Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.SE.25.01.Art016

# Evaluating the Antimicrobial Efficacy of Solvent Extracts from Gymnema Sylvestre Against Wound Pathogens

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***Abstract***

*The antibacterial activity of Gymnema sylvestre solvent extracts, including acetone, aqueous, chloroform, ethanol, and hydroxyethanol, is assessed in this study against common wound infections. G. sylvestre is a well-known traditional medicinal herb with a wide range of therapeutic uses that has drawn attention due to its antibacterial actions. The release of bioactive chemicals was optimized by the use of a systematic extraction process with several solvents. Using the agar well diffusion method, the antibacterial activity of the extract was evaluated against a variety of wound pathogens, such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus. The findings showed that the antibacterial activity of the various solvent extracts varied significantly. The extracts of ethanol, acetone, chloroform, and hydroxyethanol showed the strongest inhibitory effects, dramatically slowing the growth of the pathogens under examination. Moderate action was demonstrated by aqueous extracts against the acquired infections. These results indicate that the bioavailability of antimicrobial compounds in G. sylvestre is significantly influenced by the extraction solvent. The study emphasizes the potential of G. sylvestre as a natural antibacterial agent, especially about wound healing. The precise bioactive components causing this activity must be determined, and their mechanisms of action must be investigated, through more research. Overall, this research adds to the increasing evidence that supports the use of herbal remedies for managing wound-related infections, providing valuable insights for future therapeutic applications.*

***Keywords:*** *Antibacterial Activity, Bioactive Compound, Plant Extract, Solvent Extract, Wound Pathogen.*

## Introduction

A major worldwide health concern that is affecting the treatment of both acute and chronic microbial illnesses is antimicrobial drug resistance. The development of resistance mechanisms such as efflux pumps, enzymatic neutralization, biofilm formation, and target alterations has impeded treatment efficacy despite the effectiveness of antimicrobial drugs against diseases like tuberculosis,

***Received: 17.10.2024*** ***Accepted: 08.01.2025*** ***Published on: 31.01.2025***

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pneumonia, skin and wound infections [1]. These microbial modifications raise morbidity, mortality, and healthcare expenditures because they enable infections to resist drug stress and elude host immune responses [2]. Antimicrobial resistance is continuously highlighted by the World Health Organization as a major issue, highlighting the critical need for novel, resistant antimicrobial drugs that can defeat these sophisticated survival techniques [3]. As modern medicines are less effective, resolving this issue is critical for protecting public health using alternative medications.

Therapeutic plants are the best source of a variety of drugs, according to WHO [4]. Research on medicinal plants used in Ayurveda, Unani, homoeopathy, and Siddha suggests that these plants have a variety of active components. Plants are valuable medicines because they include phytocompounds such as flavonoids, alkaloids, tannins, and phenolics [5]. Both infectious and chronic disorders can be treated with them. Pharmaceuticals and medical supplies are mostly derived from medicinal plants [6]. Products made from medicinal plants are utilized as advanced medicines to treat life-threatening illnesses as well as home remedies to treat particular problems [7]. Several scientists from all over the world conducted research into the antibacterial qualities of medicinal plants. According to a recent exploratory survey, medicinal plants are assessed for their biological activities to find potentially novel compounds with therapeutic applications [8]. With their established antibacterial qualities, the application of phytochemicals and plant extracts can be crucial to the development of therapeutic medications [9]. Therefore, it is believed that further research on the use of plants as medicinal agents is necessary.

Throughout central and peninsular India, *Gymnema sylvestre* (Asclepiadaceace), a slow-growing, perennial, and medicinal woody climber, is a vulnerable species. It is thought that the plant is a good source of many different kinds of bioactive compounds. Many phytochemicals, including terpenoids, saponins, gymnemic acids, and gymnemasaponins, are present in the leaves of *G. sylvestre*. According to Entooru et al. [10] the essential oil extracted from *G. sylvestre* leaves shows both antibacterial and antioxidant properties. The pharmaceutical industry is seeing a rise in demand for *G. sylvestre* leaves. Gymnemic acid, the active ingredient, was isolated from leaves and is widely utilized as anti-hypercholesterolemia, anti-diabetic, and anti-sweetener. Moreover, it possesses diuretic, stomachic, and cough-suppressing qualities [11]. The historic use of *G. sylvestre* in the medical industry was thus supported by the literature review. The antibacterial activity of this medicinal plant has, however, received very little research attention.

