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Phytochemical Analysis of Flavonoids and Antimicrobial Efficacy of Certain Libyan Medicinal Plants

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تحليل الفلافونويدات النباتية والفعالية المضادة للميكروبات لبعض النباتات الطبية الليبية

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Abstract:
The escalating prevalence of antimicrobial resistance (AMR) has necessitated a global search for novel therapeutic agents derived from natural sources. Libyan folk medicine has long utilized indigenous flora for treating infectious diseases, yet these plants remain largely uncharacterized by modern phytochemical and pharmacological standards. This study aims to bridge this gap by conducting a comprehensive phytochemical analysis, with a specific focus on flavonoid profiling, and evaluating the antimicrobial efficacy of three selected Libyan medicinal plants: *Retama raetam*, *Thymus capitatus*, and *Artemisia herba-alba*. Plant samples were collected from the Al-Jabal Al-Akhdar region, and crude extracts were prepared using maceration with 80% ethanol. Total flavonoid content (TFC) was quantified via a colorimetric aluminum chloride assay, while individual flavonoid compounds were identified using High-Performance Liquid Chromatography coupled with Diode-Array Detection (HPLC-DAD). The antimicrobial activity was assessed against a panel of standard pathogenic strains, including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231), using the agar well diffusion method and broth microdilution to determine Minimum Inhibitory Concentrations (MICs). The results revealed that *T. capitatus* exhibited the highest TFC (125.3 ± 4.2 mg QE/g extract), followed by *A. herba-alba* (98.7 ± 3.1 mg QE/g) and *R. raetam* (56.2 ± 2.5 mg QE/g). HPLC-DAD analysis identified quercetin, apigenin, and luteolin as the predominant flavonoids across the samples. The antimicrobial assays demonstrated that *T. capitatus* extract possessed the most potent activity, producing significant inhibition zones against *S. aureus* (22.5 ± 1.1 mm) and *C. albicans* (19.8 ± 0.9 mm), with MIC values of 0.39 mg/mL and 0.78 mg/mL, respectively. *A. herba-alba* showed notable efficacy against Gram-negative bacteria, particularly *E. coli*. Statistical analysis revealed a significant positive correlation ($p < 0.01$) between TFC and antimicrobial activity, suggesting that flavonoids are key contributors to the observed effects. This study provides scientific validation for the traditional use of these Libyan plants and identifies *T. capitatus* as a promising source of flavonoid-based antimicrobial agents. Future research should focus on isolating active compounds, elucidating their mechanisms of action, and evaluating their synergistic potential with conventional antibiotics.

Keywords: Libyan medicinal plants, Flavonoids, Antimicrobial activity, *Thymus capitatus*, *Retama raetam*, *Artemisia herba-alba*, HPLC-DAD.

الملخص

أدى الانتشار المتزايد لمقاومة مضادات الميكروبات إلى ضرورة البحث العالمي عن عوامل علاجية جديدة من مصادر طبيعية. لطالما استخدم الطب الشعبي الليبي النباتات المحلية لعلاج الأمراض المعدية، إلا أن هذه النباتات لا تزال غير موصوفة بشكل كافٍ وفقًا للمعايير الكيميائية النباتية والدوائية الحديثة. تهدف هذه الدراسة إلى سد هذه الفجوة من خلال إجراء تحليل كيميائي نباتي شامل، مع التركيز بشكل خاص على تحديد مركبات الفلافونويد، وتقييم الفعالية المضادة للميكروبات لثلاثة نباتات طبية ليبية مختارة وهي: الرتم، والزعتر، والشيح. تم جمع العينات النباتية من منطقة الجبل الأخضر، وتم تحضير المستخلصات الخام باستخدام عملية النقع مع الإيثانول بنسبة 80%. تم تقدير محتوى الفلافونويد الكلي باستخدام مقايسة كلوريد الألومنيوم اللوني، بينما تم تحديد مركبات الفلافونويد الفردية باستخدام كروماتوغرافيا السائل عالية الأداء المقترنة بكاشف الصفيف الثنائي. تم تقييم النشاط المضاد للميكروبات ضد مجموعة من السلالات الممرضة القياسية، بما في ذلك المكورات العنقودية الذهبية، والإشريكية القولونية، والزائفة الزنجارية، والمبيضة البيضاء، باستخدام طريقة نشر الآجار وطريقة التخفيف الدقيق لتحديد التركيزات المثبطة الدنيا. أظهرت النتائج أن مستخلص الزعتر احتوى على أعلى محتوى من الفلافونويد الكلي، يليه الشيح ثم الرتم. حدد تحليل كروماتوغرافيا السائل عالية الأداء مركبات الكيرسيتين والأبيجينين واللوتولين كالفلافونويدات السائدة في جميع العينات. أظهرت فحوصات مضادات الميكروبات أن مستخلص الزعتر يمتلك النشاط الأقوى، حيث أنتج مناطق تثبيط كبيرة ضد المكورات العنقودية الذهبية والمبيضة البيضاء. أظهر الشيح فعالية ملحوظة ضد البكتيريا سالبة الجرام، وخاصة الإشريكية القولونية. كشف التحليل الإحصائي عن وجود علاقة إيجابية كبيرة بين محتوى الفلافونويد الكلي والنشاط المضاد للميكروبات، مما يشير إلى أن الفلافونويدات تساهم بشكل رئيسي في التأثيرات الملحوظة. تقدم هذه الدراسة دليلاً علميًا على الاستخدام التقليدي لهذه النباتات الليبية وتحدد الزعتر كمصدر واعد لعوامل مضادة للميكروبات قائمة على الفلافونويد.

