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Original article

Antibacterial Profile of Verbascum Speciosum Against Bacteria and Fungi Strains

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Abstract
The present study aimed to evaluate the antibacterial potential of *Verbascum speciosum* leaf extract against selected bacterial isolates. Verbascum species are known for their medicinal properties, attributed to their bioactive phytochemical components that exhibit antimicrobial, antifungal, and antiviral effects. The leaves of Verbascum speciosum were collected from Fethiye, Turkey, and subjected to ethanol 60% extraction. The activity of the extract was tested using the agar disc diffusion method against four standard Gram-negative bacteria (E.coli ATCC 25922, Enterobacter aerogenes ATCC 13048, Pseudomonas fluorescens DSMZ 50071, and Klebsiella pneumoniae ATCC 7544), and three Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, and Enterococcus faecalis ATCC 29212). Antibiotic susceptibility testing was also conducted using standard antibiotics (Vancomycin, Ceftazidime, Meropenem, Ofloxacin, and Gentamicin) to compare antibacterial efficacy. Results indicated that only Enterococcus faecalis ATCC 29212 exhibited sensitivity to the Verbascum speciosum extract at a concentration of 10µg, with an inhibition zone of 7 ± 0.1 mm. No inhibitory effects were observed against other tested bacteria. In contrast, antibiotic testing revealed varying degrees of sensitivity among bacterial isolates. Meropenem showed the strongest inhibitory effect across most strains. MIC testing further confirmed limited antimicrobial activity of the plant extract against Enterococcus faecalis. Statistical analysis showed no significant differences in antibacterial effectiveness across different plant extract concentrations, and significant differences between antibiotics and the extract's effectiveness (P-v >0.05). These findings suggest that while Verbascum speciosum contains bioactive compounds, its antibacterial potency is limited under the tested conditions, where it exhibits selective and limited antibacterial activity, primarily against Enterococcus faecalis. Further studies may be necessary to explore enhanced extraction methods or synergistic effects with conventional antibiotics.
Keywords: Verbascum Speciosum, Standard Bacteria, Antibacterial Activity,

Introduction

Medicinal plants are households to numerous phytochemicals and work as the backbone of the defense system. Phytochemicals are in various plant parts like flowers, leaves, and roots of vegetables and fruits. They play crucial roles in providing immunity along with nutrients, fibers, and protecting against various diseases. Recent research indicated that phytochemicals of medicinal plants are implicated in the biosynthesis of many new drugs [1, 2]. Verbascum species are discovered in a variety of ecological conditions; they can be found in dry, sunny, moist habitats like roadsides, stones, riverbanks, high mountains, semi-deserts, and grasslands, and they are pollinated with the help of insects and by wind [3, 4].

Verbascum is generally found in Asia, Europe, and Africa, and is present in Australia, North America, the United States, and Canada [5, 6]. Anyway, the plants of the Verbascum species comprise annual, biennial, or perennial herbs and a smaller number of small shrubs. Leaves have an alternate organization with the formation of a rosette at the base. The presence of petioles differs from plant to plant, with a simple or divided pattern. They are either serrate or crenate and can be dentate or lobed. Nearly all plants have hairs with either a simple or branched pattern or glandular or non-glandular organization, while some species are hairless. The stem is vertical in position and comprises hairs with different patterns [7].

Verbascum plants are well-known in medicinal use and as ornamentals and have chemical compounds considered to have antimicrobial, antifungal, and antiviral effects. Various members are commonly grown as ornamentals and used for decorative purposes. Previously used as medicinal herbs as a remedy for cough, diarrhea, and respiratory stimulant disorders, followed by smoking [8,9]. American researchers said that smoking dried leaves relieved asthma and other respiratory disorders. Herbalists recognize the leaf tea as a traditional remedy for respiratory congestion and hemorrhage. Other studies revealed that the strong microbial qualities of the flowers led to their use in oil infusions for treating ear infections. The roots have been used for their tonic and astringent properties to treat urinary incontinence. The seeds were also used by American Indians as a paralytic fish poison; the plant contains coumarin and rotenone, especially in seeds [10]. This study aimed to evaluate the antibacterial potential of Verbascum speciosum leaf extract against selected bacterial isolates

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Materials and Methods

Collection and preparation of plant material

Verbascum speciosum leaves were collected in May 2016 at the flowering stage from the Gölbent/Patara-Fethiye Road at coordinates 36°24'18.4"N, 29°17'49.8"E (Photo 1). The leaves were cleaned with distilled water, dried in a shaded area for a few days, and ground into fine powder in a mortar.