Solvent extracts derived from this plant are effective in fighting wound infections, which are frequently resistant to traditional antibiotics. Researchers can extract a wide variety of bioactive chemicals, such as glycosides, flavonoids, and terpenoids, which are recognized for their antimicrobial activities, by using various solvents, such as ethanol, acetone, chloroform, hydroxyethanol, and aqueous solutions. Understanding the distinct antibacterial effectiveness of these extracts against prevalent wound pathogens may facilitate the development of natural substitutes for synthetic antibiotics, consequently enhancing wound healing and decreasing the possibility of infection [12]. Therefore, the purpose of this study is to assess the antibacterial activity of *Gymnema sylvestre* solvent extracts against wound pathogens, offering insight into the plant's potential as a natural medicinal agent that can serve as a useful substitute in the management of wounds and the prevention of infections.

## Methodology

### Chemicals and Reagents

Ethanol, chloroform, aqueous, hydroxyethanol, and acetone were brought from Sigma Aldrich. HiMedia, USA provided nutrient agar (NA), Mueller-Hinton agar (MHA), and a few other necessary chemicals. Nano-pure water was used constantly during this research.

### Test Microorganisms

The reference bacterial strains, such as *Staphylococcus aureus* (MTCC 740) as Gram-positive bacteria; *Escherichia coli* (MTCC 119), *Klebsiella pneumoniae* (MTCC 530), *Pseudomonas aeruginosa* (MTCC 741) as Gram-negative bacteria were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

### Collection and Processing of Plant Materials

The leaves of *G. sylvestre* were harvested from a single plant population in Kolli Hills, Namakkal, Tamil Nadu, India, which belonged to the same age group. After collecting, the *G. sylvestre* leaves were thoroughly rinsed with distilled water to get rid of any undesired dust or debris that had adhered. After being cleaned, the leaves were drained and allowed to air dry in the shade for two to four weeks. After the leaves had fully dried, they were blended into a powder and sieved through a 20-µm mesh screen [13]. After being sieved, the powder was air-dried at 25 ± 2 °C and kept in an airtight container for further processing.

### Solvent Extraction of Active Components from G. *sylvestre*

For extraction, the collected plant sample was utilized. Using a cold maceration process for 72 hours, 25g of *G. sylvestre* leaf powder was extracted with 250ml of ethanol. Following extraction, the mixture was filtered through Whatman N0.1 filter paper to produce an extract devoid of solid particles. A rotary evaporator was then used to evaporate the solvent until it was completely dry under vacuum. The extracted crude was kept for later use at 4 °C [14]. For all other solvents, including acetone, chloroform, hydroxyethanol, and aqueous extracts, the same protocol was followed.

### Antibiotic Sensitivity Test for Isolated Strains

The agar disc diffusion method (also known as the Kirby-Bauer method) was used to assess the antibiotic sensitivity of isolated strains of *S. aureus, E. coli, K. pneumoniae,* and *P. aeruginosa*. To produce pure colonies, each bacterial isolate was first cultivated on nutrient agar. Each isolate was made into a standardized suspension using sterile saline, with the optical density adjusted to correspond with a 0.5 McFarland standard. The Mueller-Hinton agar plates were then equally covered with this suspension. Antibiotic discs containing common antibiotics such as ampicillin, ciprofloxacin, amikacin, gentamicin, and chloramphenicol were placed on the agar surface. Following an 18–24 hour incubation period at 37°C, the zones of inhibition were assessed and interpreted by the recommendations provided by the Clinical and Laboratory Standards Institute (CLSI) [15].