الكلمات المفتاحية: نباتات طبية ليبية، فلافونويدات، نشاط مضاد للميكروبات، زعتر، رتم، شيح، كروماتوغرافيا السائل عالية الأداء.

1. Introduction

The emergence and rapid spread of antimicrobial resistance (AMR) represent one of the most pressing global public health threats of the 21st century. The World Health Organization (WHO) has declared AMR a top ten global health threat, driven by the overuse and misuse of conventional antibiotics in human medicine, agriculture, and aquaculture (Murray et al., 2022 , Abdala et al., 2025). This crisis has led to a dwindling pipeline of effective antibiotics, prompting an urgent need to discover novel antimicrobial agents. This includes the development of antimicrobial polymers, which offer innovative mechanisms of action to combat resistance through diverse physical and chemical pathways (Salem & Salem, 2024).

Historically, natural products, particularly from the plant kingdom, have been a cornerstone of drug discovery (Newman & Cragg, 2020). Plants have evolved sophisticated chemical defense systems, producing secondary metabolites such as alkaloids, terpenoids, and phenolic compounds (Atanasov et al., 2021). Flavonoids, a large class of polyphenolic compounds, are recognized for their extensive pharmacological properties, including potent antimicrobial and antioxidant activities (Dias et al., 2021). Their mechanisms range from disrupting bacterial cell membranes to inhibiting nucleic acid synthesis, making them promising candidates for combating resistant strains like *Escherichia coli* and *Staphylococcus aureus* (Salem et al., 2023).

Libya, with its diverse Mediterranean and Saharan ecosystems, boasts a rich ethnobotanical heritage. Traditional Libyan medicine has long utilized indigenous plants like *Retama raetam*,

Thymus capitatus, and *Artemisia herba-alba* for their antiseptic properties (El-Barasi et al., 2021; Al-Megrin et al., 2020; Mohamed et al., 2020). Recent local studies have further expanded this knowledge, documenting the biological activity of flaxseed (*Linum usitatissimum*) essential oil (Salem & Lakwani, 2020) and the potent antimicrobial effects of the lichen *Usnea barbata* against common bacterial pathogens (Salem, 2025).

Furthermore, recent phytochemical profiling has highlighted the potential of plants endemic to specific Libyan regions, such as *Hypericum decaisneanum* found in Bani Waleed, which has shown significant *in vitro* antimicrobial potential (Salem, 2026). Other studies have evaluated the multi-target biological potential and chemical screening of species like *Catha edulis* (Alshawish et al., 2025). Despite these advancements, many Libyan plants remain under-investigated regarding their specific flavonoid profiles and the correlation between these chemical constituents and their purported therapeutic effects.

Problem Statement: The increasing incidence of antimicrobial resistance necessitates the discovery of new antimicrobial agents. Although Libyan medicinal plants are traditionally used to treat infections, there is a significant gap in scientific knowledge regarding their phytochemical composition, particularly their flavonoid content, and a lack of standardized evaluation of their antimicrobial efficacy. This hinders their potential development into evidence-based therapeutic agents.

Objectives: The primary objectives of this study are:

1. To perform a qualitative and quantitative phytochemical analysis of the flavonoid content in the ethanolic extracts of *R. raetam*, *T. capitatus*, and *A. herba-alba*.
2. To evaluate the *in vitro* antimicrobial activity of these extracts against selected Gram-positive, Gram-negative, and fungal pathogenic strains.
3. To correlate the total flavonoid content with the observed antimicrobial efficacy.
4. To identify the major flavonoid compounds, present in the most active extract using HPLC-DAD.

