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Comparative antimicrobial activity of ethanol and aqueous extracts of *Ziziphus lotus* against bacteria and fungi

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ABSTRACT

Background: Ziziphus lotus (L.) is a medicinal plant known by its potential antioxidant activities. Its fruits, leaves, and roots have been employed to treat various deseases including inflammation, gastrointestinal intoxication, anxiety, diabetes, and tuberculosis infection.
Objective: This study investigates the antibacterial and antifungal activities of aqueous and ethanol extracts from different parts of *Ziziphus lotus* (roots, stems, leaves, flowers, and fruits) against a range of bacterial and fungal strains. Eight bacterial strains (*Staphylococcus aureus*, *Micrococcus luteus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) and five fungal strains (*Candida albicans*, *Candida krusei*, *Candida neoformans*, *Aspergillus brasiliensis*, and *Aspergillus fumigatus*) were tested.
Results: The ethanol fruit extract exhibited the most potent antibacterial activity, with a minimum inhibitory concentration (MIC) of 0.012 mg/mL against *Staphylococcus aureus* and strongest antibacterial activity against *Bacillus cereus*. Leaf and flower extracts showed activity against *Enterococcus faecalis*. Aqueous extracts demonstrated moderate antibacterial effects, with the leaf extract showing an MIC of 1.23 mg/mL against *Staphylococcus. aureus*, and the flower extract being effective against *Listeria monocytogenes* and *B. cereus* (MIC of 18.75 mg/mL). For antifungal activity, the ethanol fruit extract exhibited the strongest effects, with a MIC of 0.012 mg/mL against *C. albicans*, and significant activity against *C. krusei* and *A. brasiliensis* (0.11 mg/mL). The aqueous leaf extract showed the best antifungal activity, with an MIC of 1.23 mg/mL against *C. albicans*, and notable effects against *Aspergillus* spp. (11.11 mg/mL).
Conclusion: These results confirm the traditional use of *Ziziphus lotus* in treating bacterial and fungal infections, demonstrating the activity of its extracts against a wide range of pathogens.

1. Introduction

Bacterial infections continue to pose a major global health threat, with rising concerns about antimicrobial resistance undermining the activity of existing antibiotics. Since the discovery of penicillin in 1928, antibiotics have been instrumental in controlling infectious diseases (Doğan, 2022). However, their activities is increasingly compromised due to issues such as low solubility, reduced bioavailability, and the frequent requirement for high or repeated doses (Chinemerem et al., 2022). The World Health Organization has declared antibiotic resistance a global public health emergency, with projections indicating it could result in over 50,000 deaths per day worldwide if left unaddressed (Botelho et al., 2019; Duan et al., 2024). For example, more than 95 % of *Staphylococcus aureus* (*S. aureus*) strains are now resistant to penicillin (Gajdács, 2019; Wu et al., 2021).

In light of these challenges, there is renewed interest in plant-based therapeutics as sources of novel bioactive compounds. Medicinal plants have been used for centuries in traditional healing systems, and today

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they are gaining recognition for their accessibility, affordability, and pharmacological potential (Bencheikh et al., 2023; Ouassou et al., 2021). The World Health Organization estimates that around 80 % of the global population relies on traditional medicine, largely derived from plants (Alemu et al., 2024). These medicinal properties are often attributed to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids (Sivapragasam et al., 2024). Indeed, it is estimated that 60 %–80 % of approved drugs are derived from natural sources (Dehelean et al., 2021).

Ziziphus lotus (L.) Lam., (*Z. lotus)* a Mediterranean plant belonging to the Rhamnaceae family, is one such medicinal plant with a long history of traditional use. Its fruits, leaves, and roots have been employed to treat various ailments including inflammation, pain, gastrointestinal disorders, anxiety, diabetes, and tuberculosis (Saoud et al., 2023). The plant is also valued for its nutritional and industrial applications, being consumed in forms such as tea, juice, jam, honey, and oil, and is increasingly used in cosmetics and health foods (Gorai et al., 2010; Benammar and Baghdad, 2014). Phytochemical analyses have revealed that *Z. lotus* is rich in sugars, fibers, vitamins, fatty acids, minerals, tannins, and antioxidants (Berkani et al., 2021). Key bioactive compounds identified in this species include flavonoids, triterpenic acids, polysaccharides, and betulinic acid, which contribute to its documented antioxidant, antibacterial, anti-inflammatory, hepatoprotective, and anticancer properties (Gorai et al., 2010; Yahia et al., 2020).

Previous studies on *Z. lotus* have primarily focused on the antibacterial or antioxidant activities of isolated extracts from a single part of the plant. However, limited research has systematically compared the antimicrobial potential of different plant parts (roots, stems, leaves, flowers, and fruits) using both aqueous and ethanol extraction methods. The choice of solvent plays a crucial role in extracting specific phytochemicals: water extracts more polar compounds (e.g., glycosides, polysaccharides), while ethanol is more effective for phenolics, flavonoids, and terpenoids. Evaluating both solvent types allows for a broader understanding of the plant's pharmacological profile.

This study addresses the research gap by providing a comparative evaluation of the antibacterial and antifungal activities of aqueous and ethanol extracts from five distinct parts of *Z. lotus*. To our knowledge, this is the first study to test such a comprehensive range of extracts against a panel of eight bacterial and five fungal strains, including both Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*), *Micrococcus luteus* (*M. luteus*), *Listeria monocytogenes (L. monocytogenes), Enterococcus faecalis* (*E. faecalis*), *Bacillus cereus* (*B. cereus*) and Gram-negative bacteria; *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa)* and *Salmonella typhimurium (S. typhimurium*), as well as clinically relevant fungi; *Candida albicans*, *(C. albicans) Candida krusei (C. krusei), Candida neoformans* (*C. neoformans), Aspergillus brasiliensis* (*A. brasiliensis) and Aspergillus fumigatus (A. fumigatus*).

2. Materials and methods

2.1. Materials and chemicals

Ethanol and aqueous extracts were prepared from different parts of *Z. lotus* (leaves, fruits, flowers, stems, and roots) using maceration. Each plant material was dried, ground into fine powder, and macerated separately in ethanol (80 %) or distilled water (1:10 w/v) for 48 h at room temperature (22 °C) with occasional shaking. The extracts were filtered, evaporated under reduced pressure (for ethanol), or lyophilized (for aqueous extracts), and stored at 4 °C until use. To evaluate the potential influence of the solvent, pure ethanol (95 %) was tested independently using the Agar Well Diffusion Method. A well was filled with 50 μL of pure ethanol to assess its antimicrobial activity and ensure that any observed effect could be attributed solely to the bioactive compounds in the plant extracts, rather than to the solvent itself.

Antibacterial and antifungal activities were evaluated against eight reference bacterial strains (five Gram-positive and three Gram-negative), three yeast strains, and two filamentous fungi. Microbial cultures were grown in appropriate media: Nutrient Agar and Mueller-Hinton Agar/Broth for bacteria, Sabouraud Dextrose Agar for fungi, and Yeast Peptone Dextrose (YPD) for yeasts. The minimum inhibitory concentration (MIC) was determined by the broth microdilution method following CLSI guidelines. Gentamycin (10 μg/mL) and Amphotericin B (250 μg/mL) were used as positive controls, while DMSO and sterile water served as negative controls where appropriate.

