

Antibacterial activity of Local *Verbascum* sp. extracts against nosocomial antibiotic-resistant bacteria

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Abstract

Nosocomial infections, primarily caused by bacteria, present a significant public health challenge in healthcare facilities. *Verbascum* sp. (Common Mullein), known for its medicinal properties, has been traditionally utilized to treat various ailments. This study investigates the antimicrobial activity of methanolic extracts from *Verbascum* sp. leaves against several bacterial isolates. Leaves were collected, dried, and processed to prepare methanolic extracts. The antibacterial efficacy was assessed using agar well diffusion and agar plate dilution assays to determine inhibition zones and minimum inhibitory concentrations (MIC). The results demonstrated that the methanolic extract exhibited strong antimicrobial activity against *Acinetobacter baumannii*, *Enterococcus* spp., and *Staphylococcus aureus*, with inhibition zones of 19.33 mm, 17.67 mm, and 19.67 mm, respectively. The MIC values were 125 µg/mL for both *A. baumannii* and *S. aureus*, and 250 µg/mL for *Enterococcus* spp. These findings highlight the potential of *Verbascum* species extracts as alternative treatments for drug-resistant bacterial infections in hospital settings. Notably, the extract's efficacy against these bacteria supports its use in treating infections caused by resistant strains. However, the extract showed no activity against *E. coli* and *Burkholderia gladioli*, underscoring the need for further research to understand and overcome these limitations. The findings align with previous studies, reinforcing the antimicrobial potential of *Verbascum* sp. Overall, *Verbascum* sp. leaf extract presents a promising avenue for developing new antimicrobial agents, particularly in the face of increasing antibiotic resistance and the need for alternative treatments in war-affected regions.



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Abbreviations

MHA: Mueller-Hinton agar.

CFU: Colony-Forming Unit.

DMSO: Dimethylsulfoxide.

EUCAST: The European Society of Clinical Microbiology and Infectious Diseases.

MIC: Minimum Inhibitory Concentrations.

HAIs: Hospital-acquired infections.

1 Introduction

Bacterial infections in hospitals, known as nosocomial or hospital-acquired infections (HAIs), are a significant public health problem. These infections can occur in patients during their stay in hospitals and other healthcare facilities. Common pathogens responsible for HAIs include bacteria, viruses, and fungi, which bacteria alone causing about 90% of these infections (Revelas, 2012).

Escherichia coli is a Gram-negative bacterium commonly found in the intestines of humans and animals, often associated with urinary tract infections and foodborne illnesses. *Enterococcus* spp. are Gram-positive cocci that are part of the normal intestinal flora but can cause infections in hospitalized patients, particularly in urinary tract infections and bacteremia. *S. aureus* is a Gram-positive bacterium that can cause a range of illnesses from minor skin infections to life-threatening diseases like pneumonia, meningitis, and sepsis. *A. baumannii* is a Gram-negative bacterium that is increasingly recognized for causing

severe infections in hospitalized patients, including ventilator-associated pneumonia and bloodstream infections. *B. gladioli* is a Gram-negative bacterium typically found in soil and water, and it can cause infections in immunocompromised patients (Todar 2020).

The bacterial strains *E. coli*, *Enterococcus* spp., *S. aureus*, and *B. gladioli* demonstrated resistance to Cefixime. Furthermore, *E. coli*, and *Enterococcus* spp., exhibited resistance to both Levofloxacin and Ciprofloxacin. Resistance to Cotrimoxazole was observed in *E. coli*, *B. gladioli* and *Enterococcus* spp.. Additionally, *B. gladioli*, *E. coli*, *Enterococcus* spp., and *A. baumannii* were resistant to Amikacin.

Many plants produce secondary metabolites with antimicrobial properties, which have been harnessed for centuries to treat infections and other ailments. Extracts from *Verbascum* species and other medicinal plants exhibit diverse biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects, which are crucial in developing novel therapies for persistent infections (Elshafie et al. 2023).

