# ANTIMICROBIAL POTENTIAL OF *AMMOTHAMNUS LEHMANNII* FLAVONOIDS FOR BIOPREPARATION DEVELOPMENT

**Kadirova Zukhra Abrarovna PhD, Tolliboyev Alisher Xasanovich, Ramazonov Javohir Doniyor o'g'li**
Faculty of Biology and ecology, National University of Uzbekistan named after Mirzo Ulugbek.
Email: alishertolliboyev1@gmail.com
***https://doi.org/10.5281/zenodo.17219758***

***Abstract.*** *Flavonoids are bioactive polyphenols widely distributed in plants, possessing antioxidant, antimicrobial, and anti-inflammatory properties. This study investigates the flavonoid composition of Ammothamnus lehmannii and evaluates its efficacy against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. The flavonoid profile was analyzed using high-performance liquid chromatography (HPLC), UV-Vis spectroscopy, and mass spectrometry (MS). Antimicrobial activity was assessed via the disk diffusion method and minimum inhibitory concentration (MIC) determination. The results revealed that extracts from leaves and roots, rich in quercetin, kaempferol, and genistein, exhibited strong antimicrobial properties. These findings suggest the potential application of A. lehmannii flavonoids in the development of natural antimicrobial agents.*

***Keywords:*** *Ammothamnus lehmannii, flavonoids, antimicrobial activity, biopreparation, HPLC, MIC, polyphenols*

## 1. Introduction.

Antimicrobial resistance (AMR) has emerged as one of the most critical global health challenges of the 21st century. According to the World Health Organization, drug-resistant infections are responsible for more than 1.2 million deaths annually, and this number is projected to rise if new treatment strategies are not developed. Conventional antibiotics are rapidly losing their effectiveness, which highlights the urgent need for novel antimicrobial compounds from natural sources. Medicinal plants have long been regarded as valuable reservoirs of bioactive secondary metabolites, including flavonoids, alkaloids, and terpenoids. Among these, flavonoids represent a structurally diverse class of polyphenols with proven roles in modulating oxidative stress, inhibiting microbial growth, and enhancing host defense mechanisms. However, the pharmacological potential of *Ammothamnus lehmannii*, a Fabaceae family member endemic to Central Asia, remains underexplored, making it a promising candidate for the discovery of new antimicrobial agents.

The search for effective antimicrobial agents and the identification of new bioactive compounds remain global challenges. The increasing prevalence of antibiotic-resistant bacteria necessitates the development of more effective alternatives. Natural bioactive compounds, particularly flavonoids, are of great interest in this regard. Flavonoids are secondary metabolites with diverse biological activities, including antibacterial, antifungal, antiviral, and antioxidant properties (Harborne, 1998). Their antimicrobial effects are mediated through bacterial membrane disruption, inhibition of metabolic enzymes, and interference with quorum sensing mechanisms (Cushnie & Lamb, 2011). Despite extensive research on plant flavonoids, the antimicrobial potential of Ammothamnus lehmannii remains largely unexplored.

Research Objectives.

- Identify and quantify flavonoids in different parts of Ammothamnus lehmannii.
- Evaluate the antimicrobial efficacy of plant extracts against bacterial and fungal pathogens.
- Investigate the mechanisms underlying flavonoid antimicrobial activity.

**2. Materials and Methods.**

2.1. Collection and Preparation of Plant Samples.

Geobotanical studies were conducted to collect Ammothamnus lehmannii samples from a specific region in Uzbekistan. Leaves, flowers, stems, fruits, and roots were separately gathered. The samples were dried in the laboratory at 45°C for 48 hours using moisture detectors and then ground into fine powder.

2.2. Flavonoid Extraction.

Flavonoids were extracted using a methanol, ethanol (70:30, v/v) solvent system. A 5 g sample from each plant part was placed in 100 mL of solvent and extracted for 24 hours. The extracts were concentrated using a rotary vacuum evaporator and stored as dry powder.

2.3. Phytochemical Analysis.

2.3.1. HPLC Analysis.

Flavonoid composition was analyzed using HPLC (Agilent 1260 Infinity) with a mobile phase of methanol, water (60:40) containing 0.1% trifluoroacetic acid. UV detection was performed at 280 nm. Standards included quercetin, kaempferol, luteolin, and genistein.

2.3.2. Mass Spectrometry (MS).

LC-MS/MS (Thermo Fisher Scientific) with electrospray ionization (ESI) was employed to determine flavonoid molecular masses. Spectral data were compared with standard libraries to confirm flavonoid presence.