2.2. Extraction and fractionation

The biological activity of plant extracts can indeed be influenced by multiple factors, including the time of collection and the location of the plant. For this study, fresh plant materials (leaves, flowers, fruits, roots, and stems) of *Z. lotus* were collected during the peak flowering period in May and at the fruiting stage in September, to ensure optimal levels of bioactive compounds. The flowers were harvested at full bloom, while mature fruits were collected later in the season. Roots were carefully excavated to preserve their structural integrity, and all plant parts were immediately washed with distilled water to remove debris before being dried in the shade at ambient temperature (25 °C) for 8 days, thus preserving thermolabile compounds like phenolics. Once dried, the materials were ground into fine powder, sieved (0.5 mm mesh), and stored in dark containers at room temperature (22 °C) until use.

For ethanol extraction, a total of 4 g of each powdered plant part was subjected to ethanol extraction with continuous agitation on a rotary shaker at 150 rpm for 1 h at 22 °C. After filtration (Whatman No. 1) and centrifugation at 4000 rpm (~3000×*g*) for 15 min at 4 °C. The ethanol was removed under reduced pressure using a rotary evaporator, and the resulting extracts were stored at −20 °C. For aqueous extraction, the powdered plant materials were suspended in distilled water at specific concentrations: 100 mg/mL for leaves and fruits, and 200 mg/mL for flowers, roots, and stems. The suspensions were stirred using a magnetic stirrer at 150 rpm for 1 h at room temperature (22 °C). After agitation, the mixtures were centrifuged at 5000 rpm (~4700×*g*) for 15 min, and the resulting supernatants were carefully collected. These were then frozen at −20 °C and subsequently freeze-dried (lyophilized). The dry extracts were stored in airtight containers at 4 °C until further use.

2.3. Statistical analysis

All experiments were performed in triplicate. Results are expressed as mean ± standard deviation. Statistical comparisons between groups were made using one-way ANOVA followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant. Data analysis was carried out using GraphPad Prism 5.03 (GraphPad Software, San Diego, CA, USA).

2.4. Antimicrobial activity

2.4.1. Microorganisms used

The bacterial strains used in this study, such as *S. aureus* ATCC 25923, *E. coli* ATCC 35218, and *L. monocytogenes* ATCC 19115, are well-characterized and commonly associated with pathogenicity. Although the ATCC provides certified reference strains, their pathogenic nature is linked to their ability to cause disease under suitable conditions rather than to the certification itself (Treangen et al., 2014; Butler et al., 1999; Domínguez et al., 2023). In this context, the study focused on evaluating the antibacterial and antifungal activities of *Z. lotus* extracts against a wide panel of these standard strains. Antibacterial activity was assessed against eight standard bacterial strains, including five Gram-positive strains (*S. aureus* ATCC 25923, *M. luteus* NCIMB 8166, *L. monocytogenes* ATCC 19115, *E. faecalis* ATCC 29212, and *B. cereus* ATCC 11778) and three Gram-negative strains (*E. coli* ATCC 35218, *P. aeruginosa* ATCC 27857, and *S. typhimurium* ATCC 14028). Antifungal activity was tested against five pathogenic fungal strains: *C. albicans*

ATCC 90028, *C. krusei* ATCC 6258, *C. neoformans* ATCC 14116, *A. brasiliensis* ATCC 16404, and *A. fumigatus* ATCC 204305.

2.4.2. Agar Well Diffusion Method

To assess the antimicrobial activity of the *Z. lotus* extracts, the Agar Well Diffusion Method was employed. Microbial inocula were prepared from 18- to 24 h-old cultures grown on Mueller-Hinton (MH) agar. A standardized suspension of each bacterial strain (1×10^5 CFU/mL) was evenly spread onto MH agar plates using a sterile swab. Wells (8 mm diameter) were punched into the agar with a sterile Pasteur pipette and filled with 50 μL of the respective plant extract. Gentamicin (10 μg/mL) was used as a positive control. For comparison, a well was also filled with 50 μL of gentamicin (10 μg/mL), which served as the positive control. The plates were incubated at 37 °C for 24 h, after which the zones of inhibition were measured in millimetres to evaluate antibacterial activity. All tests were performed in triplicate and independently repeated for each bacterial strain to ensure accuracy and reproducibility of the results (Mirtaghi et al., 2016).

2.4.3. The minimum inhibitory concentrations (MICs)

The minimum inhibitory concentration (MIC) of the plant extracts was determined by the broth microdilution method in 96-well microplates, following the protocol described by Eloff (1998). Each well was filled with 100 μL of the serially diluted extract and inoculated with 100 μL of standardized bacterial suspension (1×10^8 CFU/mL). The plates were then incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of the extract that inhibited visible bacterial growth. To further assess microbial inhibition, the optical density at 625 nm (OD625) was measured spectrophotometrically. A decrease in turbidity compared to the control wells indicated microbial inhibition. All tests were performed in triplicate to ensure reproducibility and reliability of the results.

2.4.4. Determination of minimum bactericidal concentrations (MBC)

To determine the minimum bactericidal concentration (MBC), aliquots (10 μL) were taken from the wells of the microdilution plates that showed no visible bacterial growth (i.e., corresponding to the MIC). These aliquots were streaked onto fresh nutrient agar plates or selective media appropriate for each bacterial strain. The plates were incubated at 37 °C for 24–48 h. The MBC was defined as the lowest concentration of the extract at which no bacterial colony was observed on the agar surface, confirming the bactericidal effect of the extract. This step allowed for the distinction between bacteriostatic and bactericidal properties of the tested plant extracts (Yusuf et al., 2022).

2.5. Antifungal activity

The antifungal activity was evaluated against *three yeast (C. Albicans ATCC 90028, C. Krusei ATCC 6258* and *C. neoformans ATCC 14116,) and two fungi (A. brasiliensis. ATCC 16404* and *A. fumigatus ATCC 204305)*.

2.5.1. Principles of fungal strain preparation

Cultivating fungi in their growth phase involves transferring a strain from an isolated colony. In this process, the strains were subcultured on yeast peptone dextrose (YPD) medium and then incubated at 37 °C. For yeast strains like *Candida*, subculturing was performed 24 h before the test, while for filamentous fungi, it required at least 48 h. Once each strain had grown on YPDA medium, a fungal suspension at a specific concentration, referred to as the inoculum, was prepared.

2.5.2. Preparation of yeast suspension

For the preparation of the inoculum, all strains were first subcultured on to Sabouraud dextrose agar at 38 °C to ensure their purity and viability. Five distinct colonies, 1 mm in diameter, were picked from a 24 h culture of *Candida* strains and suspended in 5 mL of sterile 0.145 mol/L saline solution (8.5 g/L NaCl, 0.85 % saline). The resulting suspension was vortexed for 15 s, and its turbidity was adjusted visually and with a spectrophotometer by adding either sterile saline or more colonies to match the transmittance of a 0.5 McFarland standard at 625 nm. This procedure yielded a yeast stock suspension with a concentration of 10^6 to 10^5 cell/mL, producing semi-confluent growth with most *Candida species* isolates (Njunda et al., 2012).

2.5.3. Preparation of fungal suspension of filamentous fungi

Each Petri dish is rinsed twice with 10 mL of sterile distilled water. The resulting suspension is centrifuged for 5 min at 1500 rpm/min to separate the filaments from the spores. The fungal filaments settle at the bottom of the tube, while the spores remain suspended in the water. The supernatant, containing the spores, is adjusted to a concentration corresponding to an Optical Density (OD) between 0.6 and 1 at 630 nm for *Aspergillus*. To obtain the final suspension, the inoculum is then diluted to 1/10 in sterile water.

