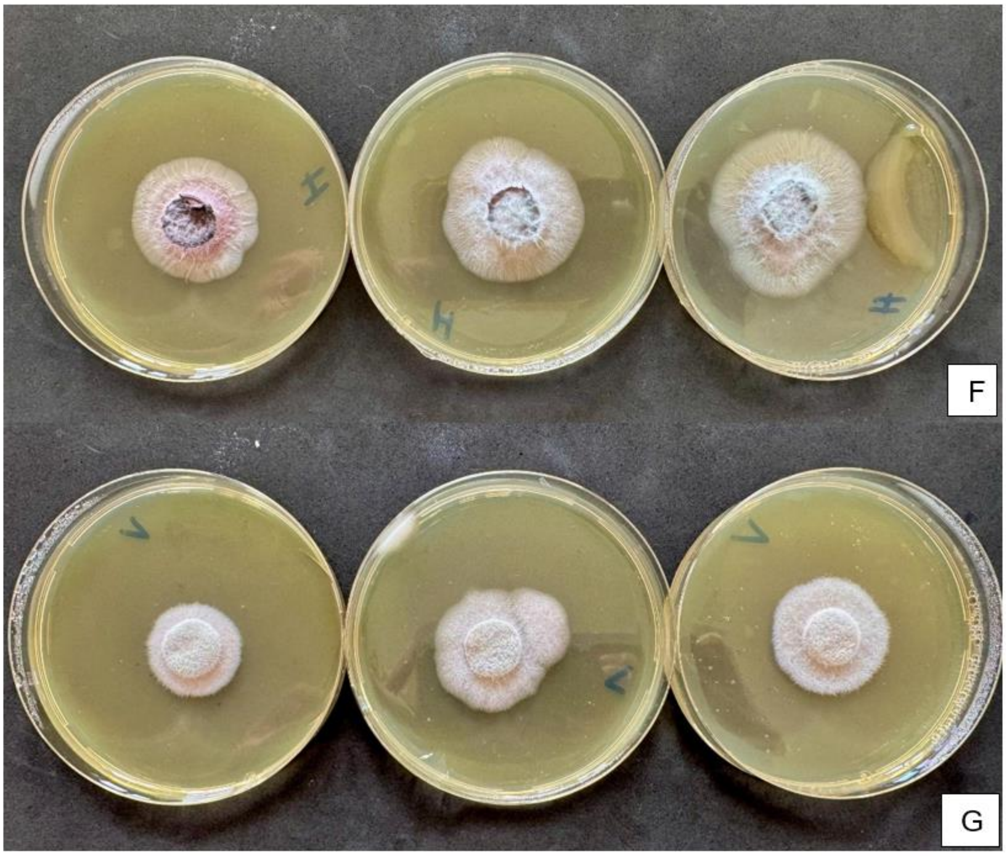

Figure 2. Inhibition of Fusarium oxysporum (F) and Verticillium dahlia (G) by the extract on day 4 of incubation.

DISCUSSION

In this study, the antimicrobial effects of different concentrations of koruk juice on various bacterial and fungal microorganisms were evaluated. Tests performed using the agar well diffusion method showed that 50% and 100% concentrations of koruk juice created significant inhibition zones against all tested bacteria. In antifungal evaluations conducted with the poisoned food technique, koruk juice was found to inhibit the growth of *Verticillium dahliae* and *Fusarium oxysporum* molds in a dose-dependent manner. However, *Aspergillus niger* showed resistance to all concentrations. These findings demonstrate that koruk juice has selective and concentration-dependent antimicrobial effects.

