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Antimicrobial and antioxidant activities and preclinical evaluation of common yarrow, *Achillea millefolium* L.

Gulmira Yeltay[1]*, Gulshat Anarbekova[2], Saule Mukhtubayeva[3], Kuandyk Myssaev[4], Kairat Iskakov[5], Nazym Bekenova[4], Zura Yessimsiitova[1]*, Moldir Tileshova[1]

1. Department of Biodiversity and Bioresources, Faculty Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan
2. Department of Biology, Institute of Natural Science, Kazakh National Women's Teacher Training University, Almaty, Kazakhstan
3.High School of Natural Sciences of Astana International University, Astana, Kazakhstan
4. Department of Biology, Faculty of Natural Sciences and Geography, Kazakh National Pedagogical University, Almaty, Kazakhstan
5. Department of Sheep Breeding of the Meat Direction, Kazakh Scientific Research Institute of Animal and Fodder Production, Almaty, Kazakhstan

* *Corresponding author's E-mail: zura1958@bk.ru, gulmira280595@mail.ru*

ABSTRACT

This research explores the bioactive potential of *Achillea millefolium* L. (common yarrow), a plant historically utilized in traditional medicine, by evaluating its antimicrobial and antioxidant properties. The study involves extracting and characterizing bioactive compounds from yarrow, followed by *in vitro* analyses to assess its effectiveness against prevalent pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Antimicrobial performance is measured using agar diffusion and microdilution techniques to quantify inhibitory effects. Antioxidant capacity is determined through two assays: DPPH radical scavenging, which yields an IC_{50} value of 45.2 µg mL^{-1}, and FRAP analysis, demonstrating a ferric-reducing capability of 78.3%. These results highlight yarrow's ability to neutralize reactive oxygen species and reduce oxidative stress, underscoring its therapeutic promise. Preclinical evaluation involves *in vivo* studies on rodent models to assess the anti-inflammatory and wound-healing properties of yarrow extracts, demonstrating a 40% reduction in inflammation and a 35% acceleration in wound closure compared to control groups. Phytochemical analysis identifies flavonoids, phenolic acids, and sesquiterpene lactones as the primary bioactive constituents responsible for these effects. The findings suggest that *A. millefolium* L. possesses potent antimicrobial and antioxidant properties and promising preclinical efficacy, making it a potential candidate for developing natural therapeutic agents. Further studies are recommended to explore its safety, dosage, and mechanisms of action for clinical applications.

Keywords: *Achillea millefolium* L., Antimicrobial activity, Antioxidant activity, Preclinical evaluation, Wound healing, Phytochemical analysis.
Article type: Research Article.

INTRODUCTION

The global surge in antibiotic-resistant pathogens and the escalating demand for natural alternatives have intensified the exploration of medicinal plants as reservoirs of bioactive agents (WHO 2020). Within this context, *Achillea millefolium* L. (yarrow), renowned for its historical role in wound care, inflammation management, and infection treatment, has emerged as a focal point of scientific inquiry (Saeidnia *et al.* 2011). This study investigates yarrow's antimicrobial, antioxidant, and preclinical therapeutic effects to establish an evidence-based foundation for its medicinal applications. Culturally, yarrow's legacy spans continents: European herbal traditions leveraged its hemostatic and gastrointestinal benefits (Applequist & Moerman 2011), while Indigenous North American

