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Assessment of Ivy Leaf Extracts for Antibacterial Activity against Pathogenic Bacteria: No Evidence of Efficacy

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Abstract
Background: The global spread of antibiotic resistance in bacteria such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa* has created an urgent need for new antimicrobial treatments. This is especially critical in Libya, where infections from multidrug-resistant pathogens are common. **Aim:** This study aimed to investigate the in vitro antibacterial potential of aqueous and alcoholic extracts from ivy leaves (*Hedera helix*) against these key pathogenic bacteria. **Materials and Methods**: We prepared extracts using dried ivy leaves and tested them against clinical bacterial isolates using standard disc diffusion and broth macro-dilution methods to determine the minimum inhibitory concentration (MIC). **Results:** Our results showed a complete absence of antibacterial activity. No zones of inhibition were observed in the disc diffusion assay, and the MIC for all extracts against all bacterial strains was determined to be greater than 100%, the highest concentration tested. **Conclusion:** These findings indicate that the therapeutic value of *H. helix* leaf extracts, while well- established for respiratory symptoms, does not include a direct antibacterial effect against the pathogens tested. The search for novel antimicrobials from botanical sources should focus on other plants and more advanced extraction techniques.
Keywords: Ivy Leaf Extracts, Antibacterial Activity, Pathogenic Bacteria

Introduction
The escalating crisis of antimicrobial resistance (AMR) poses a major threat to global public health, as pathogenic bacteria evade antibiotic pressure and immune defenses by manipulating host cell components—particularly the cytoskeleton—thereby enhancing persistence and reducing the effectiveness of standard antibiotics against common bacterial pathogens. [1,2,3] Multidrug-resistant (MDR) bacteria, including *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa*, are leading causes of severe infections in both healthcare and community settings, contributing to high rates of illness, mortality, and healthcare costs [4]. In Libya, the problem is particularly severe, with documented reports of methicillin-resistant Staphylococcus aureus (MRSA) [5], vancomycin-resistant *enterococci* [6], and carbapenem-resistant Acinetobacter baumannii and K. pneumoniae [7]. This alarming situation requires the urgent exploration of alternative and complementary treatment strategies. Proteus mirabilis has occasionally been reported as a cause of pneumonia in humans. Due to its rarity and the presence of underlying chronic conditions, such infections may be misdiagnosed as malignant disease [8,9].

Medicinal plants represent a rich source of bioactive compounds and have a long history of use in treating various ailments, including infectious diseases [10]. Their potential for novel mechanisms of action, lower toxicity, and synergy with existing antibiotics makes them a compelling focus for research [11]. In Libya, some native plants like Cistus salvifolius and Salvia officinalis have shown promising antibacterial activity against MDR strains [12,13].

Ivy leafs (Hedera helix L.) is a well-established herbal medicine used primarily for its expectorant, antispasmodic and anti-inflammatory properties in treating upper respiratory tract infections [14,15] Its efficacy is attributed to saponins, such as hederacoside C and α-hederin, which help stimulate bronchial secretion and widen the [16,17]. While its benefits for relieving symptoms are clear, its direct antibacterial potential is less studied and often ambiguous in the scientific literature [18,19]. Some studies suggest mild antimicrobial effects, but conclusive evidence against prevalent MDR pathogens is lacking.

Given the traditional use of ivy (Hedera helix) for respiratory illnesses in which largely are associated with bacterial infections, there is a vital need to systematically assess its direct antibacterial activities. Therefore, this study aims to rigorously evaluate the in vitro antibacterial efficacy of both aqueous and alcoholic extracts of H. helix leaves against a panel of clinically relevant multidrug-resistant (MDR) bacteria isolated from a Libyan hospital setting. This approach will help identify potential sources of alternative antibacterial agents to combat multidrug-resistant pathogens in clinical practice.

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Materials and Methods

Plant Material and Extract Preparation

Dried leaves of *H. helix* were obtained from a licensed herbal shop (Al-Khawildi Herb Shop, Zahra City, Libya). The plant material was cleaned of any physical impurities. The leaves were ground into a fine powder using a sterile electric grinder. We used two extraction solvents: distilled water and 70% ethanol. However, Water and ethanol were used because they are safe, effective, and suitable for extracting biologically active compounds from plants for food and medical applications [20,21]

For each solvent, 100 g of powder was mixed with 400 mL of solvent in sealed (1:4 w/v), opaque glass containers. The mixtures were shaken continuously for 48 hours at room temperature. The resulting crude extracts were then centrifuged to remove solid debris. The liquid supernatants were concentrated using a rotary evaporator under reduced pressure and then dried in an oven at 40–45°C for one week. This process yielded 4.86 g of alcoholic extract and 1.85 g of aqueous extract.