Verbascum sp. (Common Mullein), a member of the Scrophulariaceae family, is a biennial plant known for its medicinal properties. Native to Europe and Asia, it can reach heights of up to 2 meters. Traditionally, this plant has been used to treat various medical conditions such as headaches, fever, cramps, and burns. Recent studies have highlighted its potential as an antioxidant and antibacterial

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agent, making it valuable in modern medicine for combating infections (Mahdavi et al. 2020).

Common mullein has been traditionally used to treat various ailments, including inflammatory diseases and respiratory conditions such as asthma. Recent studies have revealed its potential as an antimicrobial agent, making it a valuable resource in the fight against drug-resistant bacteria often found in hospitals (Dulger et al. 2015).

The genus *Verbascum* is known for its diverse range of bioactive compounds with potential antibiotic properties. Key among these are saponins, flavonoids, and phenylethanoids. Saponins, such as verbascoside, exhibit notable antimicrobial activities, inhibiting the growth of various pathogenic bacteria (Alipieva et al. 2014; Zengin et al. 2023).

Flavonoids, including quercetin and luteolin, contribute to antimicrobial effects by disrupting microbial cell membranes and inhibiting enzyme activities (Amini et al. 2022).

One notable study by Mahdavi et al. (2020) highlights the antimicrobial effects of saponins extracted from *Verbascum thapsus*. The study reveals that these saponins are effective against a range of pathogenic microorganisms. The antimicrobial activity of saponins is attributed to their ability to interact with microbial cell membranes, leading to cell lysis and disruption of microbial growth (Mahdavi et al. 2020).

A study found that extracts from common mullein leaves exhibit strong antimicrobial activity against *Escherichia coli*, *Enterococcus faecalis*, and *Candida albicans*. The inhibition zones were 19.2, 16.8, and 16.2 mm, respectively (Dulger et al. 2015).

Methanolic extracts from *Verbascum napifolium* exhibited both anticancer and antimicrobial properties, showing efficacy against various bacterial strains. The study employed micro-well dilution assays to establish the minimum inhibitory concentrations (MIC) for different bacterial pathogens, highlighting the potent antimicrobial capabilities of the extracts (Taşkaya et al. 2023).

In another study, Methanolic extracts of *Verbascum speciosum* displayed significant antibacterial activity and have been traditionally used for treating wounds and skin disorders. The study revealed that these extracts were effective against various bacterial strains, underscoring their value in traditional medicinal practices (Pourmoslemi et al. 2023).

One of the major challenges in treating HAIs is the increasing resistance of bacteria to conventional antibiotics. This resistance has been attributed to the overuse and misuse of antibiotics, leading to the emergence of drug-resistant pathogens. These resistant strains are difficult to treat, resulting in prolonged hospital stays, higher medical costs, and increased mortality rates (Lone et al. 2024). The growing problem of antibiotic resistance necessitates the search for alternative treatments. Researchers are increasingly looking towards natural and plant-based compounds as potential sources of new antimicrobial agents. These

alternatives are seen as promising due to several key advantages over synthetic pharmaceuticals. These advantages include lower costs, easier accessibility, and a reduced frequency of side effects (Saify Nabiabad et al. 2023). Plant extracts have a long history of use in traditional medicine across various cultures for their therapeutic properties. Despite the growing antibiotic resistance among hospital-acquired bacteria, there is limited research on the efficacy of plant extracts in Rojava (a region in northern and northeastern Syria) as alternative treatments.

The aim of this research is to investigate the antimicrobial activity of *Verbascum* species against hospital-associated bacteria, addressing the need for alternative treatments due to the high cost of conventional antibiotics, restrictions on drug imports due to the blockade, increased antibiotic consumption in hospitals due to bacterial resistance, and the challenges faced in war-affected regions. This study highlights the potential of local plants as viable therapeutic options.

2 Material and methods

2.1 Plant material

Leaves of mature plants were collected from the Northern countryside of Aleppo, Fafeen area (36°13'41.8"N 37°13'41.8"E). The gathered plant materials were moved to the Biotechnology laboratory at the Institute of Science and Modern Technology.