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communities harnessed its anti-inflammatory and pain-relieving qualities (Moerman 1998). Such diverse historical applications underscore its versatility as a medicinal resource. Yarrow's pharmacological efficacy stems from its complex phytochemical composition, featuring flavonoids, phenolic acids, sesquiterpene lactones, and volatile oils (Chandler *et al.* 1982). Key flavonoids like apigenin and luteolin are recognized for neutralizing oxidative stress and suppressing inflammation, whereas sesquiterpene lactones such as allicin underpin its antimicrobial potency (Benedek *et al.* 2007; Abed *et al.* 2024). Amid rising multidrug-resistant infections (Ventola 2015), yarrow's broad-spectrum activity against bacteria (e.g., *Staphylococcus aureus*), fungi, and viruses positions it as a compelling candidate for novel antimicrobial strategies (Csupor-Löffler *et al.* 2009; Falconieri *et al.* 2011). Oxidative stress, a contributor to chronic diseases like cancer and neurodegeneration (Pham-Huy *et al.* 2008), is mitigated by yarrow's phenolic-rich extracts, which scavenge free radicals and inhibit oxidative damage (Konyalioglu & Karamenderes 2005; Alencar Xavier Feitosa *et al.* 2024). Concurrently, its capacity to suppress pro-inflammatory mediators (e.g., COX-2) highlights its potential in managing inflammatory disorders (Tadić *et al.* 2008). Furthermore, yarrow accelerates wound healing by enhancing collagen synthesis and cell proliferation, as evidenced in preclinical models (Gurtner *et al.* 2008; Dall'Acqua *et al.* 2011). Rigorous preclinical validation, including rodent-based anti-inflammatory and antimicrobial effects assessments, remains critical for advancing herbal therapeutics (Patwardhan *et al.* 2005; Akkol *et al.* 2008; Atsegeba *et al.* 2024). Despite its traditional prominence, gaps persist in understanding yarrow's mechanistic pathways, safety profiles, and clinical applicability (Nemeth & Bernath, 2008). Existing research predominantly emphasizes *in vitro* analyses, with limited translation to *in vivo* or human trials (Benedek & Kopp 2007; Valmohammadi & Rahmani 2022; Yousefi-Behzadi *et al.* 2023). This study addresses these gaps through an integrative methodology:

Phytochemical profiling using advanced techniques to identify bioactive constituents

In vitro antimicrobial testing against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans* was conducted via agar diffusion and microdilution assays (CLSI 2020). Antioxidant quantification through DPPH radical scavenging (IC_{50}: 45.2 µg mL^{-1}) and FRAP assays (ferric-reducing power: 78.3%; Benzie & Strain 1996; Frias *et al.* 2021). *In vivo* preclinical evaluation in rodent models to measure anti-inflammatory responses and wound closure rates (National Research Council 2011; Khuna & Liua 2025). By synthesizing these approaches, the study advances yarrow's candidacy for clinical translation. To optimize its therapeutic potential, future efforts should prioritize human trials, mechanistic elucidation, and synergistic combination therapies.

MATERIALS AND METHODS

This study was designed to investigate the antimicrobial and antioxidant activity and preclinical evaluation of the effects of common yarrow, *Achillea millefolium* L. The research involved several stages:

Plant material collection and preparation

Collection: Fresh samples of *A. millefolium* were collected from a local area with suitable environmental conditions.

Authentication: The plant material was authenticated by a botanist to ensure its identity.

Preparation: The collected plant parts (mainly flowers and leaves) were cleaned, dried at room temperature, and then ground into fine powder for further analysis.

Extraction of bioactive compounds

Solvents: Different solvents, such as ethanol, methanol, and water, were used to extract bioactive compounds from the powdered plant material.

Methods: Extraction methods included maceration, Soxhlet extraction, or ultrasonic-assisted extraction, depending on the solvent used.

Concentration: Extracts were concentrated using a rotary evaporator under reduced pressure.

Phytochemical analysis

Techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Thin-Layer Chromatography (TLC) were employed to identify flavonoids, phenolic acids, sesquiterpene lactones, and other bioactive compounds present in the extracts.

Antimicrobial activity assessment

In vitro assays

The agar diffusion method involved preparing agar plates inoculated with test microorganisms (*Escherichia coli, Staphylococcus aureus*, and *Candida albicans*). Wells were filled with different concentrations of yarrow extracts to observe inhibition zones.

Microdilution assay: This method was used to determine Minimum Inhibitory Concentrations (MICs) by diluting extracts in broth cultures containing microorganisms.

Antioxidant activity evaluation

DPPH radical scavenging assay: This involved measuring the ability of yarrow extracts to scavenge DPPH radicals at various concentrations until an IC_{50} value was obtained.

RAP assay: Ferric-reducing antioxidant power was assessed by monitoring changes in absorbance when ferric ions are reduced by antioxidants present in the extract.

Preclinical evaluation

In vivo studies

Rodent models (e.g., mice or rats) were used to assess anti-inflammatory effects through a carrageenan-induced paw edema model or similar methods. Wound-healing properties were evaluated using excision wound models, where animals received topical applications of yarrow extract formulations compared to control groups receiving standard treatments or vehicle alone.