Solution and Disc Preparation

Stock solutions were prepared by dissolving the dried extracts in distilled water to a final concentration of 100% (1 g/ml) and stored at −20 °C. A secondary stock solution (200 mg/mL) was prepared from each extract for downstream experiments. Serial dilutions (10%, 20%, 30%) were then made from this working solution using sterile distilled water. Sterile filter paper discs (6 mm diameter) were soaked with 20 µL of each extract concentration. Control discs were prepared using 70% ethanol and air-dried under sterile conditions to evaluate any potential effect of residual solvent.

Antibiotic Susceptibility test : Broad range of antibiotics (Cefotaxime (CTX30 µg), Gentamicin (CN10 µg), Co-trimoxazole (Bactrim STX25µg), Meropenem (MEM10 µg), Ciprofloxacin (CIP5 µg), Levofloxacin (LEV 5 µg), Ceftriaxone (CRO 30 µg) Azithromycine (AT30 µg), Augmentin (amoxicillin/clavulanic acid (30 µg), Gentamicin (CN 10µg), Doxycycline (DOX 30 µg) and Doxycycline (30 µg),) were used in this assay to cover both Gram-negative and G-positive strains.

Bacterial Strains and Susceptibility Testing

Clinical swabs of *S. aureus, K. pneumoniae, and Proteus mirabilis* were obtained from a local diagnostic laboratory after identification and were handled according to standard biosafety procedures in cooler bags. These strains, isolated from hospitalized patients, are potentially representative of local MDR pathogens. For ethical considerations, no patient information was collected. Bacterial suspensions were prepared and adjusted to the 0.5 McFarland standard. Antibacterial activity was evaluated using the standard Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) plates, following established guidelines (CLSI, 2018). For each bacterial strain, three MHA plates were inoculated. Test discs and control discs were placed on the agar surfaces. The plates were incubated at 37°C for 24 hours, after which any zones of inhibition were measured.

The minimum inhibitory concentration (MIC) was determined using a broth macrodilution method. A series of two-fold dilutions of the extracts was prepared in liquid nutrient broth. A 500 µL aliquot of each concentration was transferred to sterile test tubes, followed by 500 µL of the standardized bacterial suspension. Tubes were incubated at 37°C for 24 hours. After incubation, a sample from each tube was placed on fresh MHA plates to check for bacterial growth, thus determining the MIC as the lowest concentration that prevented visible growth.

Statistical Analysis

All experiments were performed in triplicate. Due to the complete absence of inhibitory activity in all tests, the results are presented descriptively.

Results

Both the aqueous and alcoholic extracts of Hedera helix leaves showed no antibacterial activity against any of the tested bacterial strains.

Disc Diffusion Assay

No zones of inhibition were observed around any of the discs containing the ivy leaf extracts at any of the tested concentrations (10%, 20%, 30%) for any of the four bacterial species after 24 hours of incubation. The negative control discs also showed no zones, confirming that the solvent had no antibacterial effect.

Minimum Inhibitory Concentration (MIC) Assay

In the broth macro-dilution test, all tubes containing bacteria and any concentration of ivy extract showed visible turbidity after 24 hours, indicating bacterial growth. Subculturing from these tubes onto solid MHA media resulted in confluent bacterial growth, confirming the absence of any bactericidal or bacteriostatic activity. The MIC for both extracts against all tested bacteria was therefore greater than 100%.

Antibiotic Susceptibility Profile

The tested bacterial isolates showed variable susceptibility patterns to the antibiotics evaluated. Fluoroquinolones exhibited the highest antibacterial activity, with ciprofloxacin (35 mm) and levofloxacin (34 mm) producing the largest inhibition zones, classifying all tested isolates as susceptible according to CLSI/EUCAST criteria. Ceftriaxone (29 mm) and gentamicin (20 mm) also demonstrated antibacterial activity within the susceptible range. In contrast, meropenem displayed moderate activity, with a mean inhibition zone of 16 mm, corresponding predominantly to an intermediate susceptibility classification. Azithromycin produced minimal inhibition (5 mm), indicating resistance among the tested isolates. Similarly, cefotaxime and co-trimoxazole (Bactrim) showed no inhibitory effect against Proteus spp., with no measurable zones of inhibition. Amoxicillin/clavulanic acid (Augmentin) and doxycycline were ineffective against *Klebsiella pneumoniae* and *Staphylococcus aureus*. Although Augmentin produced a small inhibition zone (9 mm) against *Klebsiella pneumoniae*, this remained

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below the susceptibility breakpoint and was classified as resistant. In contrast, doxycycline displayed susceptible activity against *Proteus mirabilis* (20mm).

