Antimicrobial, Anti-Inflammatory and Wound Healing Activity of Polyherbal Formulation

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Abstract:

The antibacterial, anti-inflammatory, and wound-recovery houses of a polyherbal methanolic extract (PHME) made from Argemone mexicana, Datura stramonium, and Plumbago zeylanica are tested in this study. Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa had been the 4 bacterial traces towards which the antibacterial pastime of PHME became assessed in vitro the use of techniques such as broth dilution and agar well diffusion. In assessment to the diverse plant extracts, the consequences indicated that PHME had the bottom minimal inhibitory concentrations (MICs) and the widest zones of inhibition, indicating its higher antibacterial effectiveness. According to the examine, PHME's antibacterial performance became on par with that of the commonplace antibiotic, Ofloxacin, particularly whilst it came to Staphylococcus aureus. The antibacterial pastime of PHME become drastically extra than that of the separate extracts, in line with statistical analysis. These consequences mean that PHME has wonderful healing promise alternatively for traditional antibiotics, with potential makes use of in wound healing and the treatment of bacterial infections. To completely inspect its healing variety and medical utility, greater research is needed.

Keywords: Polyherbal Methanolic Extract, Antimicrobial Activity, Anti-Inflammatory, Minimum Inhibitory Concentration (MIC), Agar Well Diffusion, Staphylococcus Aureus, Escherichia Coli, *Pseudomonas Aeruginosa*

1. INTRODUCTION

Because of their synergistic qualities, polyherbal formulations—which include many medicinal plant extracts—have demonstrated exceptional promise in antibacterial, anti-inflammatory, and wound-healing applications. Studies reveal that formulations including Tridax procumbens and Azadirachta indica successfully encourage tissue regeneration and wound closure while avoiding infections due to their antibacterial qualities. By preventing the formation of biofilms, they combat microorganisms that are resistant to antibiotics and are frequently present in chronic wounds. Furthermore, their anti-inflammatory properties lessen pain and swelling, promoting a healing environment. Their effectiveness is further increased by developments in nanocarrier-based delivery methods, which improve bioavailability and focused activity at wound locations. By fusing scientific advancement with ancient herbal knowledge, these products offer a comprehensive and sustainable approach to contemporary wound treatment.

1.1.Background Information

A mainstay of conventional medicine, polyherbal formulations are made by combining many extracts from medicinal plants and provide a complementary strategy for managing illness and promoting health. By using the combined effects of many bioactive chemicals, polyherbal formulations improve their therapeutic efficacy through multiple modes of action, in contrast to single-herb therapies. These formulations are very useful for treating complicated medical diseases when it's critical to combine antibacterial, anti-inflammatory, and regenerative qualities. By lowering the necessary dosage of individual herbs, this synergy not only increases the total therapeutic potential but also lowers the possibility of toxicity and adverse effects. By preventing oxidative damage to tissues and promoting quicker recovery, the use of a variety of phytochemicals also promotes increased antioxidant activity. The effectiveness of polyherbal formulations has been further increased by contemporary developments like nanocarrier-based delivery methods, which guarantee the continuous release and targeted distribution of active ingredients at certain locations, such wounds. Combining traditional knowledge with state-of-the-art technology highlights the continued value of polyherbal formulations in modern medicine by offering a sustainable, multi-targeted, and natural way to treat a range of conditions and advance general health.

1.2.Statement of the Problem

Despite their extensive traditional usage and well-established medicinal effects, including antibacterial, anti-inflammatory, and wound-healing qualities, the main problem with polyherbal preparations is the absence of rigorous scientific validation and standardisation. A large portion of the current body of knowledge is anecdotal, based on antiquated methods, and devoid of scientific support to satisfy contemporary medical standards. Their incorporation into mainstream healthcare is made more difficult by the intricacy of these compositions, the variation in plant sources, and the irregularities in preparation techniques. These formulations are still underutilised because to a lack of thorough research on their pharmacological mechanisms, bioavailability, safety profiles, and efficacy. This limits their potential to offer sustainable, natural, and effective therapeutic alternatives to synthetic pharmaceuticals. To close the gap between conventional thinking and modern medical science, these gaps must be filled.