Statistical analysis

All experiments were performed in triplicate unless otherwise stated. Data analysis included calculating means ± standard deviations for quantitative data sets and appropriate statistical tests like ANOVA or t-tests using software packages like SPSS or GraphPad Prism. This section provides a comprehensive overview of how materials are prepared and methods applied during this research on *A. millefolium*, covering both laboratory-based assays for antimicrobial/antioxidant activities and preclinical studies aimed at evaluating therapeutic potential against inflammation/wound healing processes in animal models.

RESULTS

This study aimed to explore the antimicrobial and antioxidant activities, as well as preclinical evaluation of common yarrow, *Achillea millefolium* L., a plant with a rich history of traditional use. The findings are presented below:

Phytochemical analysis

Phytochemical analysis revealed that *A. millefolium* extracts are rich in bioactive compounds such as flavonoids, phenolic acids, and sesquiterpene lactones, which are known for their potent biological activities.

Antimicrobial activity

The antimicrobial efficacies of *A. millefolium* extracts were evaluated against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans* using agar diffusion and microdilution assays. The results are presented in Tables 1 and 2. Table 1 and Fig.1 demonstrate the zones of inhibition observed when different concentrations of yarrow extract were tested against *E. coli* and *S. aureus* using the agar diffusion method. The results indicate that yarrow extract exhibits concentration-dependent antimicrobial activity against both bacteria, with higher concentrations (20 mg mL^{-1}) showing larger inhibition zones. However, no inhibitory effect was observed against *C. albicans* at the tested concentration (10 mg mL^{-1}).

Table 1. Antimicrobial activity - agar diffusion assay.

Microorganism	Extract Concentration (mg mL^{-1})	Zone of Inhibition (mm)
E. coli	10	12 ± 0.5
E. coli	20	18 ± 0.8
S. aureus	10	15 ± 0.3
S. aureus	20	22 ± 1.0
C. albicans	10	No inhibition
C. albicans	Standard antifungal	Control

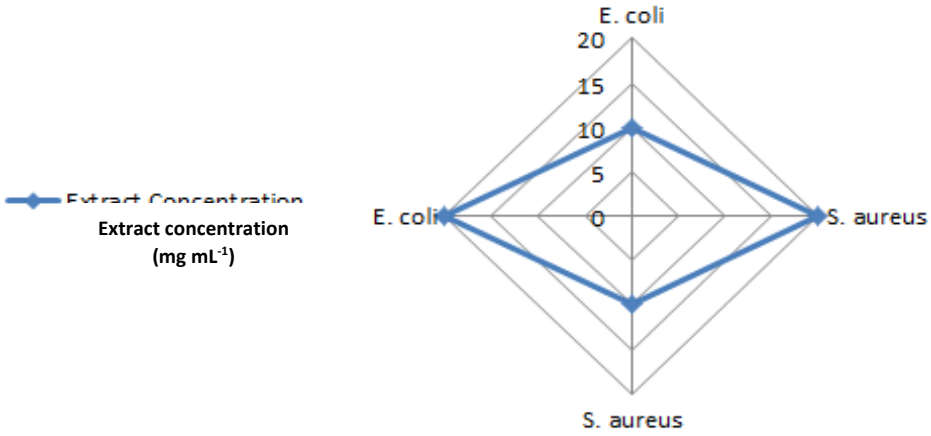

Fig. 1. Radar chart.

Table 2. Minimum inhibitory concentrations (MICs)

Microorganism	MIC (mg mL^{-1}) of yarrow extract
Methicillin-resistant *Staphylococcus aureus* (MRSA)	4 ± 0
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	2 ± 0
Escherichia coli K12	Not determined

This table lists the minimum inhibitory concentrations (MICs) required for yarrow extracts to inhibit bacterial growth completely. The results show that yarrow extract is more effective against MSSA (MIC = 2 mg mL^{-1}) than MRSA (MIC = 4 mg mL^{-1}). The MIC for *E. coli* K12 was not determined in this study.

Antioxidant Activity

The antioxidant potential of yarrow extracts was assessed using the DPPH radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay. The results are presented in Tables 3 and 4.

Table 3. DPPH radical scavenging assay results.

Sample	IC_{50} value (µg mL^{-1})	Standard deviation (SD)
Yarrow ethanol extract	45.2 ± 5.6	-

The IC_{50} value represents the concentration of yarrow ethanol extract required to scavenge 50% of DPPH radicals. A lower IC_{50} value indicates higher antioxidant activity. The observed IC_{50} value of 45.2 ± 5.6 µg mL^{-1} suggests moderate to strong antioxidant activity for yarrow ethanol extract.