2.2 Preparation of extract

After collecting the local *Verbascum* sp. leaves, they were thoroughly washed under

tap water and dried at a laboratory temperature of 24 ± 2 °C for two weeks. Subsequently, the leaves were ground into powder using a grinder. The powder was then stored in a sealed container at room temperature. Twenty grams of the dried *Verbascum* sp. leaf powder were placed in separate Erlenmeyer flasks, and 200 mL of methanol solvent was added to each flask. The flasks were covered with aluminum foil and placed on a shaker for one day. The extracts were filtered through filter paper and evaporated at 40 °C using a rotary evaporator. The extracts were collected, weighed, and stored in the refrigerator until use.

2.3 Cultivation and maintenance of test microorganisms

Certain clinical bacteria, isolated from two tertiary hospitals in Rojava, were identified using both morphological and biochemical characteristics, in addition to PCR techniques with species-specific primers. A random selection of bacterial strains was chosen from a group of samples isolated from patients who developed nosocomial infections. These bacteria include *Escherichia coli* (*E. coli*), *Enterococcus* spp., *S. aureus*, *A. baumannii*, and *B. gladioli*.

2.4 Antibacterial assay

2.4.1 Agar well diffusion assay

The bacterial inoculum suspension was evenly spread on a solid Mueller-Hinton agar (MHA) plate. Wells, 6 mm in diameter, were cut using a sterile borer. The test materials, consisting of 50 µL of methanolic extract at a concentration of 100

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mg per 1 mL, were applied. into each well. This concentration is lower than that used by Dulger, Basaran, and Ahmet Gonuz (2004), who used 200 mg/mL, comparable with Pourmoslemi et al (2023). After incubation, zones of inhibition around the wells were measured.

2.4.2 MIC determination

The minimum inhibitory concentration (MIC) values were examined for microorganisms identified as sensitive to *Verbascum* sp. extract using the Agar Dilution method (Figure 2.). The *Verbascum* sp. leaf extract was dissolved in 10% dimethylsulfoxide (Chem-lab, Belgium).

The following concentrations of the plant extract were prepared: 10000, 5000, 2500, 1250, 625, 312, and 156 µg/mL. Then 0.5 mL of each concentration was added to 9.5 mL (1:20) of solid M.H.A (HiMedia, India) at 50°C (EUCAST 2000). The final concentration of extract in plates was 500, 250, 125, 62.5, 31.2, 15.6 and 7.8 µg/mL. After that, each 10 milliliters of MHA-Extract were poured into small sterile Petri dishes (6 cm in diameter) before solidification.

The inocula were prepared from 12-hour broth cultures. The final inoculum of 1×10^4 CFU per spot was prepared by diluting a 0.5 McFarland suspension 10-fold in NaCl (Kowalska-Krochmal and Ruth, 2021). For Gram-negative bacteria, suspensions were adjusted to absorbance values of 0.1 ± 0.02 . Absorbance was measured at 600 nm using a spectrophotometer (Phylo, UV-920). Within 30 min of preparation, 1 µL of this diluted suspension was applied to the MHA media.

The plates were placed in an incubator at 35 ± 1 °C and left for 16-20 hours. The MIC was defined as the lowest concentration of that inhibited the visible growth of the microorganism. Positive controls (bacteria without extract) and negative controls (MHA only) were included to validate the results.

3 Results

The antibacterial activity of the methanolic extract of *Verbascum* sp. leaf at a concentration of 100 mg/mL is detailed in Table 1. This activity is compared with that of Imepenem and Amikacin against various bacterial isolates.

Table 1. Antibacterial Activity of Methanolic Extract of Verbascum sp. Leaf Compared with Imepenem and Amikacin Against Various Bacterial Isolates

Bacteria isolates	Inhibition zone (mm) ^a			
	Extract (100 mg/mL)	Imepenem	Amikacin	P-value
<i>A. baumannii</i> ^{*36}	19.33 ± 0.58	15.00±1.00	Nt	0.01
<i>E. coli</i> ^{*17}	0.00±0.00	18.67±0.58	Nt	-
Enterococcus spp ^{*1}	17.67±1.52	20.67±1.52	Nt	0.074
<i>S. aureus</i> ^{*35}	19.67±1.53	Nt	23.33±0.57	0.032
<i>B. gladioli</i> ^{*15}	0.00±0.00	Nt	16.33±0.58	-

^{*36, 17, 1,35}, and 15 are laboratory codes. Nt: not tested. ^a Includes 6mm well diameter.