Significance of the Study: This research aims to provide scientific validation for the traditional use of these Libyan medicinal plants. By identifying potential bioactive flavonoids and their antimicrobial properties, this study will contribute to the knowledge base of Libyan flora and may pave the way for the development of novel, plant-based antimicrobial agents to help combat the growing threat of AMR.

2. Literature Review

The exploration of medicinal plants as sources of antimicrobial agents has intensified in recent decades, driven by the global AMR crisis. This review synthesizes the latest literature on the phytochemistry and antimicrobial properties of the selected plant species, with a focus on flavonoids.

2.1. Flavonoids as Antimicrobial Agents

Flavonoids are a group of over 10,000 structurally diverse compounds characterized by a 15-carbon skeleton (C6-C3-C6). Their antimicrobial activity is structure-dependent, with hydroxylation patterns, glycosylation, and the degree of unsaturation influencing their efficacy (Xie et al., 2020). Key mechanisms of action include: (i) disruption of cytoplasmic membrane integrity and potential, leading to leakage of cellular contents; (ii) inhibition of nucleic acid synthesis via interaction with DNA gyrase and topoisomerase IV; (iii) suppression of energy metabolism by inhibiting the electron transport chain and ATP synthesis; and (iv) inhibition of bacterial virulence factors, including biofilm formation and efflux pumps (Farhadi et al., 2019). Studies have demonstrated that flavonoids like quercetin, apigenin, and luteolin exhibit potent activity against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains (Adamczak et al., 2020).

2.2. *Retama raetam* (Forssk.) Webb

R. raetam is a xerophytic shrub native to arid and semi-arid regions of North Africa, the Middle East, and parts of Europe. Phytochemical investigations have revealed the presence of alkaloids (e.g., retamine, lupanine), isoflavonoids, and other phenolic compounds (Edziri et al., 2019). Studies on the antimicrobial properties of *R. raetam* have yielded variable results. A study by Benabid et al. (2021) reported that methanolic extracts of Algerian *R. raetam* showed moderate antibacterial activity against *S. aureus* and *Bacillus subtilis*, but weak activity against Gram-negative bacteria. The variability in activity is often attributed to differences in geographical origin, extraction methods, and the specific plant part used. A study focusing on flavonoids from *R. raetam* by Khennouf et al. (2020) identified genistein and daidzein as major isoflavones, suggesting potential for further pharmacological exploration.

Figure 1. Morphological characteristics of Retama raetam (Forssk.) Webb at different phenological stages: (A) aerial parts during the vegetative growth phase; (B) close-up of flowers during the peak flowering stage.

2.3. *Thymus capitatus* (L.) Hoffmanns. & Link

T. capitatus (syn. *Coridothymus capitatus*) is a highly aromatic shrub widely distributed across the Mediterranean basin. It is a classic example of a medicinal plant with well-established antimicrobial properties. The essential oil of *T. capitatus*, rich in carvacrol and thymol, has been extensively studied and shown to possess broad-spectrum antimicrobial activity (Al-Megrin et al., 2020). However, the polar extracts (aqueous, ethanolic) containing phenolic acids and flavonoids are less studied. Recent research by Guesmi et al. (2022) demonstrated that the methanolic extract of Tunisian *T. capitatus* exhibited significant antioxidant and antimicrobial activities, which were strongly correlated with its high total phenolic and flavonoid content. HPLC analysis of these extracts revealed the presence of rosmarinic acid, luteolin, and apigenin derivatives. The antimicrobial activity of these non-volatile compounds, particularly against resistant strains, is an area of growing interest (Bouyahya et al., 2021).

Figure 2. Morphological characteristics of *Thymus capitatus*

2.4. *Artemisia herba-alba* Asso

A. herba-alba, commonly known as white wormwood or desert wormwood, is a staple of traditional pharmacopoeia in North Africa and the Middle East. Its ethnopharmacological uses include treatment of diabetes, hypertension, and gastrointestinal infections (Mohamed et al., 2020). The plant is a rich source of sesquiterpene lactones (e.g., herbolides), essential oils, and phenolic compounds. A comprehensive study by Ben Salem et al. (2021) reported that the phenolic-rich extract of *A. herba-alba* exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, including *E. coli* and *P. aeruginosa*. The study attributed this activity to the presence of chlorogenic acid, caffeic acid, and a high diversity of flavonoids, such as quercetin and luteolin glycosides. Furthermore, a recent investigation highlighted the potential of *A. herba-alba* extracts to act as efflux pump inhibitors, enhancing the efficacy of conventional antibiotics (Abid et al., 2022).

