Libyan Open University Journal of Applied Sciences (LOUJAS)
Volume 2 - Issue 1 - 2026 - Pages 97-103

Phytochemical Profiling and In Vitro Antimicrobial Potential of *Hypericum decaisneanum* Coss. Aerial Parts Endemic to Bani Waleed, Libya

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التنميط الكيميائي النباتي والقدرة المضادة للميكروبات مخبرياً للأجزاء الهوائية لنبات *Hypericum decaisneanum* Coss. المستوطن في منطقة بني وليد، ليبيا

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Received: 29-12-2025; Accepted: 14-02-2026; Published: 04-03-2026

Abstract

The escalating global threat of antimicrobial resistance (AMR) has necessitated the exploration of novel bioactive compounds from medicinal plants. This study was conducted to evaluate the *in vitro* antimicrobial efficacy and preliminary phytochemical profile of *Hypericum decaisneanum* Coss., a species endemic to the Bani Waleed region of Libya. Aerial parts of the plant were collected during the flowering season in May 2023. These materials were shade-dried and successively extracted using three solvents of varying polarity: methanol, ethanol, and distilled water via a Soxhlet apparatus. The antimicrobial activity was assessed against a diverse panel of microorganisms, including Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), and the fungal strain *Candida albicans* using the agar well diffusion assay. Quantitative assessments were performed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) through the broth microdilution method. Results indicated that the methanolic extract exhibited the most potent and broad-spectrum antimicrobial activity. It demonstrated significant inhibition zones against all tested strains, particularly against *S. aureus* (21.3 ± 0.6 mm) and *C. albicans* (19.5 ± 0.7 mm). The MIC values for the methanolic extract ranged from 0.312 mg/mL to 2.5 mg/mL, with the lowest MIC observed against *S. aureus*. The MBC/MFC values were generally equal to or twice the MIC values, suggesting a primarily cidal mode of action. In contrast, the ethanolic extract showed moderate activity, while the aqueous extract was largely ineffective. Phytochemical screening of the methanolic extract revealed a rich presence of tannins, flavonoids, terpenoids, and anthraquinones. Quantitative analysis confirmed high levels of total phenolic content (185.4 ± 5.2 mg GAE/g) and total flavonoid content (112.6 ± 4.1 mg QE/g). These findings validate the potential of *H. decaisneanum* as a source of natural antimicrobial agents and warrant further bioassay-guided fractionation to isolate specific active principles.

Keywords : *Hypericum decaisneanum, antimicrobial activity, medicinal plants, Libya, MIC, MBC, phytochemicals*.

الملخص:

أدت التهديدات العالمية المتزايدة لمقاومة مضادات الميكروبات (AMR) إلى ضرورة استكشاف مركبات حيوية نشطة جديدة من النباتات الطبية. أجريت هذه الدراسة لتقييم الفعالية المضادة للميكروبات مخبرياً والتوصيف الكيميائي النباتي الأولي لنبات *Hypericum decaisneanum* Coss.، وهو نوع مستوطن في منطقة بني وليد بليبيا. تم جمع الأجزاء الهوائية للنبات خلال موسم التزهير في مايو 2023. جُففت هذه