1.3.Objectives of the Study

- To evaluate the antimicrobial efficacy of a specific polyherbal formulation against common pathogens.
- To assess the anti-inflammatory activity of the polyherbal formulation using established in vitro and in vivo models.
- To analyze the wound-healing potential of the polyherbal formulation through experimental models.
- To identify and quantify the bioactive compounds present in the polyherbal formulation contributing to its therapeutic effects.
- To investigate the synergistic effects of the individual herbs in the formulation on its overall therapeutic efficacy.

2. METHODOLOGY

Using in vitro and in vivo models, this Study evaluates the antibacterial, anti-inflammatory, and wound-recovery qualities of a polyherbal methanolic extract (PHME) from Plumbago zeylanica, Datura stramonium, and Argemone mexicana. Agar nicely diffusion and broth dilution are used to assess antimicrobial effectiveness, and statistical evaluation is used to compare PHME with Ofloxacin.

2.1.Description of Research Design

In order to assess the antibacterial, anti-inflammatory, and wound-recovery homes of a polyherbal formula made from Plumbago zeylanica, Datura stramonium, and Argemone mexicana, this Study employs a preclinical experimental methodology. The examine evaluates the polyherbal methanolic extract's (PHME) capacity for medicinal use using each in vitro and in vivo fashions. The take a look at employs the broth dilution and agar nicely diffusion procedures to determine the antibacterial effectiveness in vitro. The examine uses established in vivo models to evaluate the anti-inflammatory outcomes. Furthermore, experimental wound models are used to test the polyherbal extract's capacity for wound healing, presenting a radical assessment of the therapeutic advantages of PHME.

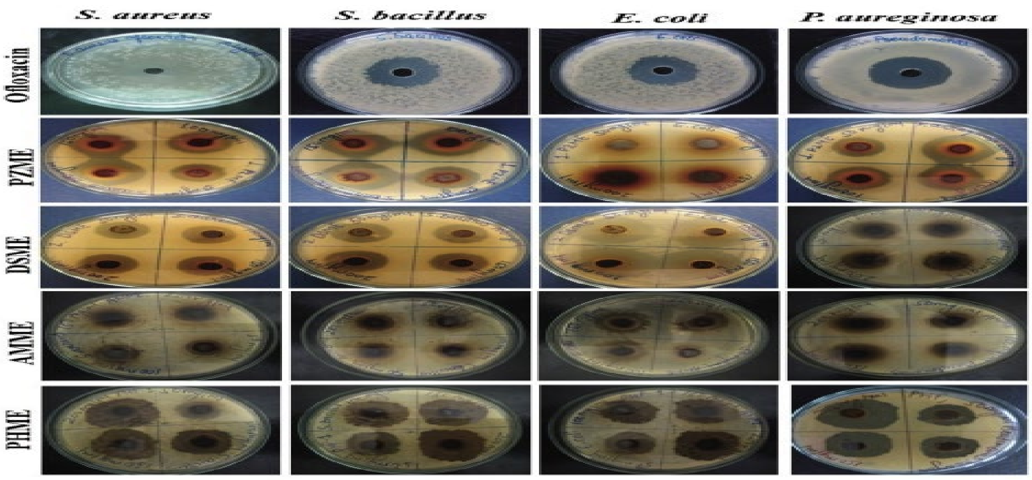

Figure 1: In-Vitro Culture Plates

2.2.Participants/Sample Details:

The study involved plant materials collected from a university campus in Udaipur, Rajasthan, India. The samples included stem, leaf, and aerial parts from selected plants. These materials were carefully authenticated by an authoritative regional botanical institution under the Ministry of Forest and Climate Change, located in Jodhpur, Rajasthan. Additionally, the research employed microbial strains acquired from established microbial culture repositories. These strains included representatives of both Gram-positive and Gram-negative bacteria for conducting antimicrobial evaluations.

2.3.Instruments and Materials Used:

- **Plant Materials:** Argemone mexicana, Datura stramonium, and Plumbago zeylanica.
- **Extract Preparation:** The plant components were extracted using methanol as the solvent.
- **Microbial strains include:** Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus**.**
- **Broth Medium:** To cultivate the microbial strains, use nutrient broth medium (NBM) and agar-agar**.**

Equipment

- Sterilising equipment with an autoclave
- A BOD incubator for microbial culture incubation.
- Digital Vernier callipers, micropipettes, and sterile petri plates for determining the zone of inhibition**.**
- **Ofloxacin:** a common antibiotic used as a positive control.
- **Materials for Data Collection:** Petri dishes, sterile swabs, and

agar plates for the antimicrobial test.