### Antibacterial Activity for Isolated Strains using Solvent Extracts from *G. sylvestre*

The agar well diffusion method was used to evaluate the efficacy of several solvent extracts like ethanol, acetone, chloroform, hydroxyethanol and aqueous extracts of *G. sylvestre* against strains of *P. aeruginosa, E. coli, S. aureus*, and *K. pneumoniae*. Mueller-Hinton agar plates were prepared and swabbed with 100 µL of each isolated microbial strain causing wound infections.

Next, using a cork borer with a 6 mm diameter, wells were made in the agar plates and filled with *G. sylvestre* solvent extracts diluted in Dimethyl sulfoxide (DMSO) at diverse concentrations of 25, 50, and 75µg/mL [16]. Amikacin and Vancomycin were kept as positive controls, whereas DMSO was used as a negative control. The diameters of the areas surrounding the wells that were free of microbial growth, known as the zones of inhibition, were measured and recorded following a 24-hour incubation period at 37°C.

### Statistical Analysis

All experiments were repeated three times in triplicate (n=3). The data was evaluated statistically using ANOVA. Results are considered significant at $p < 0.05$.

## Result

### Antibiotic Senstivity Test using Commercial Antibiotics

The results of the antibiotic sensitivity test reveal the following observations for the selected bacterial strains: *S. aureus* exhibited resistance to amikacin, ampicillin, gentamycin, and ciprofloxacin, but showed moderate sensitivity to chloramphenicol with a zone of inhibition of 16mm. *E. coli* showed varied responses, exhibiting resistance to amikacin, chloramphenicol, gentamycin, and ciprofloxacin, but moderate sensitivity to ampicillin with a zone of inhibition of 16mm. *K. pneumoniae* was resistant to amikacin, ampicillin, chloramphenicol, and gentamycin but showed mild susceptibility to ciprofloxacin with a 14mm zone of inhibition. *P. aeruginosa* displayed resistance to amikacin, ampicillin, and gentamycin, with some sensitivity to chloramphenicol (14mm) and ciprofloxacin (14mm). The studied strains exhibited notable treatment resistance, as indicated by our data, underscoring the urgent need for effective therapeutic approaches to combat these infections (Figure 1 and Table 1).

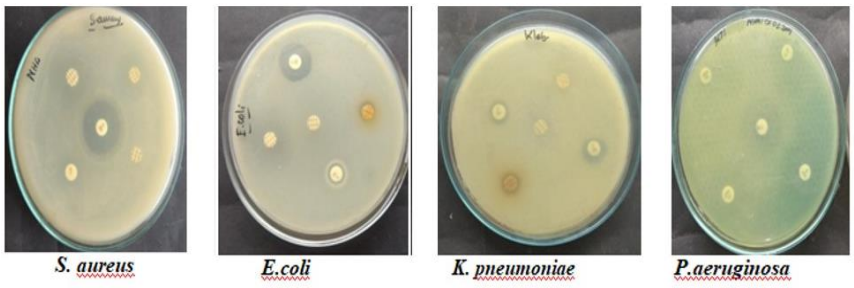


**Figure 1.** Antibiotic Senstivity Test Against Wound Pathogens

**Table 1.** ZOI in (mm) for Antibiotic Senstivity Test Against Wound Pathogens

| S.NO | Antibiotic | *S. aurues* | *E. coli* | *K.pneumoniae* | *P.aeruginosa* |
|---|---|---|---|---|---|
| **1** | Amikacin | Resistant | Resistant | 12mm | Resistant |
| **2** | Ampicillin | Resistant | 16mm | Resistant | Resistant |
| **3** | Chloramphenicol | 16mm | Resistant | Resistant | 14mm |
| **4** | Gentamycin | Resistant | Resistant | Resistant | 12mm |
| **5** | Ciprofloxacin | Resistant | Resistant | 14mm | 14mm |