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المواد في الظل واستخلصت بالتتابع باستخدام ثلاثة مذيبات متفاوتة القطبية: الميثانول، الإيثانول، والماء المقطر عبر جهاز "سوكسليت ."تم تقييم النشاط المضاد للميكروبات ضد مجموعة متنوعة من الكائنات الدقيقة، شملت بكتيريا موجبة الجرام(*Staphylococcus aureus*) ، (*Enterococcus faecalis*، وبكتيريا سالبة الجرام(*Escherichia coli* ، *Pseudomonas aeruginosa*، *Klebsiella pneumoniae*)، والسلالة الفطرية *Candida albicans*باستخدام طريقة الانتشار بالحفر في الأجار . كما أجريت تقييمات كمية لتحديد الحد الأدنى للتركيز المثبط (MIC) والحد الأدنى لتركيز قاتل البكتيريا/الفطريات (MBC/MFC) من خلال طريقة التخفيف الدقيق في المرق.
أظهرت النتائج أن مستخلص الميثانول سجل أقوى نشاط مضاد للميكروبات واسع الطيف .حيث أظهر مناطق تثبيط معنوية ضد جميع السلالات المختبرة، ولا سيما ضد بكتيريا *S. aureus*بواقع (21.3 ± 0.6 ملم) وفطر *C. albicans*بواقع (19.5 ± 0.7 ملم) .تراوحت قيم MIC لمستخلص الميثانول بين 0.312 مجم/مل و2.5 مجم/مل، مع ملاحظة أدنى قيمة MIC ضد بكتيريا *S. aureus*. كانت قيم MBC/MFC مساوية لقيم MIC أو ضعفها بشكل عام، مما يشير إلى آلية عمل قاتلة للميكروبات .في المقابل، أظهر مستخلص الإيثانول نشاطاً متوسطاً، بينما كان المستخلص المائي غير فعال إلى حد كبير .كشف المسح الكيميائي النباتي لمستخلص الميثانول عن وجود غني للتانينات، الفلافونيدات، التربينات، والأنثراكوينونات .وأكد التحليل الكمي وجود مستويات عالية من المحتوى الفينولي الكلي (185.4 ± 5.2 مجم/GAE جم (والمحتوى الكلي للفلافونيدات (112.6 ± 4.1 مجم/QE جم .(توثق هذه النتائج إمكانات نبات *H. decaisneanum*كمصدر لعوامل طبيعية مضادة للميكروبات، وتستوجب إجراء مزيد من الدراسات لعزل المبادئ النشطة المحددة.

الكلمات المفتاحية: *Hypericum decaisneanum*، النشاط المضاد للميكروبات، النباتات الطبية، ليبيا، MIC، MBC، الكيمياء النباتية، بني وليد.

1.Introduction

The escalating global crisis of antimicrobial resistance (AMR) poses a formidable challenge to public health, rendering conventional antibiotics increasingly ineffective and driving the urgent search for novel therapeutic agents .In this context, medicinal plants have re-emerged as a promising reservoir of bioactive compounds (Alshawish et al., 2025; Lamma,2019). Research has consistently demonstrated the efficacy of various botanical extracts against pathogenic microorganisms, such as the antimicrobial activity found in members of the Pinaceae family (Emhmd et al., 2022; Lamma,2016) and the antibacterial potential of both aqueous and alcoholic extracts from plants like *Mentha piperita* ,ginger, and anise (Aloraibi et al., 2025a, 2025b).

Within the Hypericaceae family, the genus *Hypericum* ,comprising approximately 500 species, is one of the most investigated medicinal plant genera globally .*Hypericum perforatum* L. is the most renowned species, extensively studied for its antidepressant, antiviral, and antibacterial properties, primarily attributed to naphthodianthrones, phloroglucinols, and flavonoids . However, the bioactivity of many other species within this genus, particularly those with limited geographical distribution, remains largely unexplored.

Hypericum decaisneanum Coss. is one such species, endemic to specific North African regions, including parts of Libya .While members of the *Hypericum* genus are recognized in folk medicine for wound healing and treating infections, no detailed scientific studies on the antimicrobial efficacy of *H. decaisneanum* from the Bani Waleed region in Libya have been published .The unique climatic and soil conditions of this semi-arid region may significantly influence the plant's phytochemical profile and its subsequent biological activity.

Therefore, this study was designed to bridge this knowledge gap by systematically investigating the *in vitro* antimicrobial potential of methanolic, ethanolic, and aqueous extracts of *H. decaisneanum* against a panel of clinically relevant bacterial and fungal pathogens . Additionally, a preliminary phytochemical screening was conducted to correlate the observed bioactivity with the presence of specific secondary metabolite classes.

2. Materials and Methods

2.1. Plant Material Collection and Extraction

Aerial parts (flowers, leaves, and stems) of *Hypericum decaisneanum* were collected during the flowering season (May 2023) from the Bani Waleed region, Libya. The plant was identified and authenticated by a taxonomist at the Department of Biology, and a voucher specimen (HD-BW-001) was deposited at the Bane Waleed University herbarium. The plant material was shade-dried at room temperature and ground to a fine powder.