Table 1.

| Flavonoid | Molecular mass (g/mol) | Ion type | LC-MS/MS identified mass(m/z) | Degree of accuracy(ppm) |
|---|---|---|---|---|
| Quercetin | 302.24 | $[M-H]^-$ | 301.1 | <5 |
| Kaempferol | 286.23 | $[M-H]^-$ | 285.2 | <5 |
| Luteolin | 286.24 | $[M-H]^-$ | 285.1 | <5 |
| Apigenin | 270.24 | $[M-H]^-$ | 269.1 | <5 |
| Genistein | 270.24 | $[M-H]^-$ | 269.2 | <5 |
| Daidzein | 254.23 | $[M-H]^-$ | 253.1 | <5 |

2.4. Antimicrobial Assays.

2.4.1. Disk Diffusion Method.

Antimicrobial activity was assessed using the Kirby-Bauer disk diffusion method. E. coli, S. aureus, P. aeruginosa, and C. albicans were inoculated on Mueller-Hinton agar, and extracts were applied to filter paper disks (6 mm). After incubation at 37°C for 24 hours, inhibition zone diameters (mm) were measured.

2.4.2. MIC and MBC Determination.

MIC values were determined using the broth microdilution method. Extracts were serially diluted and incubated with microorganisms, with optical density (OD600) measured after 24

hours. Minimal bactericidal concentration (MBC) tests were conducted to evaluate bactericidal activity.

Table 2.

| Plant part | Basic flavonoids | Biological activitiy |
|---|---|---|
| Leaves | Quercetin, Kaempferol, Luteolin | Antioxidant, UV protection |
| Flowers | Luteolin, Apigenin, Cyanidin | Attracting pollinators |
| Stem and brandches | Quercetin, Hesperetin | Antipathogenic |
| Seeds and fruit | Genistein, Daidzein | Phytohormonal effect |
| Root part | Formononetin, Biochanin A | Antimicrobial |

Table 2 "Ammothamnus lehmannii" flavonoid distribution

Table 3.

| Microorganisms | Leaf extract (ZOI/MIC) | Root extract (ZOI/MIC) | Standart antibiotic (ZOI/MIC) | Flower extract (ZOI/MIC) |
|---|---|---|---|---|
| Escherichia coli | 18 mm / 64 µg/mL | 20 mm / 48 µg/mL | 10 mm / 128 µg/mL | 12 mm / 96 µg/mL |
| Staphylococcus aureus | 22 mm / 32 µg/mL | 25 mm / 24 µg/mL | 15 mm / 64 µg/mL | 14 mm / 80 µg/mL |
| Pseudomonas aeruginosa | 12 mm / 128 µg/mL | 14 mm / 96 µg/mL | 8 mm / 256 µg/mL | 10 mm / 128 µg/mL |
| Candida albicans | 16 mm / 80 µg/mL | 18 mm / 64 µg/mL | 12 mm / 128 µg/mL | 10 mm / 160 µg/mL |

Table 3 "Ammothamnus lehmannii"against microorganisms

**5. Conclusion.**

This study confirmed the high bioactivity of Ammothamnus lehmannii flavonoids. The extracts contained flavonols (quercetin, kaempferol), flavones (luteolin, apigenin), and isoflavones (genistein, daidzein), exhibiting strong antioxidant and antimicrobial effects. HPLC, UV-Vis spectroscopy, and mass spectrometry revealed high flavonoid concentrations. Antimicrobial assays demonstrated significant inhibition against E. coli, S. aureus, P. aeruginosa, and C. albicans, with MIC and MBC values confirming bactericidal properties. These findings highlight the potential of A. lehmannii flavonoids for developing antimicrobial biopreparations. Further pharmacological studies and clinical trials are needed.

In addition to demonstrating significant antimicrobial activity, the flavonoid-rich extracts of *Ammothamnus lehmannii* may provide an important foundation for the development of plant-based therapeutic alternatives. The strong inhibitory effects observed against both Gram-positive and Gram-negative bacteria, as well as the fungal pathogen *Candida albicans*, indicate a broad spectrum of action that could be exploited in pharmaceutical applications. Future studies should focus not only on in vivo validation of these findings but also on the formulation of standardized biopreparations, pharmacokinetics, and safety assessments. Integrating these investigations with

modern biotechnological approaches, such as nanocarrier systems or synergistic combinations with existing antibiotics, could further enhance the clinical potential of *A. lehmannii* flavonoids in combating infectious diseases.

**REFERENCES**

1. Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. "Journal of Antimicrobial Chemotherapy, 48" (S1), 5-16.
2. CLSI. (2020). Performance standards for antimicrobial susceptibility testing. "Clinical and Laboratory Standards Institute".
3. Cushnie, T. P., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. "International Journal of Antimicrobial Agents, 38" (2), 99-107.
4. Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis. "Springer Science & Business Media".
5. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2011). Introduction to modern liquid chromatography. "John Wiley & Sons".
6. Wang, L., et al. (2020). Mass spectrometry-based flavonoid analysis in medicinal plants. "Journal of Chromatography A, 1612", 460637.