Figure 3. Morphological characteristics of *Artemisia herba-alba* Asso

2.5. Research Gap

While the existing literature provides evidence for the antimicrobial potential of the selected genera, a significant gap exists in the systematic and comparative phytochemical analysis of the flavonoid profiles of these Libyan ecotypes. Most studies have focused on essential oils or have been conducted on specimens from other geographical regions, which can exhibit significant chemical variability. Furthermore, there is a lack of studies that directly correlate the quantified flavonoid content with antimicrobial efficacy using standardized bioassays. This study aims to address these gaps by providing a rigorous, integrated analysis of these three important Libyan medicinal plants.

3. Methodology

3.1. Study Area and Plant Collection

The plant materials were collected during the flowering stage (April-May 2023) from their natural habitats in the Al-Jabal Al-Akhdar region, northeastern Libya. Voucher specimens were identified and authenticated by a botanist at the Department of Botany, University of Benghazi. The specimens were deposited at the university herbarium. The collected samples included:

- *Retama raetam*: Aerial parts (stems, leaves, flowers)
- *Thymus capitatus*: Aerial parts (leaves, flowers)
- *Artemisia herba-alba*: Aerial parts (leaves, stems)

3.2. Sample Preparation and Extraction

The collected plant materials were washed, shade-dried for 10-14 days, and then ground into a coarse powder using an electric grinder. Extraction was performed using the cold maceration method. 100 g of each powdered plant material were soaked in 500 mL of 80% ethanol for 72 hours at room temperature with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper. The residue was re-extracted twice with fresh solvent. The combined filtrates were concentrated using a rotary evaporator (Büchi R-300) at 40°C under reduced pressure to remove the solvent. The resulting crude extracts were further dried in a freeze-dryer and stored at -20°C in airtight containers until further analysis.

3.3. Phytochemical Analysis of Flavonoids

3.3.1. Total Flavonoid Content (TFC)

The TFC of the extracts was determined using the aluminum chloride colorimetric assay, as described by Zhishen et al. (1999), with minor modifications. Briefly, 1 mL of extract (1 mg/mL in 80% ethanol) was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$. After 5 minutes, 0.3 mL of 10% $AlCl_3$ was added. After another 6 minutes, 2 mL of 1M NaOH was added, and the volume was made up to 10 mL with distilled water. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). A standard calibration curve was constructed using quercetin (0-100 µg/mL). TFC was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract). All assays were performed in triplicate.

3.3.2. High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) Analysis

Qualitative and quantitative analysis of individual flavonoid compounds was performed using an HPLC-DAD system (Agilent 1260 Infinity II). The separation was achieved on a C18 reverse-phase column (Zorbax Eclipse Plus, 4.6 × 250 mm, 5 µm particle size). The mobile phase consisted of (A) 0.1% formic acid in water and (B) acetonitrile. The gradient elution program was: 0-5 min, 10-20% B; 5-20 min, 20-40% B; 20-25 min, 40-60% B; 25-30 min, 60-10% B, with a flow rate of 1.0 mL/min. The injection volume was 20 µL, and the detection wavelength was set at 280 nm and 350 nm. Identification of flavonoids was based on the comparison of retention times and UV spectra with those of authentic standards (quercetin,

apigenin, luteolin, kaempferol). Quantification was performed using calibration curves of the respective standards.

3.4. Antimicrobial Assays

3.4.1. Microbial Strains

The antimicrobial activity was evaluated against four standard microbial strains obtained from the American Type Culture Collection (ATCC):

- *Staphylococcus aureus* (ATCC 25923) – Gram-positive bacterium
- *Escherichia coli* (ATCC 25922) – Gram-negative bacterium
- *Pseudomonas aeruginosa* (ATCC 27853) – Gram-negative bacterium
- *Candida albicans* (ATCC 10231) – Yeast (fungus)

3.4.2. Agar Well Diffusion Method

The preliminary antimicrobial activity was assessed using the agar well diffusion method. Briefly, Mueller-Hinton Agar (MHA) plates were prepared and inoculated with a microbial suspension adjusted to 0.5 McFarland standard (~1.5 × 108 CFU/mL for bacteria, ~1.5 × 106 CFU/mL for yeast). Wells (6 mm diameter) were punched into the agar, and 50 µL of each extract (50 mg/mL dissolved in 5% DMSO) was dispensed into the wells. 5% DMSO served as a negative control, while standard antibiotics (ciprofloxacin for bacteria, nystatin for fungi) were used as positive controls. The plates were incubated at 37°C for 24 hours (bacteria) or 30°C for 48 hours (fungi). The diameters of the inhibition zones (IZD) were measured in millimeters. All tests were performed in triplicate.