2.5.4. Inhibition test in solid medium: Agar Well Diffusion Method

Petri dishes containing Sabouraud dextrose agar medium supplemented with 2 % glucose (for yeasts) are aseptically inoculated with a suspension of 10^5 cell/mL derived from a young yeast culture. Inoculation is performed by swabbing. Once the dishes are dry, the agar is punctured at the center using the upper part of a Pasteur pipette. The resulting cavities are filled with aqueous and ethanol solutions of each extract at varying concentrations: all ethanol extracts are at a concentration of 1 mg/mL, while for aqueous extracts, leaves and fruits are at 100 mg/mL, flower, stem and root are at 200 mg/mL (50 μL/well). The Petri dishes are then incubated in an oven at 30 °C for 48 h (Essid et al., 2017).

2.5.5. Determination of the minimum inhibitory concentrations (MICs) and the minimum fungicidal concentration (MFC)

To determine the minimum fungicidal concentration (MFC), fungal growth was first assessed by visual comparison with the extract-free growth control well to establish the minimum inhibitory concentration (MIC), defined as the lowest concentration of the antifungal agent at which no discernible fungal growth occurred. Appropriate controls were included: solvent control, sterility control (without microorganisms), and growth control (without extract). Subsequently, aliquots of 100 μL from wells showing complete growth inhibition (i.e., 100 % suppression) were transferred into culture tubes containing 2 mL of Sabouraud dextrose broth (Difco, Becton, Dickinson and Company, USA). The tubes were incubated at 35 °C for 10 days. Fungal growth was quantitatively assessed by measuring the optical density (OD) at 625 nm for yeasts and at 630 nm for *Aspergillus* spp. A significant increase in OD compared to the blank indicated fungal growth, whereas an OD value comparable to that of the blank confirmed fungicidal activity. The minimum fungicidal concentration (MFC) was defined as the lowest concentration of the antifungal agent at which no detectable fungal growth was observed. All experiments were conducted in triplicate (Stopiglia et al., 2011). It is important to evaluate the potential impact of the solvent used in the extraction process. Therefore, pure ethanol (95 %) was tested independently to assess its antimicrobial activity. In the Agar Well Diffusion Method, a well was filled with 50 μL of pure ethanol as a negative control. This step ensured that any observed antimicrobial effect could be attributed solely to the bioactive compounds in the extracts and not to the solvent itself.

3. Results and discussion

3.1. Antimicrobial activity

Tables 1 and 2 present the antibacterial activity of *Z. lotus* extracts, which displayed notable inhibitory effects against various bacterial strains. Aqueous extracts exhibited inhibition zone diameters ranging from 14.33 to 29 mm, whereas ethanol extracts showed diameters

Table 1
Zones of growth inhibition, the MIC and MBC values (mg/mL) of *Z. lotus* ethanol extracts against eight microorganisms.

Microorganism [a]	Inhibition zone diameters (mm)[b]						MIC (mg/mL)					MBC (mg/mL)				
	Ethanol extraction															
	Fruits	Leaves	Flowers	Stems	Roots	ATB	Fruits	Leaves	Flowers	Stems	Roots	Fruits	Leaves	Flowers	Stems	Roots
S. aureus ATCC 25923	29.33 ± 0.47	29.33 ± 0.47	28.00 ± 0.82	28.67 ± 0.97	27.00 ± 0.82	31.00[c]	0.012	0.037	0.037	0.037	0.037	0.012	0.037	0.037	0.037	0.037
M. luteus NCIMB 8166	21.00 ± 0.82	25.33 ± 0.47	21.00 ± 0.82	24.00 ± 0.82	20.00 ± 0.82	28.00 [c]	0.11	0.33	0.11	0.11	0.33	0.11	0.33	0.33	0.11	1.00
E. coli ATCC 35218	22.00 ± 0.82	_	_	16.00 ± 0.82	_	28.00 [c]	0.33	_	_	1.00	_	0.33	_	_	1	_
L. monocytogenes ATCC 19115	24.00 ± 0.82	22.33 ± 0.47	21.00 ± 0.82	24.00 ± 0.82	20.00 ± 0.82	28.00 [c]	0.11	0.33	0.33	0.33	0.33	0.11	0.33	0.33	0.33	0.33
P. aeruginosa ATCC 27857	16.33 ± 0.94	_	16.33 ± 0.94	_	_	28.00 [c]	1.00	_	1	_	_	_	_	ND	_	_
E. faecalis ATCC 29212	27.67 ± 0.94	29.00 ± 0.82	29.00 ± 0.82	30.00 ± 0.82	21.00 ± 0.82	28.00 [c]	0.11	0.037	0.037	0.041	0.33	0.33	0.037	0.037	0.037	0.33
S. typhimurium ATCC 1408	16.00 ± 0.82	_	_	_	_	26.00 [c]	1.00	_	_	_	_	1.00	_	_	_	_
B. cereus ATCC 11778	23.00 ± 0.82	28.67 ± 0.47	23.33 ± 0.47	21.00 ± 0.82	26.00 ± 0.82	29.00 [c]	0.037	0.11	0.11	0.11	0.11	0.037	0.11	0.11	0.11	0.11

Values are means of three measurements. ±, Standard deviation; ATB, antibiotics; ND, not determined.
[a] Final bacterial density was around 10^5 CFU/mL.
[b] Inhibition zone diameters (mm) produced around the wells by adding 50 µL of ethanol and aqueous extract.
[c] Gentamicin (10 µg/mL).

Table 2
Zones of growth inhibition, the MIC and MBC values (mg/mL) of *Z. lotus* aqueous extracts against eight microorganisms.

Microorganism [a]	Inhibition zone diameters (mm)[b]						MIC (mg/mL)					MBC (mg/mL)				
	Aqueous extract															
	Fruits	Leaves	Flowers	Stems	Roots	ATB	Fruits	Leaves	Flowers	Stems	Roots	Fruits	Leaves	Flowers	Stems	Roots
S. aureus ATCC 25923	29.00 ± 0.82	29.00 ± 0.82	18.33 ± 0.47	19.00 ± 0.82	24.00 ± 0.82	31.00 [c]	100	1.23	97.50	200	66.66	ND	3.7	97.5	ND	66.66
M. luteus NCIMB 8166	22.00 ± 0.82	_	20.33 ± 0.94	_	_	28.00 [c]	100	_	37.50	_	_	ND	_	37.50	_	_
E. coli ATCC 35218	18.33 ± 0.94	_	14.33 ± 0.47	_	_	28 [c]	100	_	75.00	_	_	ND	_	75.00	_	_
L. monocytogenes ATCC 19115	_	20.33 ± 0.94	25.00 ± 0.82	_	24.00 ± 0.82	31 ·00[c]	_	100	18.75	_	66.66	ND	100	18.75	_	66.66
P. aeruginosa ATCC 27857	_	_	19.00 ± 0.82	_	_	28.00 [c]	_	_	37.5	_	_	_	_	37.50	_	_
E. faecalis ATCC 29212	19.00 ± 0.82	20.67 ± 0.94	21.00 ± 0.82	_	_	29.00 [c]	100	33.33	18.75	_	_	ND	33.33	37.50	_	_
S.typhimurium ATCC 1408	14.33 ± 0.47	25.00 ± 0.82	14.33 ± 0.47	_	_	26.00 [c]	100	33.33	75.00	_	_	ND	33.33	75.00	_	_
B.cereus ATCC 11778	24.00 ± 0.82	27.66 ± 0.47	22.00 ± 0.82	19.33 ± 0.47	24.00 ± 0.82	29.00 [c]	100	33.33	18.75	200	200	ND	33.33	37.50	200	200

Values are means of three measurements. ±, Standard deviation; ATB, antibiotics; ND, not determined.
[a] Final bacterial density was around 10^5 CFU/mL.
[b] Inhibition zone diameters (mm) produced around the wells by adding 50 µL of ethanol and aqueous extract.
[c] Gentamicin (10 µg/mL).

between 16 and 30 mm. Notably, ethanol extracts demonstrated significantly lower minimum inhibitory concentrations (MICs), ranging from 0.012 to 1 mg/mL, compared to aqueous extracts, which ranged from 1.23 to 200 mg/mL.