## Antibacterial Activity of Solvent Extracts from G. *sylvestre* Against Wound Pathogens

The antibacterial activity of extracts from *G. sylvestre*, using solvents such as ethanol, acetone, chloroform, hydroxyethanol, and aqueous solutions, was systematically evaluated against various wound pathogens (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*) at different concentrations of 25µg/mL, 50µg/mL, and 75µg/mL. Among the extracts, the ethanol extract exhibited the highest zone of inhibition, measuring 20 mm against *S. aureus*, indicating strong antibacterial properties, followed by *E. coli* at 17 mm, *P. aeruginosa* at 15 mm, and the lowest zone of inhibition for *K. pneumoniae* at 14 mm (Figure 2 and Table 2). The acetone extract demonstrated a maximum zone of inhibition of 19 mm against *E. coli*, suggesting effective antibacterial activity, followed by *S. aureus* at 16 mm, *P. aeruginosa* at 15 mm, and *K. pneumoniae* at 14 mm (Figure 3 and Table 3). The chloroform extract also showed notable effectiveness, with an inhibition zone of 18 mm against *P. aeruginosa*, followed by *S. aureus* at 16 mm, *K. pneumoniae* at 15 mm, and the lowest zone of inhibition for *E. coli* at 14 mm (Figure 4 and Table 4). Additionally, the hydroxyethanol extract recorded a zone of inhibition of 17 mm against *K. pneumoniae*, reflecting its potential as an antibacterial agent, followed by *S. aureus* at 16 mm, *P. aeruginosa* at 15 mm, and the least zone of inhibition for *E. coli* at 14 mm (Figure 5 and Table 5). Lastly, the aqueous extract displayed a zone of inhibition of 16 mm against *S. aureus*, followed by *P. aeruginosa* at 15 mm, and both *E. coli* and *K. pneumoniae* at 14 mm and 13 mm, respectively (Figure 6 and Table 6). DMSO was kept as negative control for each plate. These results underscore the varying antibacterial efficacy of the different solvent extracts of *G. sylvestre*, which ranges from highest to lowest as ethanol, acetone, chloroform, hydroxyethanol, and aqueous extracts. Hence, the ethanol leaf extracts of *G. sylvestre* contain more potential antibacterial activity than the other leaf extracts.

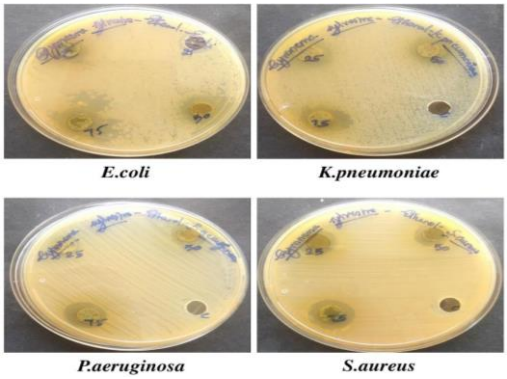


**Figure 2.** Antibacterial Activity of G. *Sylvestre* Ethanol Extract on Wound Pathogens

**Table 2.** Zone of Inhibition in (mm) for G. *Sylvestre* Ethanol Extract

| S.NO | Name of the organisms | 25µg / ml | 50µg / ml | 75µg/ ml | Negative control (DMSO) |
|---|---|---|---|---|---|
| 01 | *Escherichia coli* | 15mm | 16mm | 17mm | No Zone |
| 02 | *Klebsiella pneumoniae* | 12mm | 13mm | 14mm | No Zone |
| 03 | *Pseudomonas aeruginosa* | 13mm | 14mm | 15mm | No Zone |
| 04 | *Staphylococcus aureus* | 18mm | 19mm | 20mm | No Zone |