Photo 1: Verbascum speciosum
Gölbent/Patara-Fethiye Road at coordinates 36°24'18.4"N, 29°17'49.8"E

Plant Extraction

50g of the dried leaves powder was weighed and extracted at room temperature with 300 mL of ethanol 60% (Merck, Germany) under shaking at 100 rpm on a shaker (WiseShake, Korea) for three days [10]. To remove the solvent and concentrate the extract, the mixture was filtered with the use of a rotary evaporator (Heidolph, Germany) at a temperature of 35 to 45°C. With the use of the freeze dryer apparatus (Christ, Germany), the filtrate was completely frozen at 0.12 atm vacuum and kept at - 82 °C in a labelled and well-tight closed container.

Preparation of the extract's stock solution

Stock solutions of extracts were prepared by mixing 1g of the extract in 10 mL of absolute ethanol to end with a final concentration of 100mg/ml.

Microbial isolates

Four Gram-negative and three Gram-positive bacteria, and one fungus isolate were included in this study. The Gram-negative bacterial strains were E.coli ATCC 25922, Enterobacter aerogenes ATCC 13048, Pseudomonas fluorescens DSMZ 50071, and Klebsiella pneumoniae ATCC 7544. The Gram-positive bacterial strains were Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, and Enterococcus faecalis ATCC 29212. All isolates were obtained from the laboratory of biology at the Graduate School of Natural and Applied Science, Kastamonu University, Turkey.

Preparation of microbial suspension

A fresh microbial culture was prepared by the use of Nutrient agar for bacteria. A microbial suspension was made for each isolate by emulsifying a lopful of the fresh culture growth in 2ml of normal saline solution (0.9%), which was then adjusted to 0.5 McFarland standards (1.0 x 10^8 CFU/ml) using the Turbidimeter apparatus (Oxoid, UK), and was ready for use [11].

Antibiotic references

Five antibiotic references with different mechanisms of action were included in this study: Vancomycin 30µg, Ceftazidime 30µg, Meropenem 10µg, Ofloxacin 5µg, Gentamicin 10µg. The antibiotics were tested in this study and compared with the extract for their antibacterial activity against the tested Gram-positive and Gram-negative bacteria. All antibiotic standard references were obtained from the laboratory of biology at the Graduate School of Natural and Applied Science, Kastamomu University, Turkey.

Assessment of antimicrobial activity of the extract

The agar disc diffusion method was used to assess the antimicrobial activity of the extract against tested microorganisms. A sterile swab was immersed in the microbial suspension and distributed in all directions onto the surface of a Muller Hinton agar contained in Petri dishes [12]. In a next step sterile discs (6mm/diameter) were loaded with different volumes, 10 µL (1mg), 50 µL (5mg) and 100 µL (10mg) of the extract with a concentration of 10ug /disc**,** 50 µg/disc, and 100 µg/disc, respectively. One unloaded disc was used as a control. This was repeated with each type of tested microbes. All plates were incubated at 37°C for 18 to 24 hours, and then the inhibition zone was measured in millimeters (mm). All tests were repeated three times to ensure reliability

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Minimum Inhibitory Concentration (MIC) Test