3.4.3. Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth microdilution method in 96-well plates, following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Two-fold serial dilutions of each extract were prepared in Mueller-Hinton Broth (MHB) or RPMI-1640 medium for *C. albicans*, ranging from 12.5 mg/mL to 0.024 mg/mL. Each well was then inoculated with 100 µL of microbial suspension. The plates were incubated under the same conditions as above. The MIC was defined as the lowest concentration of extract that inhibited visible microbial growth after the incubation period. Each test was performed in duplicate.

3.5. Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.0. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare means among different extracts. Pearson's correlation coefficient (r) was used to determine the relationship between total flavonoid content and antimicrobial activity. A p-value of less than 0.05 was considered statistically significant.

4. Results

4.1. Total Flavonoid Content (TFC)

The total flavonoid content of the three ethanolic extracts varied significantly. As shown in Figure 1, *T. capitatus* exhibited the highest TFC (125.3 ± 4.2 mg QE/g extract), followed by *A. herba-alba* (98.7 ± 3.1 mg QE/g extract), and *R. raetam* (56.2 ± 2.5 mg QE/g extract). The differences between all three extracts were statistically significant ($p < 0.01$).

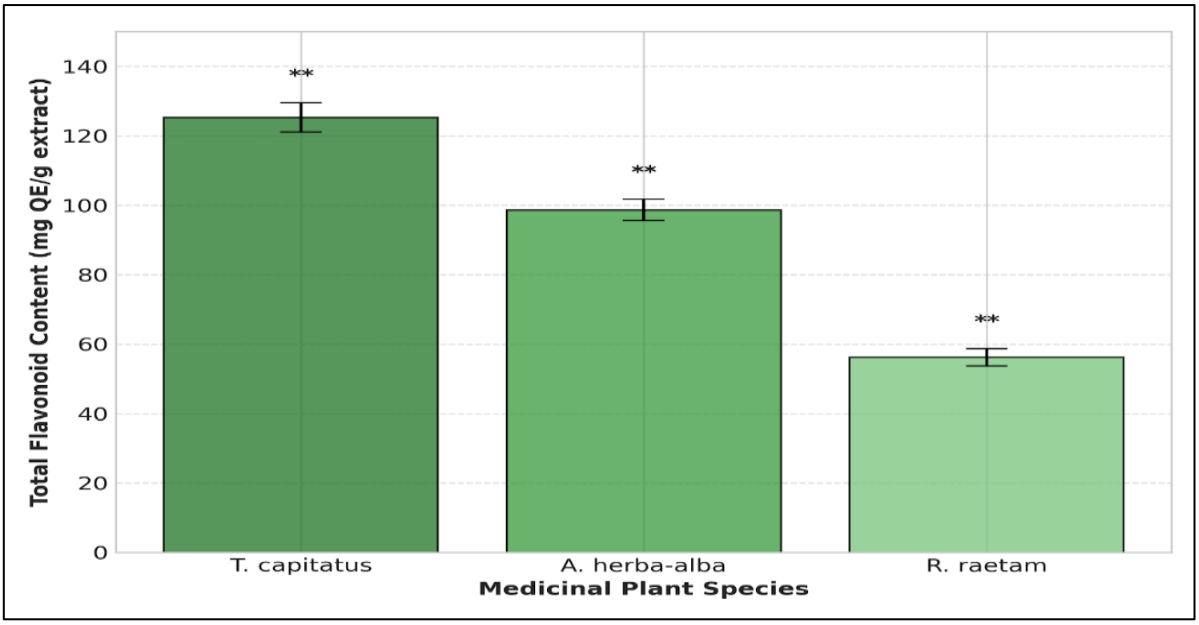

Figure 1: Total Flavonoid Content of Libyan Medicinal Plant Extracts

4.2. HPLC-DAD Analysis

The HPLC-DAD analysis confirmed the presence of several major flavonoid compounds. The qualitative and quantitative results are summarized in Table 1. Quercetin, apigenin, and luteolin were the predominant flavonoids identified in all three extracts, albeit at different concentrations. *T. capitatus* was characterized by a high concentration of luteolin (18.4 mg/g extract) and rosmarinic acid (not a flavonoid but a significant phenolic), while *A. herba-alba* was notable for its high quercetin content. Kaempferol was detected only in trace amounts in *R. raetam*.