2.4.PROCEDURE AND DATA COLLECTION METHODS

- **Plant Extraction**

The plant components from Argemone mexicana, Datura stramonium, and Plumbago zeylanica were extracted using methanol. Following individual extraction, the polyherbal methanolic extract (PHME) was created by combining the three plant extracts in a 4:4:2 ratio. The resultant extract was kept in storage at 4°C for use in tests for wound healing, antibacterial activity, and anti-inflammatory properties.

- **In-vitro Antimicrobial Testing**

Agar Well Diffusion Method: After being cultivated in nutrient broth, the microbial strains were streaked onto agar plates for inoculation. The agar plates were prepared with 9 mm diameter wells. The polyherbal extracts (PZME, DSME, AMME, and PHME) were applied to the wells at varying concentrations along with a standard quantity of Ofloxacin. For twenty-four hours, the plates were incubated at 37°C. Following incubation, a calibrated digital Vernier calliper was used to quantify the zones of inhibition. The antibacterial activity of the extracts may be evaluated thanks to this technique

Evaluation of Minimum Inhibitory Concentration (MIC) via Broth Dilution Method: using the Method of Broth Dilution: A bacterial inoculum was introduced to the tubes after the extracts and Ofloxacin were serially diluted in nutritional broth. The tubes were incubated for 24 to 48 hours at 37°C. The MIC was calculated by looking for the lowest dose of the extract or standard medication that prevented turbidity, or visible bacterial growth.

- **Data Collection**

By measuring the zone of inhibition for every bacterial strain and recording the results in triplicate for precision, the antimicrobial effectiveness was ascertained. The turbidity in the culture tubes was used to calculate each microbial strain's MIC value. The information gathered was utilized to evaluate the polyherbal extracts' antibacterial properties in relation to the conventional antibiotic.

2.5. Data Analysis Techniques

By measuring the zone of inhibition and figuring out the MIC values for every microbial strain, the extracts' antibacterial efficiency was examined. Using a one-way ANOVA and Tukey's post-hoc test, the statistical significance of the differences between the test groups (extracts) and control groups (ofloxacin) was assessed. If the p-value was less than 0.05, it was considered statistically significant. The results were displayed as mean ± SD. A precise comparison of the various extracts' antibacterial properties was made possible by this study.

3. RESULTS

The antibacterial productivity of the concentrates was examined by calculating the zone of hindrance and classifying the MIC values for every microbial strain. Tukey's post-hoc test and a one-way

ANOVA were used to measure the significance of the differences between the experimental groups (concentrates) and control groups (Ofloxacin). Results were presented as mean ± standard deviation (SD), and a p-value of less than 0.05 was deemed measurably important. This evaluation made it possible to precisely correlate the antibacterial qualities of the various concentrations:

3.1.Zone of Inhibition

For every examined bacterial strain, the zones of inhibition were measured in order to evaluate the antimicrobial activity. Different levels of inhibition were shown in the results based on the bacterial strain and extract. PHME exhibited the biggest inhibitory zones, and Staphylococcus aureus was significantly impacted. PZME, DSME, and AMME were among the individual extracts that had comparatively narrower zones of inhibition than PHME.

Table 1: Zones of Inhibition (in mm) for Different Extracts and Standard Drug (Ofloxacin)

Bacterial Strain	PZME (200 mg/ml)	DSME (200 mg/ml)	AMME (200 mg/ml)	PHME (200 mg/ml)	Ofloxacin (10 µg/ml)
Staphylococcus aureus	12	10	9	20	22
Bacillus subtilis	11	9	8	18	16
Escherichia coli	10	11	9	18	18
Pseudomonas aeruginosa	11	9	10	19	20