Among all tested samples, the ethanol fruit extract exhibited the highest antibacterial activity, with an MIC of 0.012 mg/mL against *S. aureus*. It also showed strong activity against *B. cereus*, with an MIC of 0.037 mg/mL. Similarly, ethanol extracts from leaves, stems, roots, and flowers displayed considerable activity against *E. faecalis* (MIC = 0.037 mg/mL) and *S. aureus*. Conversely, the aqueous leaf extract showed the best activity among aqueous preparations, with an MIC of 1.23 mg/mL against *S. aureus*, and moderate activity against *L. monocytogenes* and *B. cereus*. Floral aqueous extracts exhibited comparatively weaker inhibition, with MICs as high as 18.75 mg/mL.

The inhibition zone analysis further confirmed these trends. The ethanol stem extract exhibited the strongest inhibition (30 mm) against *E. faecalis*, while the ethanol fruit extract showed the lowest inhibition diameter (16 mm) against *S. typhimurium*. Aqueous extracts from fruits and leaves demonstrated significant inhibition (up to 29 mm) against *S. aureus*, whereas aqueous flower extracts exhibited the lowest inhibitory effects, particularly against *S. typhimurium* and *E. coli* (diameters as small as 14.33 mm). Overall, ethanol extracts consistently outperformed aqueous ones, confirming their superior antibacterial potential.

These findings were supported by the results of MBC tests. Ethanol extracts generally showed lower MBC values, indicating strong bactericidal activity. For example, the ethanol fruit extract had an MBC of 0.012 mg/mL against *S. aureus* and 0.037 mg/mL against *B. cereus*, while the stem extract had an MBC of 1 mg/mL against *E. coli*. In contrast, aqueous extracts exhibited significantly higher MBC values, suggesting lower bactericidal activity. The aqueous leaf extract showed MBC values of 3.7 mg/mL against *S. aureus* and 33.33 mg/mL against *S. typhimurium*, while the flower extract reached MBCs as high as 97.5 mg/mL against *S. aureus*.

To further elucidate the observed differences in antibacterial activity among *Z. lotus* extracts, we examined the phytochemical composition of each plant part as reported in the literature (Table 3). It seems that no clear correlation emerges between the nature of the extract, the concentration of key bioactive compounds, and its antibacterial activity.

The ethanol fruit extract ranked first in antibacterial activity, showing broad-spectrum activity. It is likely attributed to its richness in phenolic acids such as synapic acid, p-coumaric acid, and benzoic acid, known for their ability to disrupt bacterial membranes and inhibit enzymatic systems (El Cadi et al., 2020). In second position, the ethanol stem extract demonstrated notable antibacterial activity, particularly against *E. faecalis*. This effect may be attributed to the presence of bioactive compounds such as catechin, oleuropein, and quercetin-O-derivatives (Rached et al., 2019). Similarly, the ethanol leaf extract exhibited strong activity, likely due to its richness in quercetin, myricetin, and apigenin derivatives, which are known for their bacteriostatic and bactericidal properties (Marmouzi et al., 2019)."

The root and flower ethanol extracts also demonstrated considerable antibacterial **activity**, supporting the idea that ethanol facilitates the extraction of a wide spectrum of active secondary metabolites, including flavonoids and phenolic acids.

Among aqueous extracts, the leaf extract exhibited the highest antibacterial **activity**, likely due to the combined action of gallic acid, rutin, catechin, caffeic acid, and quercetin (Marmouzi et al., 2019). It was followed by the aqueous fruit and root extracts, which showed moderate activity, whereas the aqueous flower extract showed the lowest **activity**.

This order of antibacterial activity—ethanol fruit extract > ethanol stem extract > ethanol leaf extract > ethanol root/flower extract > aqueous leaf extract > aqueous fruit extract > aqueous root extract > aqueous flower extract—reflects the influence of both extraction solvent and phytochemical profile.

The comparison with other plant species further supports this conclusion. Plants such as *Piper nigrum*, *Carica papaya*, and *Datura stramonium*, which also contain high levels of flavonoids, alkaloids, and phenolic acids, have shown similar antibacterial profiles (Costa et al., 2017). Notably, compounds like quercetin, catechin, and kaempferol, present in both *Z. lotus* and other medicinal plants, are known to exert antibacterial activity by altering membrane permeability, inhibiting efflux pumps, and impairing bacterial DNA synthesis (Giri et al., 2020).

In summary, the ethanol extract of *Z. lotus* fruits demonstrated the most potent antibacterial activity, inhibiting the growth of eight pathogenic bacteria at concentrations ranging from 0.012 mg/mL to 1 mg/mL. These findings align with those of Yazgan (2020), who reported significant antibacterial activity of ethanol *Z. lotus* extracts against *S. typhimurium*, *Bacillus subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, with inhibition zones between 7 and 18 mm.

The superior activity of ethanol extracts is likely due to ethanol's greater ability to solubilize a wide range of bioactive constituents, particularly phenolic acids, flavonoids, tannins, and essential oils, which are known for their strong antimicrobial activity (Abdulla et al., 2016). This is supported by Mogana et al. (2020), who reported the presence of various antibacterial compounds in *Z. lotus*, such as gallic acid, catechin, quercetin, rutin, and vanillic acid. These compounds act through mechanisms including disruption of bacterial cell walls, inhibition of enzyme activity, and interference with microbial DNA replication. Consequently, ethanol extracts consistently show larger inhibition zones (18.4–21.7 mm) compared to aqueous extracts (17.6–19.3 mm).

3.2. Antifungal activity

The antifungal activity of *Z. lotus* ethanol extracts showed significant variation depending on the plant part and the fungal strain tested (Table 4). Among all plant parts, the fruit and leaf ethanol extracts exhibited the strongest antifungal activity, especially against *C. neoformans* and *C. albicans*. The fruit extract produced inhibition zones up to 28.67 ± 0.94 mm against *C. neoformans* (Fig. 1c), while the leaf extract recorded the lowest MIC value of 0.012 mg/mL against *C. albicans*. Flower and stem extracts also demonstrated moderate

Table 3
Correlation between the antimicrobial activity and phytochemical compounds found in different parts of *Z. lotus*.

Plant part	Extraction method	Key compound	Strongest activity (MIC/MFC)	Most sensitive organism	References
Root (aqueous)	Aqueous	Gallic acid, ferulic acid, caffeic acid	MIC = 66.66 mg/mL	*S. aureus*, *L. monocytogenes*	Marmouzi et al. (2019)
Leaf (aqueous)		Gallic acid, catechin, rutin, caffeic acid, quercetin	MIC = 1.23 mg/mL	*S. aureus*	
Fruit (ethanol)	Ethanol	Synapic acid, p-coumaric acid, benzoic acid Catechin, resveratrol, p-hydroxybenzoic acid	MIC = 0.012 mg/mL MIC = 0.012 mg/mL	*S. aureus*	El Cadi et al. (2020)
Branches (ethanol)		Catechin, oleuropein, quercetin-O-derivatives	MIC = 0.037 mg/mL	*S. aureus*	Rached et al. (2019)
Leaf (ethanol)		Quercetin, myricetin, apigenin derivatives	MIC = 0.037 mg/mL	*S. aureus*, *E. faecalis*	Marmouzi et al. (2019)

Table 4
Zones of growth inhibition, the MIC and MFC values (mg/mL) of *Z. lotus* ethanol extract against three yeast and two fungi.