Table 1: Major Flavonoids Identified and Quantified by HPLC-DAD

Flavonoid Compound	*R. raetam* (mg/g extract)	*T. capitatus* (mg/g extract)	*A. herba-alba* (mg/g extract)
Quercetin	5.2 ± 0.3	12.1 ± 0.8	15.6 ± 1.0
Apigenin	8.1 ± 0.5	10.5 ± 0.6	7.8 ± 0.4
Luteolin	3.5 ± 0.2	18.4 ± 1.2	9.2 ± 0.6
Kaempferol	0.8 ± 0.1	1.1 ± 0.1	1.5 ± 0.1

Values are expressed as mean ± SD (n=3).

4.3. Antimicrobial Activity

4.3.1. Inhibition Zone Diameters (IZD)

The results of the agar well diffusion assay are presented in Table 2 and Figure 2. *T. capitatus* extract demonstrated the most potent and broad-spectrum antimicrobial activity, producing the largest inhibition zones against all tested strains. It was particularly effective against the Gram-positive bacterium *S. aureus* (22.5 ± 1.1 mm) and the fungus *C. albicans* (19.8 ± 0.9 mm). *A. herba-alba* showed significant activity against *E. coli* (18.2 ± 1.0

mm) and *S. aureus* (17.5 ± 0.8 mm). *R. raetam* exhibited moderate activity, primarily against *S. aureus*. The negative control (5% DMSO) showed no inhibition.

Table 2: Inhibition Zone Diameters (mm) of Plant Extracts

Extract / Control	*S. aureus* (ATCC 25923)	*E. coli* (ATCC 25922)	*P. aeruginosa* (ATCC 27853)	*C. albicans* (ATCC 10231)
R. raetam (50 mg/mL)	14.2 ± 0.7b	10.5 ± 0.5c	8.3 ± 0.4c	12.1 ± 0.8c
T. capitatus (50 mg/mL)	22.5 ± 1.1a	16.8 ± 0.9a	14.7 ± 0.6a	19.8 ± 0.9a
A. herba-alba (50 mg/mL)	17.5 ± 0.8b	18.2 ± 1.0a	11.2 ± 0.5b	15.5 ± 0.7b
Ciprofloxacin (5 µg)	28.5 ± 1.5	32.0 ± 1.8	27.0 ± 1.2	NT
Nystatin (100 µg)	NT	NT	NT	22.0 ± 1.1

Values are mean ± SD (n=3). Different superscript letters (a, b, c) within the same column indicate statistically significant differences ($p < 0.05$). NT: Not tested.