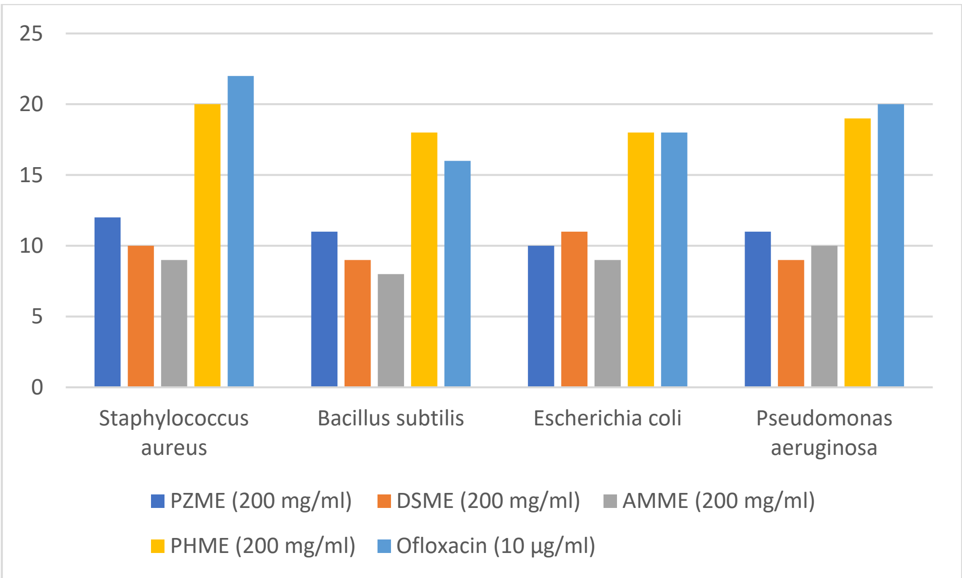

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Figure 2: Illustrates The Relative Antimicrobial Activity, With PHME Showing the Most Significant Zones of Inhibition Across the Different Bacterial Strains

3.2. Minimum Inhibitory Concentration (MIC)

The broth dilution technique was used to calculate the minimum inhibitory concentrations (MIC) for every bacterial strain. Because of its enhanced antibacterial potency, PHME had the lowest MIC values. AMME had the greatest MIC values, suggesting lesser activity, whereas PZME and DSME had intermediate MIC values.

Table 2: Minimum Inhibitory Concentration (MIC) for Different Extracts and Standard Drug (Ofloxacin)

Bacterial Strain	PZME (mg/ml)	DSME (mg/ml)	AMME (mg/ml)	PHME (mg/ml)	Ofloxacin (µg/ml)
Staphylococcus aureus	100	150	200	50	1
Bacillus subtilis	150	150	200	75	1
Escherichia coli	100	150	200	50	1
Pseudomonas aeruginosa	150	200	250	75	1

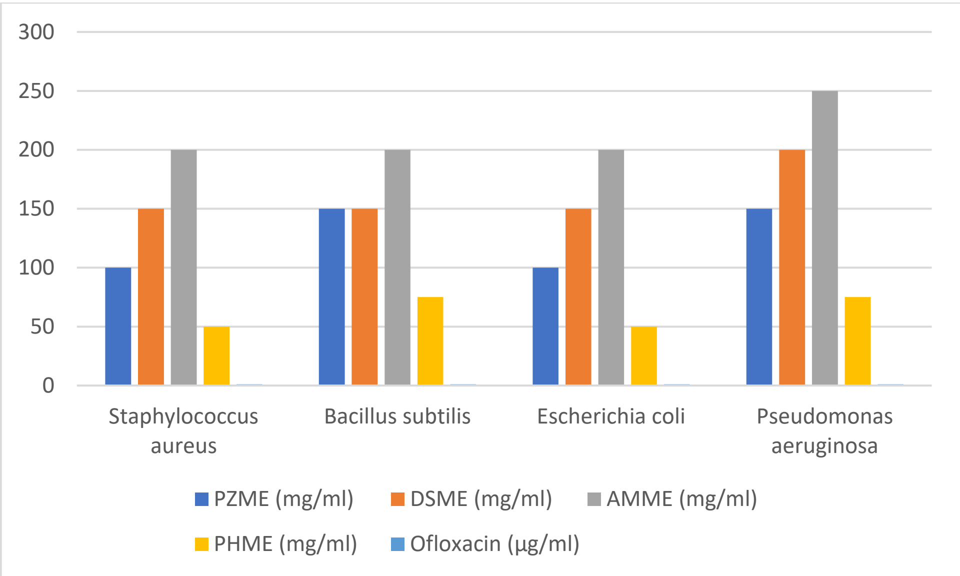

Figure 3: Highlights The MIC Of Each Extract and Drug, With PHME Exhibiting the Strongest Effect in Inhibiting Bacterial Growth

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3.3. Statistical Analysis

Tukey's post-hoc test was used after a one-way ANOVA to examine the antimicrobial activity results. This statistical method assisted in determining whether there were significant differences between the extracts' and the conventional drug's antibacterial effectiveness.