Micro-organisms [a]	Inhibition zone diameters (mm)[b]						MIC (mg/mL)					CMF (mg/mL)				
	Ethanol extract															
	Fruits	Leaves	Flowers	Stems	Roots	ATB	Fruits	Leaves	Flowers	Stems	Roots	Fruits	Leaves	Flowers	Stems	Roots
C. Albicans ATCC 90028	25.33 ± 0.47	21.33 ± 0.94	21.67 ± 0.47	24 ± 0.82	20.67 ± 0.47	31.00[c]	0.012	0.037	0.037	0.037	0.037	0.012	0.037	0.037	0.037	0.037
C. Krusei ATCC 6258	28.67 ± 0.47	24.67 ± 0.47	24.67 ± 0.47	25.67 ± 0.47	27.67 ± 0.94	28.00 [c]	0.11	0.33	0.11	0.11	0.33	0.11	0.33	0.33	0.11	1
C. neoformans ATCC 14116	28.67 ± 0.94	28.67 ± 0.94	26.67 ± 0.94	27.67 ± 0.94	20.67 ± 0.47	28.00[c]	0.33	0.33	1.00	1.00	1	0.33	-	-	1	-
A. brasiliensis. ATCC 16404	18.33 ± 0.47	17.67 ± 0.47	19.00 ± 0.82	16.67 ± 0.47	17.67 ± 0.47	28.00[c]	0.11	0.33	0.33	0.33	0.33	0.11	0.33	0.33	0.33	0.33
A. fumigatus ATCC 204305	24.67 ± 0.47	20.67 ± 0.47	20.67 ± 0.47	24.67 ± 0.47	24.67 ± 0.47	28.00 [c]	0.33	1.00	1.00	0.33	1	0.33	1	1	0.33	1

Values are means of three measurements ± standard deviation.
ATB: Antibiotic control (Amphotericin B, 250 μg/mL).
ND: Not determined.
[a] Final fungal density was around 10^5 CFU/mL.
[b] Inhibition zone diameters (mm) produced by adding 50 μL of ethanol and aqueous extract.
[c] Amphotericin B (250 μg/mL).

activity, whereas the root extract was generally less activity.

Against filamentous fungi, the flower ethanol extract was particularly active against *A. brasiliensis* with a zone of inhibition of 19 ± 0.82 mm (Fig. 1b). In addition, fruit and stem ethanol extracts exhibited the best MIC values (0.33 mg/mL) against *Aspergillus* species, further supporting their strong antifungal activity.

In contrast, the aqueous extracts, particularly from flowers and roots, showed markedly lower antifungal activity. Inhibition zones were significantly smaller (as low as 10.67 mm) and MIC values were notably higher, reaching up to 150 mg/mL (Table 5). This suggests either a reduced concentration of antifungal compounds in these parts or the lower extraction activity of water. Interestingly, the aqueous stem extract displayed moderate antifungal activity, especially against *A. brasiliensis*, with a zone of inhibition of 23 mm (Fig. 1d) and an MIC of 66.66 mg/mL, indicating the stem as a potential reservoir of water-soluble antifungal compounds.

The Minimum Fungicidal Concentration (MFC) results further support the superior activity of ethanol extracts. MFC values for ethanol extracts ranged from 0.012 mg/mL (against *C. albicans*) to 1 mg/mL (against *A. fungi*, *C. krusei*, and *C. neoformans* for various plant parts). The fruit ethanol extract was the most potent, especially against *C. albicans* and *C. krusei*, with MFCs of 0.012 and 0.11 mg/mL, respectively. In comparison, aqueous extracts displayed higher MFCs, indicating lower fungicidal activity. For example, the aqueous leaf extract against *C. albicans* had an MFC of 3.7 mg/mL, while the aqueous root extract showed an MFC of 66.66 mg/mL against *A. brasiliensis*.

The antifungal activity of *Z. lotus* appears to be strongly influenced by both the type of solvent used for extraction and the phytochemical composition specific to each plant part. Ethanol, due to its intermediate polarity, has demonstrated superior capacity to extract a wide spectrum of bioactive compounds, including flavonoids, phenolic acids, tannins, and essential oils, all of which are recognized for their antimicrobial and antifungal activity (Cowan, 1999; Dorman & Deans, 2000). In contrast, aqueous extractions tend to yield lower concentrations of these compounds, which may explain the reduced antifungal activity observed in water-based extracts (Furia et al., 2021). This solvent-dependent variability underscores the critical role of extraction methods in enhancing the availability and activity of active constituents.

Among the various parts of *Z. lotus*, ethanol extracts of the fruits exhibited the strongest antifungal activity, likely due to their richness in phenolic acids such as synapic acid, p-coumaric acid, benzoic acid, catechin, and resveratrol (El Cadi et al., 2020). These molecules are known to disrupt fungal cell membranes, interfere with enzymatic functions, and induce oxidative stress, thereby inhibiting fungal growth. Similarly, extracts from leaves, containing quercetin, myricetin, and apigenin derivatives, demonstrated notable antifungal activity, which can be attributed to the ability of flavonoids to compromise fungal cell wall integrity and inhibit spore germination (Marmouzi et al., 2019). Extracts obtained using an ethanol water mixture (70:30, v/v), particularly from the branches, also showed significant antifungal activity, further supporting the contribution of polyphenols to fungal inhibition (Rached et al., 2019). In contrast, aqueous extracts from leaves and roots, though containing compounds like gallic acid, caffeic acid, ferulic acid, and rutin, were comparatively less active, likely due to the lower solubility of these compounds in water and the reduced extractability of less polar molecules (Marmouzi et al., 2019).

The antifungal mechanism of activity of *Z. lotus* has been attributed to the disruption of fungal cell membrane integrity, increased permeability, leakage of intracellular contents, and inhibition of spore germination, all primarily driven by its phenolic constituents. (Brijesh et al., 2009; Borges et al., 2013; Bouchra et al., 2003). Essential oils and polyphenolic extracts have also been shown to induce morphological changes such as vesiculation, mycelial lysis, and damage to fungal cell walls (Sharma & Tripathi, 2006). Additional studies have highlighted the role of phenolics and sterols in altering key biological pathways, ultimately leading to growth inhibition or apoptosis in fungal cells

