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# PHARMACOLOGICAL EVALUATION OF WOUND HEALING ACTIVITY OF KYLLINGA POLYPHYLLA IN EXPERIMENTAL RATS

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## ABSTRACT

The present study aimed to evaluate the wound healing potential of the methanolic extract of *Kyllinga polyphylla* whole plant using experimental rat models. The plant material was extracted using the Soxhlet method, yielding 5.60% crude extract. Preliminary phytochemical screening revealed the presence of bioactive constituents such as alkaloids, flavonoids, phenolic compounds, proteins, and triterpenoids. Quantitative analysis showed high total phenolic content (77.8 mg gallic acid equivalents/g) and total flavonoid content (62.5 mg rutin equivalents/g), indicating strong antioxidant potential. The antioxidant activity assessed by the DPPH assay demonstrated moderate free radical scavenging activity, with a maximum inhibition of 60.63% and an $IC_{50}$ value of 48.68 µg/mL. Herbal gel formulations containing 1% and 2% extract were prepared and evaluated for physicochemical properties, which showed acceptable pH, viscosity, spreadability, and good homogeneity. Skin irritation studies confirmed the safety of the formulations. Antimicrobial evaluation revealed that the 2% gel exhibited the highest activity, with a zone of inhibition of 15 mm. In vivo wound healing activity assessed using the excision wound model demonstrated significant enhancement in wound contraction and reduction in epithelialization time in extract-treated groups compared to the control, with the 2% formulation showing superior efficacy. Overall, the findings suggest that *Kyllinga polyphylla* possesses promising antioxidant, antimicrobial, and wound healing properties, supporting its traditional use and potential development as a natural topical wound healing agent.

**KEYWORDS:** *Kyllinga polyphylla*; Wound healing; Antioxidant activity; Herbal gel; Phytochemical screening; Excision wound model; Antimicrobial activity.

## INTRODUCTION

Wound healing is an essential physiological process that restores the integrity and function of damaged tissues through a well-orchestrated sequence of events, including hemostasis, inflammation, proliferation, and remodeling. Any disruption in these phases may lead to delayed healing, chronic wounds, or excessive scar formation. Factors such as microbial infection, oxidative stress, poor vascularization, metabolic disorders, and prolonged inflammation significantly impair the healing process. Chronic non-healing wounds represent a major burden on healthcare systems worldwide, particularly in elderly and diabetic populations.[1-3]

Conventional wound healing therapies, including antibiotics, antiseptics, and growth factor–based treatments, have shown variable success and are often associated with limitations such as drug resistance, cytotoxicity to healthy tissues, high cost, and restricted availability. These drawbacks have stimulated growing interest in the exploration of alternative therapeutic agents, particularly from natural sources. Medicinal plants have been extensively used in traditional medicine for centuries to treat wounds and skin disorders, owing to their biocompatibility, minimal side effects, and broad spectrum of pharmacological activities.[4-6]

*Kyllinga polyphylla* (family: Cyperaceae) is a perennial herb widely distributed in tropical and subtropical regions and traditionally used in folk medicine for the treatment of inflammation, ulcers, wounds, and skin infections. Previous phytochemical investigations of

*Kyllinga* species have revealed the presence of flavonoids, phenolics, alkaloids, terpenoids, and essential oils, which are known to exhibit antioxidant, anti-inflammatory, antimicrobial, and collagen-enhancing properties—key mechanisms involved in wound repair. Antioxidants play a crucial role in wound healing by scavenging reactive oxygen species that otherwise delay tissue regeneration and collagen synthesis.[4-8]

Despite the ethnomedicinal importance of *Kyllinga polyphylla*, scientific studies validating its wound healing efficacy are scarce. There is a lack of systematic pharmacological evaluation using standardized experimental models. Therefore, the present study was undertaken to evaluate the wound healing activity of the whole-plant extract of *Kyllinga polyphylla* in experimental rats. The study aimed to assess its effects on wound contraction, epithelialization, and tissue regeneration, thereby providing experimental evidence to support its traditional use and to explore its potential as a safe and effective natural wound healing agent.

The study aimed to pharmacologically assess the wound healing activity of *Kyllinga polyphylla* whole plant extract using experimental rat models. *Kyllinga polyphylla* is traditionally known for its medicinal uses, including treatment of wounds, inflammation, and infections. The plant contains various phytoconstituents such as alkaloids, flavonoids, phenolic compounds, proteins, carbohydrates, steroids, and triterpenoids, which are recognized for their therapeutic properties including antioxidant, antimicrobial, and anti-inflammatory effects.[10-14]

The purpose of this study was to scientifically validate the traditional claims of *Kyllinga polyphylla* in wound management by investigating its effects on wound contraction, epithelialization, and antimicrobial activity in controlled experimental settings. The extract was prepared through Soxhlet extraction, and its phytochemical composition and antioxidant potential were thoroughly analyzed before conducting in vivo wound healing and in vitro antimicrobial assays.[14-15]

This research was performed to bridge the gap between traditional knowledge and modern scientific evidence, supporting the development of effective, natural wound healing agents. The future prospects of this study include isolating and characterizing the specific bioactive compounds responsible for the wound healing effects, optimizing formulation techniques, and ultimately developing standardized herbal preparations for clinical use. This approach not only promotes safer, cost-effective therapies but also encourages the sustainable utilization of medicinal plants like *Kyllinga polyphylla* in healthcare.[14-19]

**Collection and Authentication of Plant Material**

*Kyllinga polyphylla* whole-plant specimens were collected from the medicinal plant garden of the Pinnacle Biomedical Research Institute (PBRI), Bhopal, Madhya Pradesh. The plant material was taxonomically identified and authenticated by Dr. Jagrati Tripathi, Department of Botany, Government College Khimlasa, Sagar. After authentication, the collected plant material was thoroughly washed and shade-dried at room temperature for a period of 2–3 weeks to preserve thermolabile phytoconstituents. The dried plant material was then pulverized into a fine powder using an electric grinder and stored in airtight containers for further analysis.