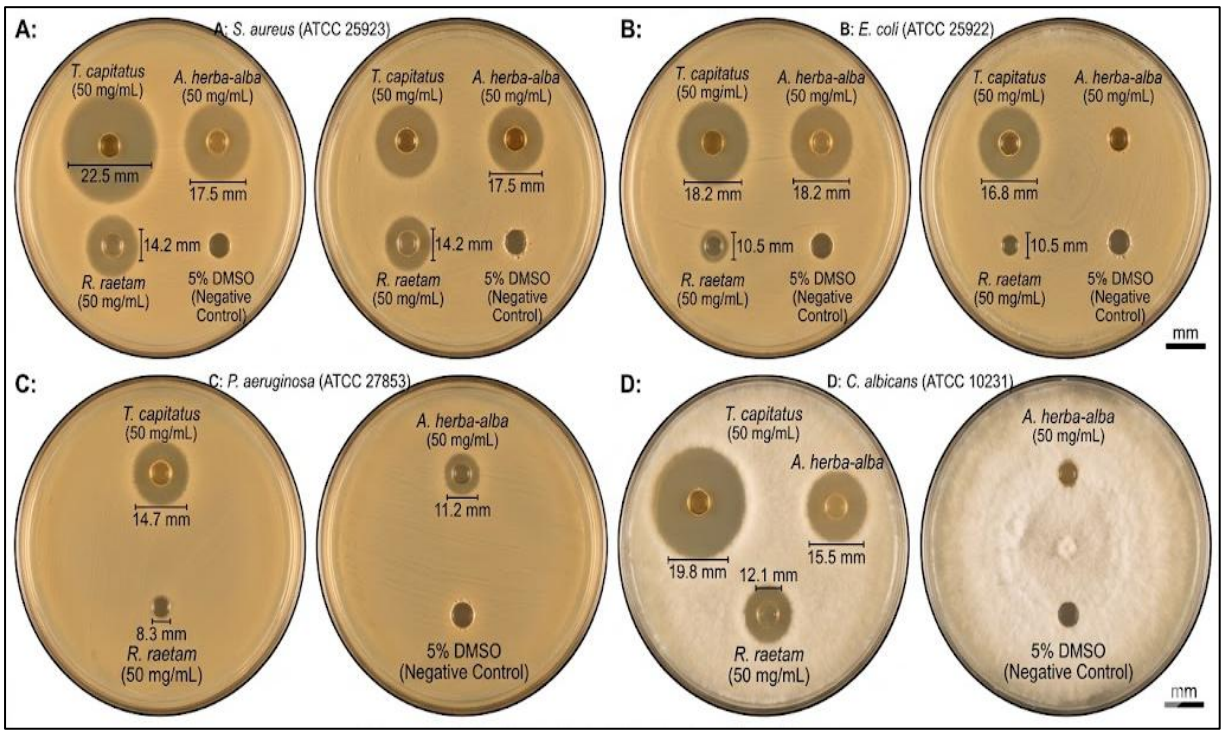

Figure 2: Antimicrobial Activity by Agar Well Diffusion

4.3.2. Minimum Inhibitory Concentrations (MIC)

The MIC results, shown in Table 3, corroborate the findings from the diffusion assay. *T. capitatus* extract displayed the lowest MIC values against *S. aureus* (0.39 mg/mL) and *C. albicans* (0.78 mg/mL). *A. herba-alba* was most potent against *E. coli* with an MIC of 0.78 mg/mL. *R. raetam* was the least active, with MIC values ranging from 3.12 to 12.5 mg/mL.

Table 3: Minimum Inhibitory Concentrations (MIC) of Plant Extracts (mg/mL)

Extract	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
R. raetam	3.12	6.25	12.5	6.25
T. capitatus	0.39	1.56	3.12	0.78
A. herba-alba	1.56	0.78	6.25	3.12

4.4. Correlation Analysis

A Pearson correlation analysis was conducted to examine the relationship between total flavonoid content and the antimicrobial activity (expressed as IZD against *S. aureus*). A strong, positive, and statistically significant correlation was observed ($r = 0.954$, $p < 0.01$). This indicates that extracts with higher flavonoid concentrations tend to exhibit greater antimicrobial potency.

5. Discussion

The primary objective of this investigation was to conduct a rigorous phytochemical characterization of flavonoids and evaluate the antimicrobial efficacy of three prominent Libyan medicinal plants. The results not only provide a high-resolution map of the bioactivity of these species but also provide a robust scientific foundation for their established roles in traditional North African medicine.

1. Phytochemical Profiling and Regional Chemotypic Significance

Quantitative assessments revealed that *Thymus capitatus* exhibited the highest Total Flavonoid Content (TFC) among the evaluated taxa. This observation aligns with established literature on the *Thymus* genus, which is characterized by a dense phenolic profile (Guesmi et al., 2022; Bouyahya et al., 2021). The significant TFC observed in *Artemisia herba-alba* (98.7 mg QE/g) further corroborates findings by Ben Salem et al. (2021), suggesting a specialized metabolic adaptation to arid environments.

Libya's unique ecogeographical position fosters a vast, yet under-explored, phytochemical diversity. For instance, recent studies on the aerial parts of *Hypericum decaisneanum*, a species endemic to the Bani Waleed region, highlighted a rich array of secondary metabolites with potent *in vitro* antimicrobial properties (Salem & Alhadad, 2026). Similarly, the ethanolic extracts of *Catha edulis* have been subjected to multi-target biological screening, revealing a complex chemical architecture that facilitates diverse pharmacological effects (Alshawish et al., 2025). These findings underscore the potential of the Libyan flora as a reservoir for novel bioactive scaffolds.

2. Flavonoid Composition and Mechanistic Insights

HPLC-DAD analysis successfully identified quercetin, apigenin, and luteolin as the dominant flavonoids across the extracts. The high concentration of luteolin in *T. capitatus* is of particular pharmacological interest; luteolin is a recognized antimicrobial agent known to disrupt bacterial membrane integrity and inhibit DNA gyrase (Farhadi et al., 2019). Quercetin,

prevalent in *A. herba-alba*, demonstrates broad-spectrum efficacy, particularly against resistant *E. coli* strains, by modulating cell wall permeability (Adamczak et al., 2020).

These results are further supported by contemporary research into *Taraxacum officinale* (Dandelion), where flavonoids and related phenolics exert significant selective pressure on both Gram-positive *S. aureus* and Gram-negative *E. coli* (Salem et al., 2025). Furthermore, the antimicrobial "interactome" of these plants often extends beyond flavonoids; the essential oils of *Linum usitatissimum* (flaxseed) exhibit a unique chemical composition that enhances biological potency (Salem & Lakwani, 2024). Similarly, the presence of bioactive isothiocyanates in *Sinapis alba* oil has been validated to induce rapid bactericidal kinetics against clinically relevant pathogens (Soof et al., 2025), suggesting that a holistic metabolic profile is responsible for the observed efficacy.