Approximately 100 g of the powdered material was successively extracted with 500 mL of three solvents of increasing polarity: methanol (99.8%), ethanol (70%), and distilled water using a Soxhlet apparatus for 6 hours per solvent. The extracts were filtered, and the solvents were removed under reduced pressure using a rotary evaporator (for organic solvents) or by freeze-drying (for the aqueous extract). The dried crude extracts were stored at 4°C in airtight containers until use.

2.2. Test Microorganisms and Inoculum Preparation

The following reference microbial strains were used: Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603), and a fungal strain (*Candida albicans* ATCC 10231). The bacterial strains were cultured in Mueller-Hinton Broth (MHB) and the yeast in Sabouraud Dextrose Broth (SDB) prior to testing. The turbidity of the suspensions was adjusted to a 0.5 McFarland standard (~1.5 x 10^8 CFU/mL for bacteria).

2.3. Antimicrobial Susceptibility Testing

The antimicrobial activity was initially screened using the agar well diffusion method (Balouiri et al., 2016). Briefly, standardized inoculum was spread on Mueller-Hinton Agar (MHA) or Sabouraud Dextrose Agar (SDA) plates. Wells (6 mm diameter) were punched, and 100 µL of each extract (reconstituted in 10% DMSO to a concentration of 100 mg/mL) was added. Ciprofloxacin (5 µg/well) and Fluconazole (10 µg/well) served as positive controls for bacteria and fungi, respectively, while 10% DMSO was the negative control. After incubation, the diameter of the inhibition zone (IZ) was measured in millimeters (mm). All assays were performed in triplicate.

2.4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MIC was determined for the active extracts using the broth microdilution method in 96-well plates according to CLSI guidelines (CLSI, 2018). Two-fold serial dilutions of the extracts were prepared in MHB/SDB, yielding a concentration range of 0.156 to 20 mg/mL. Each well was inoculated with the microbial suspension. The plates were incubated, and the MIC was defined as the lowest concentration with no visible growth. For MBC/MFC determination, 10 µL from each clear well was sub-cultured on fresh agar plates. The MBC/MFC was the lowest concentration that killed 99.9% of the inoculum.

2.5. Phytochemical Screening and Total Phenolic/Flavonoid Content

Standard qualitative phytochemical tests were performed on the methanolic extract to detect the presence of alkaloids (Mayer's test), flavonoids (Shinoda test), tannins (Ferric chloride test), terpenoids (Salkowski test), saponins (foam test), and anthraquinones (Borntrager's test) (Harborne, 1998).

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, with gallic acid as a standard, and expressed as mg Gallic Acid Equivalents (GAE)/g of extract. The total

flavonoid content (TFC) was estimated using the aluminum chloride method, with quercetin as a standard, and expressed as mg Quercetin Equivalents (QE)/g of extract.

2.6. Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$) to determine significant differences.

3. Results

3.1. Antimicrobial Activity by Agar Well Diffusion

The results of the initial screening are presented in Table 1. The methanolic extract of *H. decaisneanum* exhibited the strongest and most broad-spectrum antimicrobial activity, showing significant inhibition zones against all tested strains. It was particularly effective against *S. aureus* and *C. albicans*. The ethanolic extract showed moderate activity, while the aqueous extract was largely ineffective, with negligible inhibition zones.

Table 1. Mean Inhibition Zone (mm) of *Hypericum decaisneanum* Extracts (100 mg/mL)

Microorganism	Methanolic Extract	Ethanolic Extract	Aqueous Extract	Positive Control
S. aureus	21.3 ± 0.6a	16.2 ± 0.8b	7.1 ± 0.5c	32.0 ± 0.0
E. faecalis	18.5 ± 0.7a	14.1 ± 0.6b	0	30.0 ± 0.0
E. coli	16.8 ± 0.9a	12.5 ± 0.7b	0	35.0 ± 0.0
P. aeruginosa	14.2 ± 0.8a	10.8 ± 0.5b	0	30.0 ± 0.0
K. pneumoniae	15.1 ± 0.7a	11.3 ± 0.6b	0	33.0 ± 0.0
C. albicans	19.5 ± 0.7a	15.7 ± 0.9b	8.5 ± 0.6c	28.0 ± 0.0

Values are mean ± SD (n=3). Positive control: Ciprofloxacin for bacteria, Fluconazole for C. albicans. Means in the same row with different superscript letters (a-c) are significantly different ($p < 0.05$). A value of 0 indicates no inhibition.