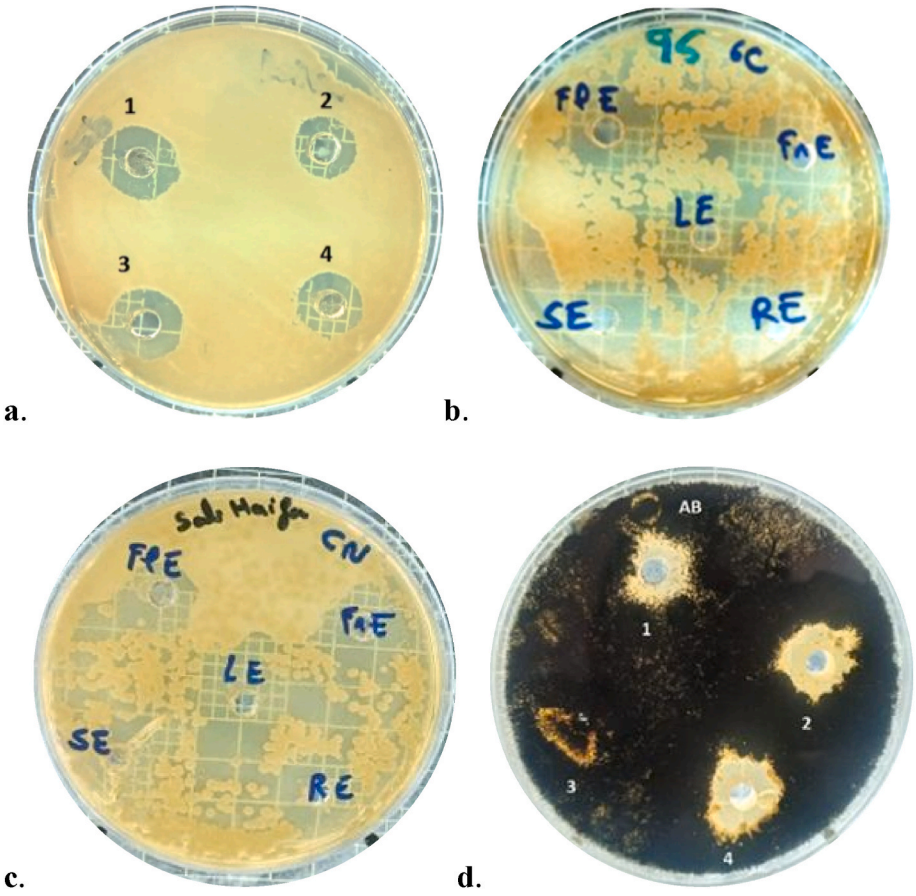

Fig. 1. Inhibition zones of *Z. lotus* extracts against fungal strains (a) Inhibition zone of ethanol extracts (1 mg/mL) against *C. albicans* (ATCC 90028): 1 – fruit, 2 – leaf, 3 – stem, 4 – flower. (b) Inhibition zone of ethanol extracts (1 mg/mL) against *C. krusei* (ATCC 6258): FrE – fruit extract, LE – leaf extract, SE – stem extract, FlE – flower extract. (c) Inhibition zone of ethanol extracts (1 mg/mL) against *C. neoformans* (ATCC 14116): FrE – fruit extract, LE – leaf extract, SE – stem extract, FlE – flower extract.(d) Inhibition zone of aqueous extracts against *A. brasiliensis* (ATCC 16404):1 – stem (200 mg/mL), 2 – leaf (100 mg/mL), 3 – fruit (100 mg/mL), 4 – flower (200 mg/mL).

(Kamelé et al., 2019).

Despite the traditional use of *Z. lotus* in folk medicine, research specifically targeting its antifungal activity remains relatively scarce. However, the promising results observed, particularly from ethanol-based extracts, highlight its potential as a natural antifungal agent. Table 6 summarizes the relationship between plant part, extraction method, phytochemical profile, and antifungal activity, providing a comparative overview of *Z. lotus* alongside other medicinal plants such as *Piper nigrum*, *Carica papaya*, and *Datura stramonium*.

4. Conclusion

This study compared the antibacterial and antifungal properties of ethanol and aqueous *Z. lotus* extracts, demonstrating that ethanol extracts were significantly more effective. The lower minimum inhibitory concentrations and minimum bactericidal/fungicidal concentrations of ethanol extracts, especially against *S. aureus*, highlight their potent antimicrobial activity. Ethanol's ability to solubilize a wide range of bioactive compounds, including phenolic acids, flavonoids, and tannins, contributes to this enhanced activity by disrupting microbial cell membranes and inhibiting essential metabolic processes.

In contrast, aqueous extracts required higher doses to achieve comparable results, suggesting that some bioactive compounds may be less soluble in water. These findings underscore the potential of ethanol *Z. lotus* extracts as natural, eco-friendly alternatives to synthetic antimicrobials. Further research is needed to identify and characterize the active components and explore their synergistic effects, with applications in both medicine and agriculture.

While standard protocols were used and all data were converted into mass-based measurements to enable comparison across plant parts, it is important to note that this study did not include detailed chemical characterization of the extracts. Given that the chemical composition of *Z. lotus* varies throughout the year and across different organs, the specific bioactive profile of the extracts used here may differ from those reported in other studies.

CRediT authorship contribution statement

Haifa Dhif: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Jalila ben Salah-Abbès:** Writing – review & editing. **Ahmed Al-Amiery:** Writing – original draft, Methodology, Formal analysis. **Kamel Chaieb:** Writing – review & editing, Supervision. **Mara Calleja-Gómez:** Writing – review & editing, Visualization, Validation, Conceptualization. **Houda Berrada:** Writing – review & editing, Validation, Conceptualization. **Samir Abbès:** Writing – review & editing, Supervision.

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Table 5
Zones of growth inhibition MIC and MFC values (mg/mL) of *Z. lotus* aqueous extract against three yeast and two fungi.

Micro-organisms [a]	Inhibition zone diameters (mm)[b]						MIC (mg/mL)				CMF (mg/mL)					
	Aqueous extract															
	Fruits	Leaves	Flowers	Stems	Roots	ATB	Fruits	Leaves	Flowers	Stems	Roots	Fruits	Leaves	Flowers	Stems	Roots
C. Albicans ATCC 90028	27.33 ± 0.47	27.00 ± 0.82	13.67 ± 0.47	17.67 ± 0.47	24.33 ± 0.47	31.00 [c]	100	1.23	150	200	66.66	ND	3.7	100	ND	66.66
C. Krusei ATCC 6258	27.67 ± 0.47	-	10.67 ± 0.47	-	-	28.00 [c]	100	-	150	-	-	ND	-	100	-	-
C. neoformans ATCC 14116	18.33 ± 0.94	-	-	-	-	17.00 [c]	100	-	-	-	-	ND	-	ND	-	-
A. brasiliensis. ATCC 16404	17.33 ± 0.94	21.00 ± 0.82	-	23 ± 0.82	17 ± 0.82	11.00 [c]	100	100	-	66.66	66.66	ND	100	ND	ND	66.66
A. fumigatus ATCC 204305	11.33 ± 0.94	10.67 ± 0.47	10.67 ± 0.47	10.67 ± 0.47	10.67 ± 0.47	12.00 [c]	33.33	11.11	150	66.66	200	ND	ND	100	ND	ND

Values are means of three measurements ± standard deviation.
ATB: Antibiotic control (Amphotericin B, 250 μg/mL).
ND: Not determined.
[a] Final fungal density was around 10^5 CFU/mL.
[b] Inhibition zone diameters (mm) produced by adding 50 μL of ethanol and aqueous extract.
[c] Amphotericin B (250 μg/mL).

Table 6
Correlation between bioactive compounds identified in different parts of *Z. lotus* and their antimicrobial activities (antibacterial and antifungal).