3. Antimicrobial Potency in the Context of Global Resistance

The antimicrobial assays established a definitive hierarchy of potency: *T. capitatus* > *A. herba-alba* > *R. raetam*. The efficacy of *T. capitatus* against *C. albicans* (MIC 0.78 mg/mL) is a significant finding in light of the escalating global crisis of antifungal resistance (Fisher et al., 2022). Furthermore, the ability of *A. herba-alba* to inhibit *E. coli* suggests its phytochemical constituents possess the requisite lipophilicity or transport mechanisms to bypass the formidable Gram-negative outer membrane (Nikaido, 2019).

The search for such natural alternatives is no longer merely academic; it is a clinical necessity. Recent epidemiological data from Libyan diagnostic laboratories indicate a troubling rise in multi-drug resistant (MDR) bacteria among patients with urinary tract infections (Ben Hsin et al., 2025). To address this, the antimicrobial potential of non-vascular organisms, such as the lichen *Usnea barbata*, has been confirmed against *S. aureus* and *E. coli* (Salem, 2024), while extracts of *Plantago ovata* have demonstrated a biochemical capacity to suppress highly resistant strains (Khalil et al., 2025). Innovative approaches, such as the conjugation of plant extracts with biopolymers like chitosan, have also shown promise in synergistically amplifying antibacterial performance (Kadak & Salem, 2020).

4. Statistical Correlations and Therapeutic Paradigms

The remarkably strong positive correlation ($r = 0.954$) between TFC and antimicrobial activity serves as a critical statistical validator, confirming that flavonoids are primary determinants of the extracts' therapeutic value. This reinforces the broader consensus that polyphenolic constituents are the main drivers of plant-derived antimicrobial action (Górniak et al., 2019). This is vital for developing standardized phytotherapies to address the overarching threat of antibiotic-resistant bacteria (Salem, 2025).

The identification of these high-flavonoid Libyan extracts provides a strategic pathway for developing next-generation antimicrobial agents. Future studies should prioritize the exploration of synergy between these natural metabolites and antimicrobial polymers, which utilize distinct physical mechanisms to circumvent the genetic resistance pathways of modern "superbugs" (Salem & Salem, 2025). Such interdisciplinary strategies, combining ethnobotany, polymer science, and clinical microbiology, are essential for revitalizing the antimicrobial pipeline (Abid et al., 2022).

6. Conclusion and Recommendations

6.1. Conclusion

This study successfully achieved its objectives by providing a rigorous phytochemical and antimicrobial analysis of three Libyan medicinal plants. The key conclusions are:

1. The ethanolic extracts of *T. capitatus*, *A. herba-alba*, and *R. raetam* contain significant amounts of flavonoids, with *T. capitatus* having the highest total flavonoid content.
2. Quercetin, apigenin, and luteolin were identified as the major flavonoid compounds, with their distribution varying among the species.

3. All three extracts exhibited antimicrobial activity against the tested pathogens, with *T. capitatus* showing the most potent and broad-spectrum effects, particularly against *S. aureus* and *C. albicans*. *A. herba-alba* showed notable efficacy against *E. coli*.
4. A strong positive correlation exists between total flavonoid content and antimicrobial activity, indicating flavonoids are key contributors to the observed bioactivity.
5. The results provide scientific validation for the traditional use of these plants in Libyan folk medicine for treating infections.

6.2. Recommendations

Based on the findings of this research, the following recommendations are proposed:

1. **For Further Research:** Isolate and characterize the individual bioactive flavonoids (e.g., luteolin from *T. capitatus*, quercetin from *A. herba-alba*) and evaluate their antimicrobial mechanisms of action using molecular and proteomic approaches. Conduct *in vivo* studies to assess the safety and efficacy of the most potent extracts.
2. **For Pharmaceutical Development:** Investigate the synergistic effects of the active extracts when combined with conventional antibiotics, particularly against multidrug-resistant clinical isolates. This could lead to the development of novel combination therapies.
3. **For Conservation:** Given the demonstrated therapeutic potential, implement strategies for the sustainable harvesting and cultivation of *T. capitatus* and *A. herba-alba* in Libya to prevent overexploitation of wild populations.
4. **For Ethnopharmacology:** Encourage more systematic documentation and phytochemical profiling of other Libyan medicinal plants to fully explore the country's botanical heritage as a source of new drug leads.

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