For the evaluation of the active extract, and starting with a concentration of 100mg/ml, nine serial dilutions 1:2 at were prepared; 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml 1.5625mg/ml, 0.7813mg/ml, and 0.3906mg/ml. The microdilution was performed in 96-well microtiter plates. In brief, fresh overnight cultures were diluted in Mueller Hinton Broth at a density adjusted to a 0.5 McFarland turbidity, where the final inoculum was 5 x 10^8 CFU/ml of bacterial colony. The wells numbered 1 – 10 were designed to test the extract activity. A 100ul of each of the serial dilutions of extract concentrations was added to each of the 10 wells (1 – 10 wells), followed by adding 10ul of the adjusted prepared bacterial suspension to each well (1 – 10 wells). Well no. 11 was designed as a positive well containing only the bacterial suspension, and well 12 was designed as a negative control well containing only Mueller Hinton Broth. This was done for all tested bacterial isolates. All plates were covered and incubated for 24 hours at 37°C for 24 hrs. In this study, the MIC was the lowest concentration of plant extracts that exhibited no growth of the organism in the wells by visual reading [13]. This step was done under aseptic technique.

Statistical Analysis

Mean and standard deviation were calculated in this study with use of Excel. ANOVA was used to determine the p-value, which was considered significant when $p>0.05$.

Results

The antimicrobial activity of the extract of Verbascum speciosum was evaluated against a panel of bacterial isolates using the agar well diffusion method, and the results are summarized in (Table 1). Among all tested bacterial strains, only Enterococcus faecalis ATCC 29212 showed sensitivity to the Verbascum speciosum extract at the highest concentration tested (100 µL), with an inhibition zone measuring 7 ± 0.1 mm (Photo 1). No inhibitory effects were observed for the other bacterial isolates, including Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, Enterobacter aerogenes ATCC 13048, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 7544, and Pseudomonas fluorescens DSMZ 50071, at any of the tested extract concentrations (10 µL, 50 µL, and 100 µL). The minimum inhibitory concentration assay was done only for Enterococcus faecalis and the results showed that 100mg/ml is the lowest concentration of the extract that can inhibit the growth of this bacterium.

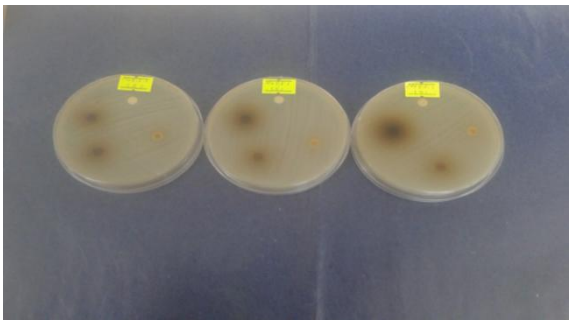

Photo 1: The Plate shows the Inhibition zone of Verbascum speciosum against Enterococcus faecalis

Table 1: Inhibition zone of Verbascum speciosum against all tested bacteria

Tested bacteria	**Mean of inhibition zones in diameter (mm) ± standard deviation**		
	10 µL (1µgl)	50 µL (5µg/µl)	100 µL (10µg)
Enterococcus faecalis ATCC 29212	0	0	7±0.1
Staphylococcus aureus ATCC 25923	0	0	0±0.1
Staphylococcus epidermidis DSMZ 20044	0	0	0±0.1
Enterobacter aerogenes ATCC 13048	0	0	0±0.1
E.coli ATCC 25922	0	0	0±0.1
Klebsiella pneumonia ATCC 7544	0	0	0±0.1
Pseudomonas fluorescens DSMZ 50071	0	0	0±0.1