The methanolic extract of Verbascum sp. leaves exhibits notable antibacterial activity. The study's findings reveal that this extract has varying efficacy against different bacterial strains.

In the case of Enterococcus spp, the extract shows an inhibition zone of 17.67±1.52 mm, while Imepenem demonstrates a slightly higher activity with an inhibition zone of 20.67±1.52 mm. The P-value of 0.074 suggests that the extract is nearly as effective as Imipenem against this bacterium, with the difference being statistically insignificant.

Regarding *S. aureus*, the extract displays an inhibition zone of 19.67±1.53 mm (Figure 1), which is notably greater than the 23.33 ±0.57 mm observed with Amikacin. The P-value of 0.032 indicates that the difference in antibacterial activity between the methanolic extract and Amikacin is

statistically significant, supporting the efficacy of the Verbascum sp. extract against this *S. aureus*. Overall, the Verbascum sp. leaf extract is effective against *A. baumannii*, Enterococcus spp, and *S. aureus*. However, it does not show activity against *E. coli* and *B. gladioli*. The significant P-values for *S. aureus* and marginal values for *A. baumannii* and Enterococcus spp suggest that the extract could be a viable alternative or complement to conventional antibiotics for these bacteria. Nevertheless, its lack of effectiveness against certain strains limits its broader applicability.

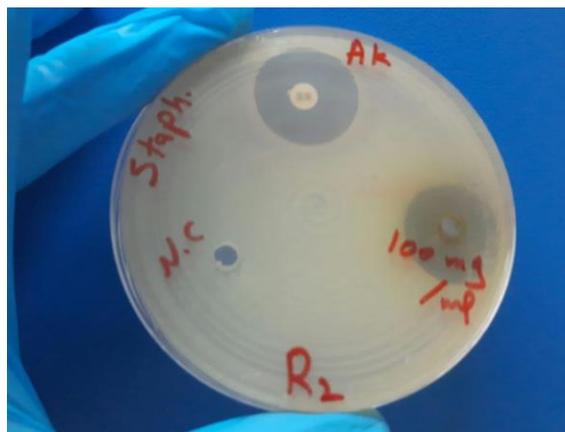


Figure 1. The application of the plant extract against *S. aureus* bacteria. "N.C." indicates the negative control, and "R2" refers to repeat number 2.

The results demonstrated that the extract exhibited varying degrees of inhibitory activity, as summarized in (Table 2.)

Table 2. The Minimum Inhibitory Concentrations (MIC) of Various Bacterial Isolates using methanolic extract of *verbascum sp.*.

Bacteria isolate	MIC ($\mu\text{g/mL}$)
<i>A. baumannii</i>	125
Enterococcus spp	250
<i>S. aureus</i>	125

The inhibitory effects of the *Verbascum* extract at different concentrations using agar plate dilution is provided in figure 2.

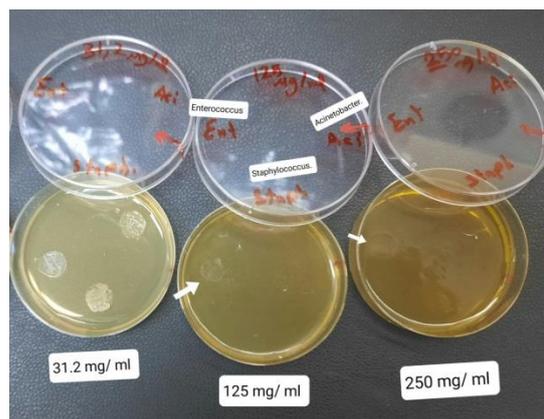


Figure 2. Provides a visual representation of the inhibitory effects of the *Verbascum* extract at different concentrations. The abbreviations "aci," "ent," and "staph" correspond to *A. baumannii*, *Enterococcus* species, and *S. aureus*, respectively.