Plant part	Identified bioactive compounds	Observed antimicrobial activity (Present study)	Potential mechanism of activity	Literature references
Fruits	Flavonoids (quercetin, kaempferol), phenolic acids, catechins	Strong antibacterial activity (e.g., MIC = 0.012 mg/mL against *S. aureus*) and antifungal activity (*C. albicans*)	Membrane disruption, enzyme inhibition	Albouchi et al., 2013; Giri et al., 2020
Leaves	Rutin, luteolin, tannins	Moderate antifungal activity against *C. neoformans* and *A. brasiliensis*	Fungal cell wall disruption	China et al. (2012)
Flowers	Licoflavone, sterols	Activity against *C. krusei* and *A. brasiliensis*	Mycelial lysis, inhibition of spore germination	Babii et al. (2016)
Stems	Flavonols, catechins	Moderate antibacterial activity against *P. aeruginosa* and antifungal against *A. fumigatus*	Inhibition of bacterial efflux pumps (e.g., NorA)	Vipin et al. (2020)
Roots	Alkaloids, phenolic compounds	Weak antifungal activity	Low water solubility of active compounds	Abdulla et al. (2016)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Abdulla, G., Abdel-Samie, M. A., & Zaki, D. (2016). Evaluation of the antioxidant and antimicrobial effects of *ziziphus* leaves extract in sausage during cold storage. *Pakistan Journal of Food Sciences, 26*(1), 10–20.

Albouchi, F., Hassen, I., Casabianca, H., & Hosni, K. (2013). Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of Ailanthus altissima (mill.) swingle leaves. *Suid-Afrikaanse Tydskrif Vir Plantkunde [South African Journal of Botany], 87*, 164–174. https://doi.org/10.1016/j.sajb.2013.04.003

Alemu, M., Asfaw, Z., Lulekal, E., Warkineh, B., Debella, A., Sisay, B., & Debebe, E. (2024). Ethnobotanical study of traditional medicinal plants used by the local people in habru district, north wollo zone, Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 20*(1), 4. https://doi.org/10.1186/s13002-023-00644-x

Babii, C., Bahrin, L. G., Neagu, A.-N., Gostin, I., Mihasan, M., Birsa, L. M., & Stefan, M. (2016). Antibacterial activity and proposed action mechanism of a new class of