Pseudomonas aeruginosa, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*, which were used as test bacteria in the study, showed sensitivity to 50% and 100% concentrations of koruk juice. At these concentrations, *P. aeruginosa* and *E. coli* formed inhibition zones of 14 mm and 18 mm, respectively; *B. cereus* formed zones of 14 mm and 22 mm; and *E. faecalis* formed zones of 12 mm and 14 mm. Low concentrations (10% and 15%) failed to inhibit the growth of the test bacteria. These findings suggest that the ineffectiveness of the low concentrations might be due to the insufficient density of phenolic compounds in koruk juice to penetrate the microbial cell wall or membrane and interact with target structures [11-12]. It is known that the effectiveness of plant-derived antimicrobial substances is often dose-dependent, and they fail to show a significant effect on microorganisms when they fall below a certain threshold [13]. In particular, phenolic compounds can alter membrane permeability, denature proteins, and interfere with essential enzymatic pathways, which requires a minimum active concentration to be effective. A weak effect was observed against *E. faecalis* only at a 20% concentration of koruk juice. For the *S. aureus* strain, the effect of the 100% concentration of koruk juice was similar to that of the antibiotic disc used as a positive control. These findings can be attributed to the high levels of natural antimicrobial compounds in unripe grapes, such as polyphenols (especially flavan-3-ols), flavonoids, and organic acids [14].

The study also used the poisoned food technique to evaluate the antifungal effect of koruk juice against three different mold species. It was determined that *Verticillium dahliae* was more sensitive to koruk juice, and its growth was significantly suppressed in a concentration-dependent manner. While this mold formed a colony of 69 mm in diameter in the negative control, it grew to diameters of 49 mm, 51 mm, and 36 mm at koruk juice concentrations of 25%, 50%, and 100%, respectively. While *Fusarium oxysporum* grew to 85 mm in the negative control, it showed a growth diameter of 74 mm, 57 mm, and 53 mm at concentrations of 25%, 50%, and 100%, respectively. These results suggest that the phenolic and polyphenolic compounds in koruk juice may have both fungistatic and fungicidal effects. However, the literature has reported that koruk pomace extracts can both halt the growth of (fungistatic) and cause lethal inhibition (fungicidal) against phytopathogenic fungi such as Fusarium, Botrytis, and Penicillium in a concentration-dependent manner [15]. In contrast, *Aspergillus niger* remained resistant to all concentrations, reaching 90 mm and covering the entire Petri plate by the end of the second day. The absence of any mold growth on the positive control plates containing cycloheximide supports the reliability of the method.

The antimicrobial effect of koruk juice on bacterial pathogens has been demonstrated through in vitro studies using various extraction methods and concentrations. Numerous studies have tested koruk juice or other koruk products using the agar well diffusion method and observed that they form significant inhibition zones against a variety of Gram-positive and Gram-negative bacteria. For example, Parekh and Chanda [16] reported that an aqueous extract obtained from unripe grapes showed strong antibacterial activity against *Alcaligenes faecalis*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, as well as *Proteus vulgaris*. These effects are believed to be associated with the organic acids (particularly malic acid) and phenolic compounds present in verjuice. Organic acids can inhibit microbial growth by lowering the environmental pH, while phenolic compounds may disrupt cell membranes and inhibit enzymatic activities. Such mechanisms likely contribute to the observed antibacterial and antifungal activities of the extract. This data supports the potential use of koruk juice as a natural antimicrobial agent to prevent bacterial contamination. However, the literature on the effect of koruk juice on fungal species is quite limited. The existing findings, though scarce, suggest that koruk juice may also be effective against some fungal strains. For example, a study by Simonetti et al. [15] evaluated koruk juice against *Candida* spp. strains and reported a 37% reduction in mature *Candida* biofilms.

This study demonstrated that the extract obtained from unripe grapes (*Vitis vinifera L.*) exhibits notable antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus* *faecalis*, and antifungal activity, particularly against *Fusarium oxysporum* and *Verticillium dahliae*. These findings suggest potential applications of the extract as a natural antimicrobial agent in food preservation and agricultural disease management. However, the study was limited by the use of a single replicate for each treatment and the evaluation of a limited number of microorganisms. Future research should involve multiple replicates, a broader range of microbial species, and additional analytical techniques to better elucidate the mechanisms underlying its antimicrobial effects and to assess its effectiveness under practical application conditions.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Conflicts of Interest

There is no conflict of interest for the publication of this article.

Ethics committee approval

Ethics committee approval is not required in this study. The study was conducted following the international declaration, guidelines, etc.

Referee Evaluation Process

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