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Antibiotic susceptibility testing was also performed to assess the baseline sensitivity of the bacterial isolates to selected standard antibiotics (Table 2). The tested antibiotics included vancomycin (VA, 30 µg), ceftazidime (CAZ, 30 µg), meropenem (MEM, 10 µg), ofloxacin (OFX, 5 µg), and gentamicin (CN, 10 µg). Among Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923 exhibited high sensitivity to Meropenem, with an inhibition zone of 30 ± 0.2 mm, followed by Ofloxacin (24 ± 0.2 mm) and Gentamicin (22 ± 0.2 mm). Vancomycin also demonstrated inhibitory activity, with a zone diameter of 17 ± 0.2 mm. Similarly, *Staphylococcus epidermidis* DSMZ 20044 showed the greatest sensitivity to Meropenem (30 ± 0.2 mm) and Ofloxacin (25 ± 0.2 mm), while it was less responsive to Vancomycin (7 mm) and more moderately responsive to Gentamicin (14 ± 0.1 mm).
Enterococcus faecalis ATCC 29212 exhibited moderate sensitivity to Meropenem (15 ± 0.1 mm) and Ofloxacin (14 ± 0.1 mm), with no observable response to Vancomycin or Gentamicin. Among Gram-negative isolates, *Enterobacter aerogenes* ATCC 13048 showed variable responses to the tested antibiotics, with maximum inhibition observed for Meropenem (25 ± 0.1 mm), followed by Ofloxacin (23 ± 0.1 mm) and Gentamicin (21 ± 0.1 mm).
Ceftazidime also showed some inhibitory effect (20 ± 0.2 mm). In contrast, Escherichia coli ATCC 25922 exhibited resistances to Ofloxacin (0 ± 0.1 mm) but remained sensitive to Meropenem (30 ± 0.2 mm), Ceftazidime (14 ± 0.1 mm), and Gentamicin (20 ± 0.1 mm). Klebsiella pneumoniae ATCC 7544 showed the strongest response to Meropenem (22 ± 0.1 mm) compared to Ceftazidime (0 ± 0.1 mm, indicating resistance) and Gentamicin (10 ± 0.2 mm). Pseudomonas aeruginosa DSMZ 50071 exhibited high sensitivity to Meropenem (30 ± 0.2 mm) and moderate sensitivity to Ceftazidime (11 ± 0.1 mm) and Gentamicin (20 ± 0.2 mm), although its response to Ceftazidime was relatively weak. All data are expressed as means and standard deviations.

Table 2. Inhibition zone (mm) of antibiotics against the tested bacteria

Tested bacteria	**Mean of inhibition zones in diameter (mm) ± standard deviation**				
	VA 30µg	**CAZ 30µg**	**MEM 10µg**	**OFX 5µg**	**CN 10µg**
Enterococcus faecalis ATCC 29212	-	-	15±0.1	14±0.1	-
Staphylococcus aureus ATCC 25923	17±0.2	-	30±0.2	24±0.2	22±0.2
Staphylococcus epidermidis DSMZ 20044	7	18±0.1	30±0.2	25±0.2	14±0.1
Enterobacter aerogenes ATCC 13048	ND	20±0.2	25±0.1	23±0.1	21±0.1
E.coli ATCC 25922	ND	14±0.1	30±0.2	0±0.1	20±0.1
Klebsiella pneumonia ATCC 7544	ND	0±0.1	22±0.1	20±0.1	10±0.2
Pseudomonas aeruginosa DSMZ 50071	ND	11±0.1	30±0.2	18±0.1	20±0.2

(ND): Not done (-): No effect (VA): Vancomycin 30µg (CAZ): Ceftazidime 30µg (MEM): Meropenem 10µg (OFX): Ofloksazin 5µg (CN): Gentamicin 10µg

Discussion

The results demonstrated that only Enterococcus faecalis ATCC 29212 is the only one exhibited sensitivity to the extract of Verbascum speciosum at the highest concentration tested (100 µL), with an inhibition zone measuring 7 ± 0.1 mm. No inhibitory effects were observed for the other tested strains, including Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, Enterobacter aerogenes ATCC 13048, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 7544, and Pseudomonas fluorescens DSMZ 50071, across all concentrations. This limited antimicrobial activity suggests that Verbascum speciosum extract may possess selective antibacterial properties, specifically targeting certain Gram-positive bacteria such as Enterococcus faecalis, but is ineffective against other common pathogens under the tested conditions.
Antibiotic susceptibility testing revealed variable responses among the bacterial isolates to the selected standard antibiotics. Among Gram-positive bacteria, S. aureus ATCC 25923 showed the highest sensitivity to Meropenem, followed by Ofloxacin and Gentamicin. Vancomycin also demonstrated inhibitory activity, albeit to a lesser extent. These findings align with previous studies indicating the efficacy of carbapenems like Meropenem against Gram-positive cocci, particularly methicillin-sensitive Staphylococcus aureus (MSSA) strains [14].
Similarly, Staphylococcus epidermidis DSMZ 20044 demonstrated strong sensitivity to Meropenem and Ofloxacin, while showing minimal response to Vancomycin and moderate sensitivity to Gentamicin. This