synthetic tricyclic flavonoids. *Journal of Applied Microbiology, 120*(3), 630–637. https://doi.org/10.1111/jam.13048
Benammar, C., & Baghdad, C. (2014). Antidiabetic and antioxidant activities of *zizyphus lotus L* aqueous extracts in wistar rats. *Journal of Nutrition & Food Sciences, s8*. https://doi.org/10.4172/2155-9600.s8-004
Bencheikh, N., Radi, F. Z., Fakchich, J., Elbouzidi, A., Ouahhoud, S., Ouasti, M., Bouhrim, M., Ouasti, I., Hano, C., & Elachouri, M. (2023). Ethnobotanical, phytochemical, toxicological, and pharmacological properties of *ziziphus Lotus* (L.) lam.: A comprehensive review. *Pharmaceuticals, 16*(4). https://doi.org/10.3390/ph16040575
Berkani, F., Serralheiro, M. L., Dahmoune, F., Mahdjoub, M., Kadri, N., Dairi, S., Achat, S., Remini, H., Abbou, A., Adel, K., & Madani, K. (2021). *Ziziphus Lotus* (L.) lam. Plant treatment by ultrasounds and microwaves to improve antioxidants yield and quality: An overview. *NAJFNR, 5*(12), 53–68. https://doi.org/10.51745/najfnr.5.12.53-68
Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance, 19*(4), 256–265. https://doi.org/10.1089/mdr.2012.0244
Botelho, J., Grosso, F., & Peixe, L. (2019). Antibiotic resistance in *Pseudomonas aeruginosa* - Mechanisms, epidemiology and evolution. *Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy, 44*(100640), Article 100640. https://doi.org/10.1016/j.drup.2019.07.002
Bouchra, C., Achouri, M., Idrissi Hassani, L. M., & Hmamouchi, M. (2003). Chemical composition and antifungal activity of essential oils of seven Moroccan labiatae against botrytis cinerea pers: Fr. *Journal of Ethnopharmacology, 89*(1), 165–169. https://doi.org/10.1016/s0378-8741(03)00275-7
Butler, D. L., Jakielaszek, C. J., Miller, L. A., & Poupard, J. A. (1999). *Escherichia coli* ATCC 35218 as a quality control isolate for susceptibility testing of *Haemophilus influenzae* with haemophilus test medium. *Antimicrobial Agents and Chemotherapy, 43*(2), 283–286. https://doi.org/10.1128/AAC.43.2.283
China, R., Mukherjee, S., Sen, S., Bose, S., Datta, S., Koley, H., Ghosh, S., & Dhar, P. (2012). Antimicrobial activity of Sesbania grandiflora flower polyphenol extracts on some pathogenic bacteria and growth stimulatory effect on the probiotic organism *Lactobacillus acidophilus*. *Microbiological Research, 167*(8), 500–506. https://doi.org/10.1016/j.micres.2012.04.003
Chinemerem Nwobodo, D., Ugwu, M. C., Oliseloke Anie, C., Al-Ouqaili, M. T. S., Chinedu Ikem, J., Victor Chigozie, U., & Saki, M. (2022). Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *Journal of Clinical Laboratory Analysis, 36*(9), Article e24655. https://doi.org/10.1002/jcla.24655
Costa, A. F. S., Almeida, F. C. G., Vinhas, G. M., & Sarubbo, L. A. (2017). Production of bacterial cellulose by Gluconacetobacter hansenii using corn steep liquor as nutrient sources. *Frontiers in Microbiology, 8*, 2027. https://doi.org/10.3389/fmicb.2017.02027
Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564–582. https://doi.org/10.1128/CMR.12.4.564
Dehelean, C. A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., Cretu, O. M., & Pinzaru, I. (2021). Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. *Molecules, 26*(4), 1109. https://doi.org/10.3390/molecules26041109
Doğan, A. (2022). The problem of resistance in antibiotics and new treatments. *Health Science*, 47. Efe Akademi Yayınları.
Domínguez, A. V., Ledesma, M. C., Domínguez, C. I., Cisneros, J. M., Lepe, J. A., & Smani, Y. (2023). In vitro and in vivo virulence study of *Listeria monocytogenes* isolated from the Andalusian outbreak in 2019. *Tropical Medicine and Infectious Disease, 8*(2), 58. https://doi.org/10.3390/tropicalmed8020058
Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*(2), 308–316. https://doi.org/10.1046/j.1365-2672.2000.00969.x
Duan, M., Qiu, C., Huang, X., Sun, L., He, X., Wang, Z., Yue, H., Wang, K., Qi, Y., Peng, S., Shi, X., Xi, Z., Tong, M., Ding, X., Hou, Y., & Zhao, Y. (2024). Novel biaryloxazolidinone derivatives with broad-spectrum antibacterial activity, favorable drug-like profiles and in vivo efficacy against linezolid-resistant staphylococcusaureus. *European Journal of Medicinal Chemistry, 273*(116493), Article 116493. https://doi.org/10.1016/j.ejmech.2024.116493
El Cadi, H., Bouzidi, H. E. L., Selama, G., El Cadi, A., Ramdan, B., Oulad, Y., Majdoub, E., Alibrando, F., Dugo, P., Mondello, L., & others. (2020). Physico-chemical and phytochemical characterization of Moroccan wild jujube *zizyphus lotus (L.)* fruit crude extract and fractions. *Molecules, 25*(21), 5237. https://doi.org/10.3390/molecules25215237
Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica, 64*(8), 711–713. https://doi.org/10.1055/s-2006-957563
Essid, R., Hammami, M., Gharbi, D., Karkouch, I., Hamouda, T. B., Elkahoui, S., Limam, F., & Tabbene, O. (2017). Antifungal mechanism of the combination of Cinnamomum verum and Pelargonium graveolens essential oils with fluconazole against pathogenic candida strains. *Applied Microbiology and Biotechnology, 101*(18), 6993–7006. https://doi.org/10.1007/s00253-017-8442-y
Furia, E., Beneduci, A., Malacaria, L., Fazio, A., La Torre, C., & Plastina, P. (2021). Modeling the solubility of phenolic acids in aqueous media at 37 °C. *Molecules, 26*(19), 6500. https://doi.org/10.3390/molecules26196500
Gajdács, M. (2019). The continuing threat of methicillin-resistant Staphylococcus aureus. *Antibiotics, 8*(2), 52. https://doi.org/10.3390/antibiotics8020052
Giri, K., Shrestha, B. K., Shakya, J., Sah, S. N., & Khanal, H. (2020). Antibacterial effect of green tea extract against multi drug resistant Escherichia coli isolated from urine sample of patients visiting tertiary care hospital of eastern Nepal. *International Journal of Applied Sciences and Biotechnology, 8*(1), 45–51. https://doi.org/10.3126/ijasbt.v8i1.27313
Gorai, M., Maraghni, M., & Neffati, M. (2010). Relationship between phenological traits and water potential patterns of the wild jujube *ziziphus Lotus* (L.) lam. In southern Tunisia. *Plant Ecology & Diversity, 3*(3), 273–280. https://doi.org/10.1080/17550874.2010.500337
Kamelé, K. Y., Clémentine, K.-K. A., Carole, K. A., Mesmin, K. Y., Honora, T. B. F., & Kablan, T. (2019). Activité Antifongique In Vitro des Extraits de Cinq Plantes Locales sur Colletotrichum Higginsianum, Fusarium Oxysporum et Rhizopus Stolonifer, Agents Pathogènes de la Papaye (*Carica Papaya* L.) et de la Tomate (Solanum Lycopersicum L.). *European Scientific Journal, 15*(9). https://doi.org/10.19044/esj.2019.v15n9p304
Marmouzi, I., Kharbach, M., El Jemli, M., Bouyahya, A., Cherrah, Y., Bouklouze, A., … Faouzi, M. E. A. (2019). Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in *zizyphus Lotus* leaves and fruits. *Industrial Crops and Products, 132*, 134–139. https://doi.org/10.1016/j.indcrop.2019.02.007
Mirtaghi, S. M., Torbati Nejad, P., Mazandarani, M., Livani, F., & Bagheri, H. (2016). Evaluation of antibacterial activity of Urtica dioica L. leaf ethanol extract using agar well diffusion and disc diffusion methods. *Majallah-i-ʻulūm-i āzmāyishgāhī, 10*(5), 15–21. https://doi.org/10.18869/acadpub.mlj.10.5.15. Department of Laboratory Sciences, Department of Laboratory Sciences, Department of Biology, Department of Plant Sciences, & Department of Microbiology.
Mogana, R., Adhikari, A., Tzar, M. N., Ramliza, R., & Wiart, C. (2020). Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* miq. Against bacterial clinical isolates. *BMC Complementary Medicine and Therapies, 20*(1), 55. https://doi.org/10.1186/s12906-020-2837-5
Njunda, A. L., Nsagha, D. S., Assob, J. C., Kamga, H. L., & Teyim, P. (2012). *In Vitro* antifungal susceptibility patterns of *Candida albicans* from HIV and AIDS patients attending the nylon health district hospital in douala, Cameroon. *Journal of Public Health in Africa, 3*, e2. https://doi.org/10.4081/jphia.2012.e2
Ouassou, H., Bouhrim, M., Kharchoufa, L., Imtara, H., Daoudi, N. E., Benoutman, A., Bencheikh, N., Ouahhoud, S., Elbouzidi, A., & Bnouham, M. (2021). Calralluma europaea (guss) n.E.Br.: A review on ethnomedicinal uses, phytochemistry, pharmacological activities, and toxicology. *Journal of Ethnopharmacology, 273*(113769), Article 113769. https://doi.org/10.1016/j.jep.2020.113769
Rached, W., Barros, L., Ziani, B. E. C., Bennaceur, M., Calhelha, R. C., Heleno, S. A., Alves, M. J., Marouf, A., & Ferreira, I. C. F. R. (2019). HPLC-DAD-ESI-MS/MS screening of phytochemical compounds and the bioactive properties of different plant parts of zizyphus *lotus* (L.) desf. *Food & Function, 10*(9), 5898–5909. https://doi.org/10.1039/c9fo01423c
Sharma, N., & Tripathi, A. (2006). Fungitoxicity of the essential oil of citrus sinensis on post-harvest pathogens. *World Journal of Microbiology and Biotechnology, 22*(6), 587–593. https://doi.org/10.1007/s11274-005-9075-3
Sivapragasam, G., Devi, T. G., Ragavan, N. D., Suryadevara, N., Sridewi, N., Al Obaid, S., Alharbi, S. A., & Arulselvan, P. (2024). A comparative phytochemical characterization of *Moringa oleifera* plant parts by different solvent extraction. *Indian Journal of Pharmaceutical Education, 58*(2s), s648–s659. https://doi.org/10.5530/ijper.58.2s.69
Stopiglia, C. D. O., Vieira, F. J., Mondadori, A. G., Oppe, T. P., & Scroferneker, M. L. (2011). In vitro antifungal activity of dihydroxyacetone against causative agents of dermatomycosis. *Mycopathologia, 171*(4), 267–271. https://doi.org/10.1007/s11046-010-9370-x
Treangen, T. J., Maybank, R. A., Enke, S., Friss, M. B., Diviak, L. F., Karaolis, D. K., Koren, S., Ondov, B., Phillippy, A. M., Bergman, N. H., & others. (2014). Complete genome sequence of the quality control strain *Staphylococcus aureus* subsp. *aureus* ATCC 25923. *Genome Announcements, 2*(e01110–14). https://doi.org/10.1128/genomeA.01110-14
Vipin, C., Saptami, K., Fida, F., Mujeeburahiman, M., Rao, S. S., Athmika, Arun, A. B., & Rekha, P. D. (2020). Potential synergistic activity of quercetin with antibiotics against multidrug-resistant clinical strains of *Pseudomonas aeruginosa*. *PLoS One, 15*(11), Article e0241304. https://doi.org/10.1371/journal.pone.0241304
Wu, Q., Sabokroo, N., Wang, Y., Hashemian, M., Karamollahi, S., & Kouhsari, E. (2021). Systematic review and meta-analysis of the epidemiology of vancomycin-resistance *Staphylococcus aureus* isolates. *Antimicrobial Resistance and Infection Control, 10*(1), 101. https://doi.org/10.1186/s13756-021-00967-y
Yahia, Y., Benabderrahim, M. A., Tlili, N., Bagues, M., & Nagaz, K. (2020). Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *ziziphus mill.* Species. *PLoS One, 15*(5), Article e0232599. https://doi.org/10.1371/journal.pone.0232599
Yazgan, H. (2020). Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. *Lebensmittel-Wissenschaft und -Technologie-Food Science and Technology, 130*(109669), Article 109669. https://doi.org/10.1016/j.lwt.2020.109669
Yusuf, B., Abraha, B., Salih, K., Abdurahman, A., Alemu, S., Wondimu, A., & Bayu, Y. (2022). In vitro antibacterial evaluation of four selected medicinal plants against *Staphylococcus aureus* isolated from bovine mastitis in mieso district, west hararghe zone, Oromia regional state, Ethiopia. *The Open Microbiology Journal, 16*(1), Article e187428582201140. https://doi.org/10.2174/1874285822160101140