4 Discussion

In the study, the methanolic extract of *Verbascum sp.* leaves demonstrated varying degrees of antibacterial activity against several bacterial strains. Notably, the extract was effective against *A. baumannii*, *Enterococcus spp.*, and *S. aureus*, as evidenced by inhibition zones of 19.33 mm, 17.67 mm, and 19.67 mm, respectively. For *Enterococcus spp.*, the extract showed an inhibition zone of 17.67 ± 1.52 mm, while Imipenem demonstrated a slightly higher activity with an inhibition zone of 20.67 ± 1.52 mm. The P-value of 0.074 suggests that the extract is nearly as effective as Imipenem against this bacterium, with the difference being statistically insignificant.

The MIC values further highlighted the efficacy of the extract, with 125 $\mu\text{g/mL}$ for *A. baumannii* and *S. aureus*, and 250 $\mu\text{g/mL}$ for *Enterococcus spp.*. These results align with previous studies, such as the findings by Yabalak et al. (2022), which demonstrated significant antimicrobial

activity of *Verbascum pseudoholotrichum* extracts against various bacteria, including *Enterococcus faecalis*, with a MIC of approximately 45.5 µg/mL (Yabalak et al. 2022). Additionally, the study by Dulger, Basaran, and Ahmet (2004) found no antimicrobial activity against *E. coli* with *Verbascum gypsicola* extract, consistent with our current findings (Dulger et al. 2004).

The extract exhibited no activity against *E. coli* and *B. gladioli*. This lack of effectiveness is consistent with the findings of Nofouzi et al. (2016), who also reported resistance of *E. coli* to similar extracts.

Arslan (2002) found that among the three *Verbascum* species studied, only *Verbascum bombyciferum* displayed antimicrobial activity against *E. coli*. However, all three species showed significant activity against *S. aureus*. The species examined were *Verbascum olympicum*, *Verbascum prusianum*, and *Verbascum bombyciferum*.

The observed resistance of *E. coli* to the current plant extract can be attributed to the source of the bacterial strain. The *E. coli* used in this study was isolated from hospital settings and demonstrated resistance to various antibiotics, such as cotrimoxazole, levofloxacin, ciprofloxacin, and cefixime. This suggests that the hospital-derived strain may possess unique resistance mechanisms or exhibit a higher level of antibiotic resistance compared to strains used in earlier studies.

Similar to the rarity of *B. gladioli* bacteria in the medical field, it is expected that research on *Verbascum* sp. against *Burkholderia* sp. is limited. In our study, we investigated the antimicrobial effects of *Verbascum* sp. extracts against

Burkholderia sp. and found no significant activity.

5 Conclusion

The methanolic extract of *Verbascum* sp. leaves demonstrated significant antibacterial activity against several hospital-acquired bacterial strains, including *A. baumannii*, *Enterococcus spp.*, and *S. aureus*. The observed inhibition zones and MIC values highlight the potential of *Verbascum* sp. extract as an alternative or complementary treatment to conventional antibiotics, especially given the growing issue of antibiotic resistance. Notably, the extract's efficacy against these bacteria supports its use in treating infections caused by resistant strains. However, the extract showed no activity against *E. coli* and *B. gladioli*, underscoring the need for further research to understand and overcome these limitations.

The findings align with previous studies, reinforcing the antimicrobial potential of *Verbascum* species. However, the resistance of *E. coli* observed in this study can be attributed to the hospital-derived strain's unique resistance mechanisms, indicating a need for targeted strategies against such resilient bacteria. Additionally, the study highlights the scarcity of research on *Verbascum* sp. against rare pathogens like *B. gladioli*, suggesting an area for future exploration.

Overall, *Verbascum* sp. leaf extract presents a promising avenue for developing new antimicrobial agents, particularly in the face of increasing antibiotic resistance and the need for alternative treatments in war-affected regions. Further studies should focus on enhancing the extract's efficacy and expanding its spectrum of activity to

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include a broader range of bacterial pathogens.

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