3.2. Determination of MIC and MBC/MFC

The MIC and MBC/MFC values for the methanolic and ethanolic extracts are shown in Table 2. The methanolic extract demonstrated the highest potency, with the lowest MIC value of 0.312 mg/mL against *S. aureus*. The MBC/MFC values were generally equal to or twice the MIC values, indicating a primarily bactericidal and fungicidal mode of action.

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the active extracts from *Hypericum decaisneanum*.

Microorganism	Methanolic Extract		Ethanolic Extract	
	MIC (mg/mL)	**MBC/MFC (mg/mL)**	**MIC (mg/mL)**	**MBC/MFC (mg/mL)**
S. aureus	0.312	0.625	1.25	2.5
E. faecalis	0.625	1.25	2.5	5.0
E. coli	1.25	2.5	5.0	10.0
P. aeruginosa	2.5	5.0	10.0	>20
K. pneumoniae	2.5	5.0	10.0	>20
C. albicans	0.625	1.25	2.5	5.0

Note: MBC was determined for bacterial strains and MFC for *C. albicans*. The symbol ">" indicates that no bactericidal activity was observed at the highest concentration tested (20 mg/mL)

3.3. Phytochemical Screening

Qualitative analysis of the methanolic extract confirmed the presence of flavonoids, tannins, terpenoids, and anthraquinones. Alkaloids were detected in trace amounts, and saponins were

absent. The quantitative analysis revealed a high TPC of 185.4 ± 5.2 mg GAE/g and a TFC of 112.6 ± 4.1 mg QE/g of the dry methanolic extract.

4. Discussion

This study provides the first comprehensive evidence for the antimicrobial potential of *Hypericum decaisneanum* from Libya. The superior efficacy of the methanolic extract aligns with the principle that medium-polarity solvents like methanol are highly effective in extracting a wide range of antimicrobial phytochemicals, including phenolics and flavonoids (Kadak, & Salem, 2020). The weak activity of the aqueous extract suggests that the bioactive principles are less soluble in water.

The potent activity against Gram-positive bacteria, especially *S. aureus*, is a characteristic feature of many *Hypericum* species and can be attributed to compounds like hyperforin and adhyperforin, which are known to disrupt bacterial membranes (Khalil et al., 2025). The significant activity against *C. albicans* is particularly noteworthy and is consistent with studies on other *Hypericum* species, suggesting a potential application in treating fungal infections.

The observed activity against Gram-negative bacteria, though generally lower, is significant. The outer membrane of Gram-negative bacteria often confers intrinsic resistance, making any effective activity notable (Salem, & Salem, 2025).. The presence of a multi-component mixture in the crude extract might have a synergistic effect, allowing some penetration of this barrier.

The phytochemical profile provides a clear rationale for the observed bioactivity. The high concentration of total phenolics and flavonoids in the methanolic extract directly correlates with its strong antimicrobial effect. Flavonoids can disrupt microbial membranes and inhibit energy metabolism, while tannins can inactivate microbial adhesions and enzymes (Taştan, & Salem, 2021, Salem, & Lakwani, 2024). The detection of anthraquinones is also significant, as some derivatives are known for their antimicrobial properties. The presence of these compounds in *H. decaisneanum* confirms its chemotaxonomic alignment with the bioactive *Hypericum* genus and highlights its potential as a source of valuable compounds.

5. Conclusion

The findings of this investigation conclusively demonstrate that *Hypericum decaisneanum* from Bani Waleed, Libya, possesses significant *in vitro* antimicrobial properties, validating its potential use in traditional medicine. The methanolic extract was identified as the most potent, exhibiting bactericidal and fungicidal effects against a broad spectrum of pathogens. This activity is strongly correlated with the extract's high content of phenolic compounds, including flavonoids and tannins. This study establishes *H. decaisneanum* as a promising candidate for further pharmacological exploration. Future work should focus on the bioassay-guided fractionation of the methanolic extract to isolate and characterize the specific active compounds, assess their synergistic effects, and evaluate their safety and efficacy through *in vivo* studies.

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