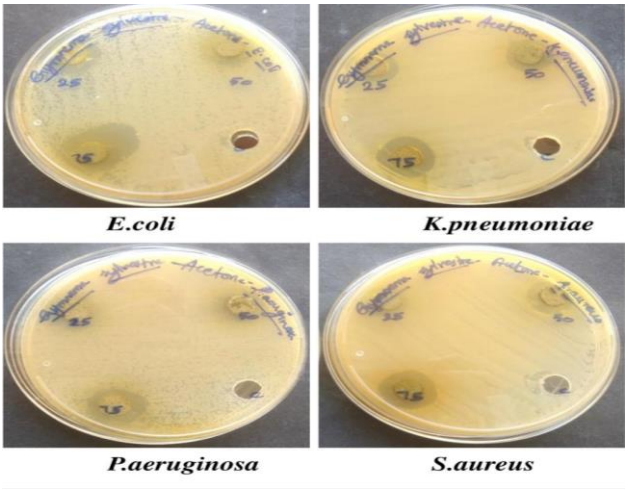


**Figure 3.** Antibacterial Activity of *G. Sylvestre* Acetone Extract on Wound Pathogens

**Table 3.** Zone of Inhibition in (mm) for G. *Sylvestre* Acetone Extract

| S.NO | Name of the organisms | 25µg / ml | 50µg / ml | 75µg/ ml | Negative control (DMSO) |
|---|---|---|---|---|---|
| 01 | *Escherichia coli* | 17mm | 18mm | 19mm | No Zone |
| 02 | *Klebsiella pneumoniae* | 12mm | 13mm | 14mm | No Zone |
| 03 | *Pseudomonas aeruginosa* | 13mm | 14mm | 15mm | No Zone |
| 04 | *Staphylococcus aureus* | 14mm | 15mm | 16mm | No Zone |

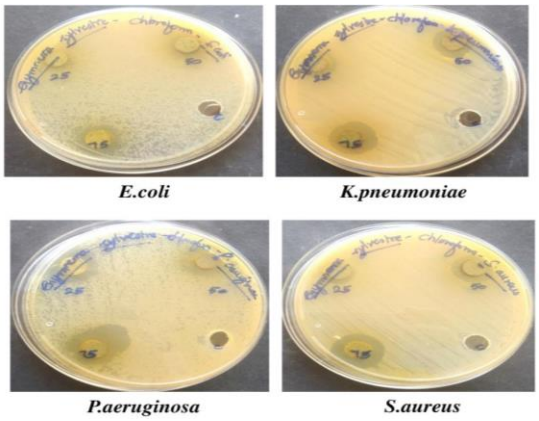


**Figure 4.** Antibacterial Activity of G. *Sylvestre* Chloroform Extract on Wound Pathogens

**Table 4.** Zone of Inhibition in (mm) for G. *Sylvestre* Chloroform Extract

| S.NO | Name of the organisms | 25µg / ml | 50µg / ml | 75µg/ ml | Negative control (DMSO) |
|---|---|---|---|---|---|
| 01 | *Escherichia coli* | 12mm | 13mm | 14mm | No Zone |
| 02 | *Klebsiella pneumoniae* | 13mm | 14mm | 15mm | No Zone |
| 03 | *Pseudomonas aeruginosa* | 16mm | 17mm | 18mm | No Zone |
| 04 | *Staphylococcus aureus* | 14mm | 15mm | 16mm | No Zone |

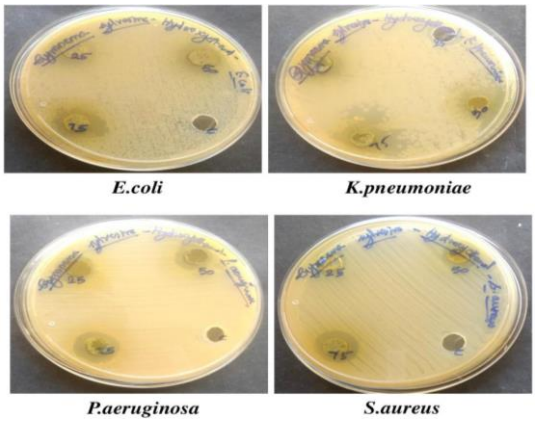


**Figure 5.** Antibacterial Activity of G. *Sylvestre* Hydroxyethanol Extract on Wound Pathogens

**Table 5.** Zone of Inhibition in (mm) for G. *Sylvestre* Hydroxyethanol Extract

| S.NO | Name of the organisms | 25µg / ml | 50µg / ml | 75µg/ ml | Negative control (DMSO) |
|---|---|---|---|---|---|
| 01 | *Escherichia coli* | 12mm | 13mm | 14mm | No Zone |
| 02 | *Klebsiella pneumoniae* | 15mm | 16mm | 17mm | No Zone |
| 03 | *Pseudomonas aeruginosa* | 13mm | 14mm | 15mm | No Zone |
| 04 | *Staphylococcus aureus* | 14mm | 15mm | 16mm | No Zone |