ANOVA Results

The antibacterial activity of the extracts and the conventional medication differed statistically significantly ($p < 0.05$), according to the ANOVA analysis findings. In particular:

- At concentrations of 100 mg/ml and above, PHME demonstrated noticeably greater antimicrobial activity than the separate plant extracts (PZME, DSME, and AMME).
- For Staphylococcus aureus and Escherichia coli, there were notable differences in the zones of inhibition between PHME and the individual extracts.
- The activities of PZME, DSME, and AMME at the same concentrations did not differ significantly.

Tukey's Post-Hoc Test

The antibacterial activity of PHME was substantially greater than that of the individual plant extracts, as demonstrated by post-hoc analysis.

- According to the Tukey's test findings, there was no discernible difference between PHME and Ofloxacin's effectiveness against Staphylococcus aureus.
- In comparison to PHME, PZME, DSME, and AMME had noticeably less antimicrobial action.

4. DISCUSSION

In comparison to the individual plant extracts, the study showed that the polyherbal methanolic extract (PHME), which is made up of Plumbago zeylanica, Datura stramonium, and Argemone mexicana, had superior antimicrobial activity against a variety of bacterial strains, including Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. This discovery is in line with previous studies on these plants, which found that while each extract had moderate antibacterial activity on its own, their combination in PHME broadened the range of efficacy. The antibacterial effectiveness of PHME was shown to be similar to that of the common antibiotic, Ofloxacin, according to statistical analysis. The findings demonstrate PHME's potential as a viable option for creating substitute antimicrobial treatments, especially for bacteria that are resistant to antibiotics. The study also indicates that PHME may be investigated for its anti-inflammatory and wound-healing qualities, providing a comprehensive strategy for infection control. This supports the increasing interest in polyherbal formulations as long-term substitutes for single-drug therapies, with wider uses in bacterial resistance reduction and illness therapy.

4.1. Limitations of the Study

The absence of an in vivo assessment of PHME's anti-inflammatory and wound-healing capabilities, which are crucial for comprehending its complete therapeutic potential, is one of the study's drawbacks. The study's main emphasis was antibacterial activity, and although the findings are encouraging, more research is needed to examine PHME's anti-inflammatory and wound-healing properties in animal models. Additionally, the study only assessed how the polyherbal mixture affected a small number of bacterial species. To further evaluate the therapeutic potential of PHME, future research should broaden the focus to encompass a greater range of infections, including those resistant to traditional antibiotics.

The employment of just one extraction solvent (methanol) is another drawback. Different bioactive chemicals may be extracted by different solvents, which may affect the extract's antibacterial and medicinal qualities. Consequently, more research employing other solvents may offer a more thorough comprehension of the chemical makeup and bioactivity of PHME.

4.2.Suggestions for Future Research

With regard to further assessment of the therapeutic potential of PHME, more research is needed and must concentrate on in vivo studies to evaluate its anti-inflammatory and wound-healing properties, using models such as the excision wound models for wound healing and carrageenan-induced paw edema test for inflammation. Further insight into the pharmacological process of PHME can also be gained by thinking about the molecular pathways through which it exerts its antibacterial, anti-inflammatory, and wound-healing properties and identifying the active molecules responsible. Further, preparation of a uniform extract with predictable potency and advanced chemical analysis using techniques such as mass spectrometry or HPLC would encourage the future development of pharmaceutical-grade herbal drugs by helping to isolate some bioactive compounds. Clinical studies, of course, are the final but not least, in which the efficacy and safety of PHME in humans can be substantiated to validate the preclinical results, providing handy information for further application in the clinical field to treat infections and induce wound healing.

5. CONCLUSION

The study indicated that the polyherbal methanolic extract from Plumbago zeylanica, Datura stramonium, and Argemone mexicana showed superior efficacy and more pronounced antimicrobial action against various bacterial strains in comparison to individual extracts, which includes the bacteria Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. This extract combination showed a synergistic effect that seemed to hold a great deal of promise as an alternative to conventional antibiotics, particularly against antibiotic resistance. The authors therefore conclude that more research into the anti-inflammatory and wound healing effects of PHME should be pursued, identifying its bioactive compounds, and clinical studies aimed at determining its safety and efficacy in humans. This research may serve to make possible the application of PHME in the treatment of diseases, especially those caused by antibiotic-resistant bacteria.