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pattern of resistance to Vancomycin is notable, as coagulase-negative Staphylococci are increasingly recognized as opportunistic pathogens with potential for glycopeptide resistance [15]. Enterococcus faecalis ATCC 29212 exhibited moderate sensitivity to meropenem and Ofloxacin, with no observable response to Vancomycin or Gentamicin. This intrinsic resistance to Vancomycin in some enterococcal species has been previously documented, highlighting the challenge in treating infections caused by these organisms. This intrinsic resistance to Vancomycin in some enterococcal species has been previously documented, highlighting the challenge in treating infections caused by these organisms [16].

Among Gram-negative isolates, E. aerogenes ATCC 13048 showed maximum inhibition with Meropenem, followed by Ofloxacin and Gentamicin. Ceftazidime also showed some inhibitory effect, suggesting its potential utility in treating infections caused by this organism. In contrast, E. coli ATCC 25922 was resistant to Ofloxacin but remained sensitive to Meropenem, Ceftazidime, and Gentamicin. Fluoroquinolone resistance in E. coli is a growing concern globally, often linked to the overuse and misuse of these agents in both human and veterinary medicine [17].

Klebsiella pneumoniae ATCC 7544 showed the strongest response to Meropenem compared to Ceftazidime (0 ± 0.1 indicating resistance) and Gentamicin. This finding underscores the increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing Klebsiella pneumoniae strains, which are typically resistant to third-generation cephalosporins such as Ceftazidime [18].

Finally, Pseudomonas aeruginosa DSMZ 50071 exhibited high sensitivity to Meropenem and moderate sensitivity to Ceftazidime and Gentamicin. While Carbapenems remain a mainstay for treating Pseudomonas aeruginosa infections, emerging resistance mechanisms such as metallo-beta-lactamases pose a significant threat to their continued efficacy [19]. In line with this study, several studies have shown that Verbascum species exhibit selective antimicrobial activity, often more effective against Gram-positive bacteria than Gram-negative strains [11]. Also, another study done by Al-Tawfiq and Hidron [20], reported continued high susceptibility of Staphylococcus species to carbapenems like Meropenem, especially in hospital settings where resistance is monitored closely, and this is in line with this study, which showed high sensitivity of Staphylococcus and Staphylococcus epidermidis to Meropenem. Furthermore, this study found that Klebsiella pneumoniae was resistant to ceftazidime, a finding that matches with a review written by Rawat and Nair [21] that highlights the rising trend of extended-spectrum beta-lactamase (ESBL) production among K. pneumoniae isolates, rendering them resistant to third-generation cephalosporins like ceftazidime. Other studies reported a discrepancy; unlike this study's result, which found that Pseudomonas fluorescens DSMZ 50071 is highly sensitive to meropenem (30 mm), while in contrast, ECDC data 2023 indicate that Pseudomonas aeruginosa shows rising resistance to meropenem in clinical settings, particularly in ICU patients, with resistance rates exceeding 20% in some countries.

Although this study showed that Enterococcus. faecalis showed no response to vancomycin, but many recent clinical isolates remain largely susceptible to vancomycin unless they carry the vanA gene. The meta-analysis reports that although VRE is increasing, a large proportion of E. faecalis isolates remain vancomycin-sensitive, suggesting that resistance may be strain-specific or context-dependent [22].

Conclusion

In summary, Verbascum speciosum extract exhibits selective and limited antibacterial activity, primarily against Enterococcus faecalis. The standard antibiotics tested confirmed meropenem as the most potent agent overall, although resistance patterns highlight the ongoing challenge of antimicrobial resistance among clinically relevant pathogens. Further studies are warranted to explore the active constituents of the extract and their potential synergistic effects with conventional antibiotics.

Conflict of interest. Nil

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