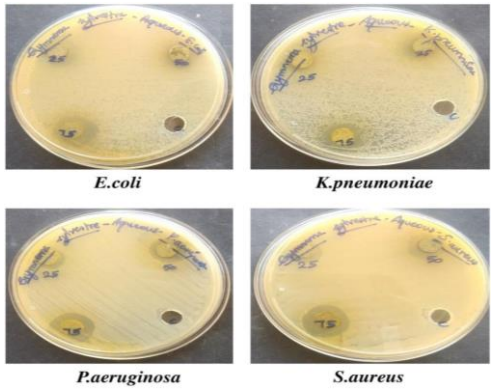


**Figure 6.** Antibacterial Activity of G. *Sylvestre* Aqueous Extract on Wound Pathogens

**Table 6.** Zone of Inhibition in (mm) for G. *Sylvestre* Aqueous Extract

| S.NO | Name of the organisms | 25µg / ml | 50µg / ml | 75µg/ ml | Negative control (DMSO) |
|---|---|---|---|---|---|
| 01 | *Escherichia coli* | 12mm | 13mm | 14mm | No Zone |
| 02 | *Klebsiella pneumoniae* | 11mm | 12mm | 13mm | No Zone |
| 03 | *Pseudomonas aeruginosa* | 13mm | 14mm | 15mm | No Zone |
| 04 | *Staphylococcus aureus* | 14mm | 15mm | 16mm | No Zone |

## Discussion

Antibiotics are one of the most significant weapons in the fight against bacterial infections, and they have offered enormous benefits to human health since their development. Microbial resistance, which is rapidly increasing, compromises the efficacy of any treatment agent, leaving the future of

antimicrobial drug use questionable [17]. Plants have been utilized as medications and treatments for a variety of ailments throughout history. These medications are used as prototypes to generate more effective and less dangerous therapies. As a result, a comprehensive investigation of medicinal plants' potential against diseases could bring some relief to the world's growing health problem [18].

In order to broaden the spectrum of antimicrobial drugs derived from natural sources, the current study has been designed to elevate traditional uses of medicinal herbs, such as *Gymnema sylvestre*, to the level of a primary treatment technique. A preliminary assessment for its antibacterial properties using solvent extracts revealed broad spectrum efficacy, consistent with prior research on medicinal plants [19]. *P. aeruginosa* is a well-known example of a "multidrug resistant (MDR) pathogen" because of its widespread distribution, innately sophisticated mechanisms for resisting antibiotics, and link to severe diseases [20]. While *K. pneumoniae* is a well-known multidrug-resistant pathogen that causes severe pneumonia, urinary tract and bloodstream infections, and is frequently linked to healthcare settings [21], *S. aureus* is linked to skin infections and food poisoning [22]. Even though *E. coli* is a common intestinal resident, it has the potential to turn pathogenic and cause a variety of diseases, including urinary tract infections and more severe ailments like sepsis, particularly when it takes on multidrug-resistant strains [23]. The significant sensitivity of these pathogens to the plant extract thus gave the study a lot of support. This prompted additional physiochemical parameter improvement as well as the identification, isolation, and investigation of relevant phytoconstituents.

The substances found in crude extracts of *G. sylvestre* are predominantly terpenes, alcohols, hydrocarbons, alkaloids, and their derivatives. The crude extracts from *G. sylvestre* leaves were analysed using GCMS to detect components. Several investigations utilizing GCMS have shown the effect of different solvents on isolating phytochemical elements with therapeutic properties from crude extracts of medicinal plants. The main compounds detected in the crude extracts of *G. sylvestre*, including inositol and 1-deoxy-found in methanol extract, The compounds 2-Pentanone, 3,3,4,4-tetramethyl, Tetratriacontane, and Hexadecane were identified in extracts of methanol and ethanolic extracts, and benzene, chloroform, ethyl acetate, and hexane, respectively. Subramanian et al. [14] identified compounds that are either chemically or biologically active: eicosane from benzene, chloroform, and ethyl acetate extracts; heneicosane from benzene, ethyl acetate, and hexane extracts; phthalic acid, di(2-propylpentyl) ester from hexane and methanol extracts; squalene, phytol, n-Hexadecanoic acid, and stigmasterol found in extracts. Therefore, the plant's reported antibacterial activity is probably caused by bioactive compounds found in the crude extracts of *G. sylvestre*, such as inositol, Tetratriacontane, 2-Pentanone, Eicosane, Hexadecane, Phthalic acid ester, Heneicosane, Phytol, Squalene, stigmasterol and n-Hexadecanoic acid. These substances, discovered in various solvent extracts, have known chemical and biological properties that contribute to the therapeutic efficacy of *G. sylvestre* against pathogenic microorganisms.