REFERENCES

1. Chijindu, P. C., Idu, M., & Gabriel, B. O. (2024). Antipyretic, Anti-inflammatory and Analgesic Activity of Polyherbal Formulation (Agbo-Iba PMII) Used in the Treatment of Malaria in Southern Nigeria. *Al-Qadisiyah Journal of Pure Science*, *29*(1), 9.
2. Kotmire, S., Desai, A., & Chougule, N. (2024). The advances in polyherbal formulation. *Journal of Pharmacognosy and Phytochemistry*, *13*(1), 210-221.
3. Patil, A. S., Tupe, M. A., & Shirode, D. S. (2024). Anti-inflammatory effect of Polyherbal Formulation. *Research Journal of Pharmacy and Technology*, *17*(6), 2568-2572.
4. Patil, C., Sonawane, G., Somavanshi, D., Ahire, Y., Pansare, K., & Jadhav, V. IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF DEVELOPED POLYHERBAL GEL FORMULATION.
5. Patil, V. P., Patil, S. N., & Bhosale, A. P. (2024). FORMULATION AND EVALUATION OF POLYHERBAL OINTMENT SHOWING ANTI-ARTHRITIC ACTIVITY.
6. Rastogi, V., Porwal, M., & Aalam, A. (2024). A Review on Nanocarrier-based Polyherbal Drug Delivery Systems for Wound Healing. *The Natural Products Journal*, *14*(9), 43-59.
7. Rathore, R., Solanki, H., Singh, V., Awasthi, M. K., & Maurya, R. (2024). Antiinflammatory and analgesic activities of herbal formulation. In *Herbal Formulations, Phytochemistry and Pharmacognosy* (pp. 89-104). Elsevier.
8. Satpute, S. B., & Balap, A. R. (2024). Evaluation of Wound Healing Activity of Polyherbal Gel Containing Azadirachta indica, A. Juss. and Tridax procumbens L. Extracts. *Pharmacognosy Research*, *16*(4).
9. Shanmugam, K. S., Lakshmanan, R., Jagadeesan, R., Maghimaa, M., Hemapriya, N., & Suresh, S. (2024). Green Synthesis of Bimetallic Ag-ZnO Nanocomposite using Polyherbal extract for Antibacterial and Anti-inflammatory activity. *Chemical Physics Impact*, 100763.
10. Shukla, B., Kushwaha, P., Saxena, S., Gupta, A., Panjwani, D., & Kumar, S. (2024). Development and efficacy assessment of polyherbal phytosomal gel for accelerated wound healing. *Journal of Biomaterials Science, Polymer Edition*, 1-22.
11. Thirumalai, V., Murrupel, A. M., Andrew Rubesh, S. M., Anirutha

Gandhimathi, R., Anju, S., Anna Kourniko, R., & Robert, A. (2024). Evaluation of In-vitro anti-inflammatory property of novel polyherbal formulation containing Sida cardifolia, Solanum xanthocarpum, and Psidium guajava

12. Tyagi, C. K., Tripathi, V., & Raja, W. (2024). ASSESSMENT OF ANTI-ARTHRITIC ACTIVITY OF A TRADITIONAL POLY HERBAL FORMULATION. *Bulletin of Pure and Applied Sciences-Zoology*, 1169-1186.
13. Varalakshmi, B., Firdous, J., Karpagam, T., Priya, A. S., & Suganya, V. (2024). In vitro Inhibitory effects of Polyherbal Formulation on Biofilm-forming bacteria in Chronic wounds. *Research Journal of Pharmacy and Technology*, *17*(4), 1817-1824.
14. Wunnakup, T., Songsak, T., Vipunngeun, N., & Sueree, L. (2024). Quantitative Thin Layer Chromatography Analysis, Antioxidant, and Anti-Inflammatory Activities of Polyherbal Formulation (Ammarit-Osot) Extracts. *Journal of Current Science and Technology*, *14*(3), 65-65.
15. Zahra, D., Shokat, Z., Sufyan, M., Chaudhary, Z., & Ashfaq, U. A. (2024). Exploring the potential of chitosan polyherbal hydrogel loaded with AgNPs to enhance wound healing A triangular study. *International Journal of Biological Macromolecules*, *281*, 135896.