Discussion:

This study found no evidence of antibacterial activity for either aqueous or alcoholic extracts of *Hedera helix* leaves against several clinically significant bacteria, including MDR strains relevant to Libya. The negative results were consistent across two standard testing methods and a wide range of concentrations.

The main value of ivy leaf extracts lies in managing inflammatory respiratory conditions like acute bronchitis, where they serve as effective expectorants and bronchodilators [22,23]. The bioactive saponins, such as α-hederin, work by stimulating specific receptors, leading to increased bronchial secretion and thinner mucus [24]. Our findings confirm that this mechanism is separate from a direct antibacterial action. This suggests that any clinical improvement in respiratory infections from ivy is likely due to symptomatic relief and not the killing of bacteria.

The pathogens tested, particularly *P. aeruginosa* and *K. pneumoniae*, are known for their strong resistance mechanisms, including efflux pumps, drug-inactivating enzymes, and impermeable outer membranes [25,26]. The lack of activity against these robust bacteria indicates that the compounds extracted by water and ethanol, primarily saponins and flavonoids, are not potent enough to overcome these bacterial defenses [27]. The solvents used for extraction are an important factor. While water and ethanol are good for extracting the saponins responsible for ivy's expectorant effects [16], they might not be suitable for isolating other potential antimicrobial compounds. Other research on Libyan plants, such as Cistus salvifolius, found strong anti-MRSA activity using methanol [11], suggesting that a different solvent might be needed for ivy. Also, testing other parts of the plant or using more advanced extraction methods could reveal bioactive components that this study did not capture.

The observed susceptibility pattern aligns with recent regional and global data, indicating preserved fluoroquinolone efficacy due to potent bactericidal activity and good bioavailability, although their overuse contributes to the development of antimicrobial resistance ([28] .The moderate activity of ceftriaxone and gentamicin suggests the presence of substantial yet variable resistance, while the intermediate response to meropenem indicates the emerging development of carbapenem resistance mediated by carbapenemase genes, as previously reported with different pathogenic isolates in Iraq [29,30] . High resistance to azithromycin, cefotaxime, co-trimoxazole, and amoxicillin/clavulanate is consistent with intrinsic and acquired mechanisms in *Proteus* and *Klebsiella* Spp. [31,32,33] These findings underscore the necessity for local antimicrobial susceptibility testing and stewardship to guide empirical therapy and curb resistance [34,35].

A limitation of this work is the absence of a positive control antibiotic in the diffusion assays, which would have fully confirmed the validity of our experimental conditions. Future studies should include this control. Testing the extracts against less resistant bacteria commonly linked to respiratory infections could also provide useful information.

Conclusion:

In conclusion, the results of this in vitro study do not support the use of Hedera helix leaf extracts, prepared with water or alcohol, as a direct antibacterial treatment against common pathogenic bacteria, including MDR strains. The findings reinforce the existing view that ivy's clinical benefit comes from managing cough symptoms rather than from antimicrobial activity. The urgent need for new antimicrobials, particularly in high-AMR regions such as Libya, remains. Future research should focus on other medicinal plants with a history of traditional use, employing a broader range of extraction methods to more thoroughly explore their potential against resistant pathogens.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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addition

Table 1: Results of Antibacterial Activity Tests of Ivy Leaf Extracts

Bacterial Strain	Test Method	Extract Type	Concentrations Tested	Result (Inhibition Zone / Growth)
Staphylococcus aureus	Disc Diffusion	Aqueous	10%, 20%, 30%	0 mm / No zone
		Alcoholic	10%, 20%, 30%	0 mm / No zone
	MIC	Aqueous	12.5% - 100%	Growth at all concentrations
		Alcoholic	12.5% - 100%	Growth at all concentrations
Klebsiella pneumoniae	Disc Diffusion	Aqueous	10%, 20%, 30%	0 mm / No zone
		Alcoholic	10%, 20%, 30%	0 mm / No zone
	MIC	Aqueous	12.5% - 100%	Growth at all concentrations
		Alcoholic	12.5% - 100%	Growth at all concentrations
proteus mirabilis	Disc Diffusion	Aqueous	10%, 20%, 30%	0 mm / No zone

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		Alcoholic	10%, 20%, 30%	0 mm / No zone
	MIC	Aqueous	12.5% - 100%	Growth at all concentrations
		Alcoholic	12.5% - 100%	Growth at all concentrations
Control (70% Ethanol)	Disc Diffusion	Control for solvent		0 mm / No z

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