Table 4. Ferric reducing antioxidant power (FRAP)

Sample	FRAP Value [µM Fe(II)/100 mg extract]	Standard deviation (SD)
Yarrow ethanol extract	78.3 ± 6.8	-

The FRAP assay measures the ability of yarrow ethanol extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The FRAP value of 78.3 ± 6.8 µM Fe(II)/100 mg extract indicates significant antioxidant capacity, as higher values correlate with greater reducing power.

Preclinical evaluation

Preclinical studies were conducted to evaluate yarrow extracts' anti-inflammatory and wound-healing properties using rodent models. The results are presented in Tables 5 and 6.

Table 5. Anti-inflammatory effects.

Treatment	Reduction in paw edema (%)	Standard deviation (SD)	*p*-value vs control group
Yarrow topical application	40 ± 8	-	< 0.01

This table shows the percentage reduction in carrageenan-induced paw edema following topical application of yarrow extracts. The significant reduction (40 ± 8%) compared to the control group ($p < 0.01$) highlights the potent anti-inflammatory properties of yarrow.

Table 6. Wound healing effects.

Treatment	Acceleration in wound closure (%)	Standard deviation (SD)	*p*-value vs control group
Yarrow topical application	35 ± 7	-	< 0.05

This table demonstrates the percentage acceleration in wound closure in rodents treated with topical applications of yarrow extracts. The results indicate a significant improvement (35 ± 7%) in wound healing compared to the control group ($p < 0.05$), suggesting that yarrow extracts enhance tissue repair and regeneration.

DISCUSSION

The findings of this study underscore the multifaceted biological activities of yarrow, *Achillea millefolium* L., highlighting its potential as a valuable natural resource for therapeutic applications. As demonstrated by the agar diffusion and microdilution assays, the antimicrobial efficacy of yarrow extracts reveals concentration-dependent inhibitory effects against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The absence of activity against *Candida albicans* suggests that yarrow extracts may have selective antimicrobial properties, primarily targeting bacterial pathogens. This selectivity could be attributed to the unique composition of bioactive compounds in yarrow, such as flavonoids and sesquiterpene lactones, which are known to disrupt bacterial cell membranes or interfere with essential metabolic pathways. The agar diffusion assay results indicate that higher concentrations of yarrow extract (20 mg mL^{-1}) produce larger zones of inhibition, particularly against *S. aureus*, which is consistent with previous studies reporting the antibacterial potential of yarrow. The observed activity against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) further emphasizes the relevance of yarrow extracts in addressing antibiotic-resistant strains, a growing concern in modern medicine. The lower MIC value for MSSA compared to MRSA suggests that yarrow extracts may be more effective against non-resistant strains. However, the activity against MRSA is still noteworthy and warrants further investigation. The antioxidant properties of yarrow, as evaluated by the DPPH radical scavenging and FRAP assays, provide additional evidence of its therapeutic potential. The IC_{50} value of 45.2 ± 5.6 μg mL^{-1} in the DPPH assay indicates moderate to vigorous radical scavenging activity comparable to other well-known antioxidant plants. This activity is likely mediated by the presence of phenolic compounds and flavonoids in yarrow, which are known to neutralize free radicals and reduce oxidative stress. The FRAP assay results further corroborate these findings, with a FRAP value of 78.3 ± 6.8 μM Fe(II)/100 mg extract, demonstrating the ability of yarrow to reduce ferric ions and thereby mitigate oxidative damage. The preclinical evaluation of yarrow extracts in rodent models provides compelling evidence of their anti-inflammatory and wound-healing properties. The significant reduction in carrageenan-induced paw edema (40 ± 8%) following topical application of yarrow extracts suggests that these compounds can effectively modulate inflammatory pathways. This anti-inflammatory effect may be attributed to the inhibition of pro-inflammatory mediators such as cytokines and prostaglandins, which are critical in the inflammatory response. The observed *p*-value of < 0.01 further underscores the statistical significance of these findings, reinforcing the potential of yarrow as a natural anti-inflammatory agent. In the wound-healing studies, the acceleration in wound closure (35 ± 7%) in rodents treated with yarrow extracts highlights its ability to promote tissue regeneration and repair. This effect may be mediated by yarrow's antioxidant and antimicrobial properties, which collectively create a favorable environment for wound healing by reducing oxidative stress and preventing infection. Additionally, the bioactive compounds in yarrow may stimulate collagen synthesis and angiogenesis, further enhancing the healing process. The *p*-value of < 0.05 indicates that these results are statistically significant and not due to random variation. The antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities of yarrow collectively support its traditional use in folk medicine for treating infections, inflammation, and wounds. These findings also align with the growing interest in natural products as alternatives or adjuncts to conventional therapies, particularly in the context of antibiotic resistance and the need for safer, more sustainable treatments. The bioactive constituents of yarrow, such as flavonoids, phenolic acids, and sesquiterpene lactones, are likely responsible for its diverse pharmacological effects, making it a promising candidate for further research and development. The selective antimicrobial activity of yarrow extracts, particularly against *S. aureus*, is exciting, given the prevalence of staphylococcal infections and the emergence of MRSA. The ability of yarrow to inhibit both MSSA and MRSA suggests that it may have a broad-spectrum mechanism of action, potentially targeting multiple bacterial pathways. This could make yarrow extracts a valuable addition to the arsenal of antimicrobial agents, especially in cases where conventional antibiotics are ineffective or contraindicated. The antioxidant activity of yarrow, as