Ramadass et al. [11] evaluated the antibacterial activity of ethanolic and chloroform leaf extracts of *G. sylvestre* was evaluated against several pathogenic bacteria. The results demonstrated significant inhibitory effects, particularly against *S. aureus, E. faecalis, S. enterica, and E. coli*, with zones of inhibition ranging from 23 to 26 mm. Arora and Sood, [19] demonstrated the antibacterial efficacy of *G. sylvestre* solvent extracts against both gram-positive and negative bacteria. Among the tested solvents, ethyl acetate was

identified as the most effective extractant, with *K. pneumoniae* 1 (31.5 mm) and *S. epidermidis* (25.5 mm) showing the highest sensitivity among Gram-negative and positive bacteria, respectively. Gunasekaran et al. [24] assessed the antibacterial potential of *G. sylvestre* using methanolic extracts, revealing significant antimicrobial activity against both *P. aeruginosa* and *S. aureus*, as well as antifungal activity against *F. oxysporum*. These findings emphasize the broad-spectrum antimicrobial properties of *G. sylvestre*, particularly in methanolic extracts. Solanki and Arora [25-28] isolated and characterized antimicrobial peptides from the leaves of *G. sylvestre*, demonstrating significant antibacterial activity against bacteria associated with diabetic foot infections. The peptides exhibited zones of inhibition ranging from 18 to 22 mm against *S. aureus, E. faecalis, E. coli, K. pneumoniae,* and *P. aeruginosa*, highlighting the potential of *G. sylvestre* as a source of antimicrobial agents for combating infections in diabetic patients.

*Gymnema sylvestre* leaf solvent extract was pharmacologically evaluated and found to have powerful antibacterial properties, making it an attractive eco-friendly strategy for wound infection prevention. Its extract contains a variety of active secondary metabolites, some of which may function synergistically. However, further research is required to explain the mechanism underlying this effect. This work could serve as a foundation for future research into the biological and pharmacological effects of *G. sylvestre* leaf extract.

## Conclusion

In conclusion, the evaluation of the antibacterial efficacy of solvent extracts from *Gymnema sylvestre* against wound pathogens has demonstrated promising results, with the ethanol extract showing particularly potent antibacterial activity. Among the various solvents tested, the ethanol extract exhibited a zone of inhibition (ZOI) of approximately 20mm against multiple wound-associated pathogens. This level of inhibition indicates its significant potential in restricting the growth of harmful bacteria often implicated in wound infections. The strong antibacterial properties of the ethanol extract suggest that it may be an effective natural agent for combating infections in wound care, providing a possible alternative to conventional treatments. The findings support further research into the bioactive components of *G. sylvestre* responsible for this activity and highlight its potential for inclusion in antimicrobial formulations, particularly for treating infections caused by resistant or persistent bacteria associated with wound healing complications. As many bacteria have become multidrug-resistant, conventional antibiotic therapy has become less effective and is often associated with side effects. Bioactive compounds of plant which play a key role in the plant defence system. These bioactive compounds can be a better alternative as they inhibit the growth of microorganisms by forming ion channels in microbial membranes, thereby disrupting their function and offering a novel approach in fighting bacterial infections.

## Acknowledgement

The authors extend their acknowledgment to the Department of Botany, Aringar Anna Government Arts College, Namakkal, Tamil Nadu, India for providing the necessary facilities.

## Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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