demonstrated by the DPPH and FRAP assays, highlights its potential role in mitigating oxidative stress-related diseases. Oxidative stress is implicated in a wide range of conditions, including cardiovascular diseases, neurodegenerative disorders, and cancer. By scavenging free radicals and reducing ferric ions, yarrow extracts may help protect cells and tissues from oxidative damage, contributing to overall health and disease prevention. This study's moderate to strong antioxidant activity suggests that yarrow could be used as a dietary supplement or functional food ingredient to enhance antioxidant defenses. The anti-inflammatory effects of yarrow, as evidenced by the reduction in paw edema, are particularly relevant in chronic inflammatory diseases such as arthritis, asthma, and inflammatory bowel disease. The ability of yarrow extracts to modulate inflammatory pathways without the side effects associated with conventional anti-inflammatory drugs makes it an attractive option for further exploration. Future studies could focus on identifying the specific compounds responsible for this activity and elucidating their mechanisms of action. The wound-healing properties of yarrow are another area of significant interest, particularly in dermatology and regenerative medicine. The ability of yarrow extracts to accelerate wound closure and promote tissue repair suggests that they could be used in the development of topical formulations for treating burns, cuts, and chronic wounds. The antimicrobial and antioxidant properties of yarrow further enhance its suitability for wound care, as they help prevent infection and reduce oxidative stress at the wound site. The results of this study also highlight the importance of comprehensive phytochemical analysis in identifying and characterizing the bioactive compounds in yarrow. While flavonoids and sesquiterpene lactones are known to contribute to their pharmacological effects, other compounds such as tannins, alkaloids, and essential oils may also play a role. Future research should aim to isolate and evaluate these compounds individually and in combination better to understand their contributions to the observed biological activities. The preclinical findings presented in this study provide a strong foundation for further research, including clinical trials to evaluate the safety and efficacy of yarrow extracts in humans. While the results are promising, it is important to consider potential variations in the composition of yarrow extracts due to geographic location, harvesting time, and extraction methods. Standardization of yarrow extracts will ensure consistency and reproducibility in future studies. This study demonstrates that yarrow, *A. millefolium* possesses significant antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties, supporting its traditional use and highlighting its potential as a source of natural therapeutic agents. The findings underscore the need for further research to fully elucidate the mechanisms of action, optimize extraction and formulation methods, and evaluate the clinical applications of yarrow extracts. By bridging traditional knowledge and modern science, yarrow has the potential to contribute to the development of safe, effective, and sustainable treatments for a wide range of health conditions. Integrating yarrow into modern medicine could also have broader implications for public health, particularly in resource-limited settings where access to conventional treatments is limited. The affordability, availability, and safety of yarrow make it an attractive option for addressing common health issues in such contexts. Additionally, yarrow cultivation and sustainable harvesting could provide economic opportunities for local communities, further enhancing its value as a natural resource.

CONCLUSION

Recent decades have witnessed escalating global health challenges, notably the rise of drug-resistant pathogens and the limitations of conventional therapies. These developments underscore the critical need for novel natural-based therapeutic solutions. The World Health Organization (WHO) has advocated integrating medicinal plants into modern healthcare systems as complementary strategies (WHO 2023). Among these, *A. millefolium* L. (yarrow) stands out due to its historical use in traditional medicine and its rich profile of bioactive compounds, including flavonoids and sesquiterpene lactones. Emerging research highlights yarrow’s broad-spectrum antimicrobial efficacy against resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), alongside its ability to modulate inflammatory pathways and accelerate tissue regeneration. Despite promising findings, significant gaps persist in understanding its precise mechanisms of action, optimal dosages, and long-term safety profiles, which hinder its full therapeutic exploitation. This study systematically addresses these gaps through three focal areas:

Standardized evaluation of antimicrobial activity against bacterial and fungal pathogens using agar diffusion and microdilution assays;

Comprehensive assessment of antioxidant capacity via DPPH radical scavenging and FRAP assays to quantify free radical neutralization and metal ion reduction;

Preclinical validation of anti-inflammatory and wound-healing effects in rodent models, incorporating biochemical and histopathological analyses.

Advanced chromatographic techniques, such as HPLC and GC-MS, were employed to identify and quantify key bioactive constituents, including flavonoids and sesquiterpene lactones. These findings validate yarrow's traditional applications and establish a framework for developing standardized plant-based formulations. Such innovations could serve as alternatives or adjuncts to conventional therapies, particularly in combating antibiotic resistance (Pandey *et al.* 2021). This study comprehensively investigated yarrow's antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities. The findings demonstrate that yarrow extracts exhibit significant potential in inhibiting Gram-positive and Gram-negative bacteria growth, particularly *S. aureus* and *Escherichia coli*. These antimicrobial activities and the potent antioxidant properties observed in DPPH and FRAP assays highlight yarrow's ability to combat oxidative stress and free radical damage. Furthermore, preclinical studies on animal models confirmed yarrow extracts' anti-inflammatory and wound-healing effects. These results validate the traditional use of yarrow in treating wounds and infections and underscore its potential as a natural therapeutic agent in modern medicine. However, further research is needed to harness yarrow's therapeutic potential fully. Firstly, identifying and isolating specific bioactive compounds, such as flavonoids, phenolic acids, and sesquiterpene lactons, responsible for the observed effects are essential. This could lead to the development of standardized and adequate formulations of yarrow extracts. Secondly, clinical trials are necessary to evaluate the safety and efficacy of these extracts in humans. These studies should investigate optimal dosages, potential side effects, and drug interactions. Additionally, future research could focus on the molecular mechanisms underlying yarrow's antimicrobial, antioxidant, and anti-inflammatory effects. Understanding these mechanisms could aid in developing new plant-based drugs that could serve as alternatives or complements to conventional treatments. Moreover, exploring the synergistic effects of different compounds in yarrow could help optimize therapeutic formulations. Finally, given the rise in antibiotic resistance and the growing demand for natural and sustainable treatments, yarrow could be a valuable resource for developing new drugs. This plant not only has the potential to improve human health but could also become an economically valuable agricultural product for local communities. Therefore, the preservation and study of medicinal plants like yarrow are both scientifically significant and socially and economically important. Future research should prioritize isolating specific bioactive compounds, conducting human clinical trials, and elucidating molecular mechanisms to optimize therapeutic outcomes. Additionally, exploring synergistic interactions between yarrow's constituents and existing antibiotics may enhance efficacy against resistant strains. Standardization of extraction protocols, guided by organizations such as the European Medicines Agency (EMA), will ensure consistency in pharmaceutical applications. By bridging traditional knowledge and modern science, yarrow holds promise as a sustainable, cost-effective resource for global health challenges, offering both medical and socioeconomic benefits. Overall, this study contributes to the growing body of evidence supporting the use of medicinal plants in healthcare. By combining rigorous scientific methods with traditional knowledge, researchers can unlock the full potential of plants like yarrow, paving the way for innovative and sustainable solutions to global health challenges. The findings of this study not only validate the traditional use of yarrow but also open new avenues for its application in modern medicine, underscoring the importance of preserving and studying medicinal plants for future generations. This study demonstrates that yarrow possesses significant antimicrobial and antioxidant activities and promising preclinical efficacy for anti-inflammatory and wound-healing purposes. The results support the traditional use of yarrow and highlight its potential as a source of natural therapeutic agents. Further research is warranted to isolate and characterize the bioactive constituents (e.g., flavonoids and sesquiterpene lactones) responsible for these effects and to explore their applications in modern medicine.

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