**Soxhlet Extraction of Plant Material**

The powdered plant material was subjected to Soxhlet extraction following a standard protocol. The dried powder was placed in a thimble within the Soxhlet apparatus, and extraction was carried out using methanol as the solvent. The solvent was continuously heated, vaporized, condensed, and allowed to percolate repeatedly through the plant material, facilitating efficient extraction of bioactive compounds. Upon completion of extraction, the solvent was removed by evaporation to obtain a concentrated crude extract. The percentage yield of the extract was calculated using the following formula:

$$\%\text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

**Preliminary Phytochemical Screening**

The methanolic extract of *Kyllinga polyphylla* was subjected to qualitative phytochemical screening using standard chemical tests to identify major classes of phytoconstituents. Tests were performed for saponins, tannins, phenolic compounds, triterpenoids, steroids, carbohydrates, glycosides, proteins, amino acids, flavonoids, and alkaloids using established protocols. The presence of these phytochemicals was confirmed based on characteristic color changes or precipitate formation in each test.

**Quantitative Estimation of Phytochemicals**

Following qualitative screening, the extract was analyzed for total phenolic content (TPC) and total flavonoid content (TFC).

**Total Phenolic Content (TPC)**

TPC was determined using the Folin–Ciocalteu method. The extract was reacted with Folin–Ciocalteu reagent followed by sodium carbonate, and the reaction mixture was incubated for 60 minutes at room temperature. Absorbance was measured at 760 nm using a UV–Visible spectrophotometer. Gallic acid was used as the standard, and results were expressed as gallic acid equivalents (GAE).

**Total Flavonoid Content (TFC)**

TFC was estimated using a colorimetric aluminum chloride method. The extract was mixed with aluminum

chloride and sodium acetate, incubated at room temperature, and absorbance was measured at 510 nm. Rutin was used as the reference standard, and results were expressed as rutin equivalents (mg/g of dry extract).

**In Vitro Antioxidant Activity (DPPH Assay)**

The antioxidant activity of the extract was evaluated using the DPPH free radical scavenging assay. Various concentrations of the extract (20–100 µg/mL) were prepared and mixed with DPPH solution. The mixtures were incubated in the dark for 30 minutes, and absorbance was measured at 517 nm. Percentage inhibition was calculated using the formula:

$$\%\text{Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

**Preparation of Herbal Gel Formulation**

Herbal gel formulations containing *Kyllinga polyphylla* extract were prepared using Carbopol 940 and carboxymethyl cellulose as gelling agents. Carbopol was dispersed in warm water and allowed to swell, while carboxymethyl cellulose and methyl paraben were dissolved separately. Both solutions were combined under continuous stirring, and triethanolamine was added to adjust pH. The extract was incorporated at concentrations of 1% and 2% to obtain different formulations. Propylene glycol was added as a permeation enhancer, and the gel was stirred until a homogeneous formulation was obtained.

**Evaluation of Gel Formulations**

The prepared gel formulations were evaluated for pH, spreadability, viscosity, and extrudability to ensure suitability for topical application. An acute dermal toxicity study was also conducted to confirm the safety of the extract when applied to the skin.

**Antimicrobial Activity (Well Diffusion Method)**

The antimicrobial activity of the formulations was assessed using the agar well diffusion method against *Escherichia coli*. Different concentrations of the formulations were placed into wells created on nutrient agar plates inoculated with bacterial culture. After incubation, the zone of inhibition was measured to evaluate antimicrobial efficacy.

**Pharmacological Evaluation of Wound Healing Activity**

Healthy Wistar albino rats (250–350 g) were used for the study following approval from the Institutional Animal Ethics Committee (IAEC). Excision wounds were created under anesthesia on the dorsal surface of the rats. Animals were divided into control, test, and standard treatment groups. The gel formulations were applied topically once daily, and wound contraction was monitored at regular intervals. The percentage of wound contraction and the period of epithelialization were recorded to assess wound healing efficacy.

**RESULT AND DISCUSSION**

The present study evaluated the pharmacological potential of the methanolic extract of *Kyllinga polyphylla* whole plant, with particular emphasis on its wound healing efficacy in experimental rats. Soxhlet extraction of the plant material yielded 5.60% (w/w) crude extract, indicating the moderate efficiency of methanol in extracting bioactive phytoconstituents. Methanol is widely recognized for its ability to solubilize polar and semi-polar compounds, which likely contributed to the recovery of pharmacologically active constituents.

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, proteins, and triterpenoids. These classes of phytochemicals are well documented for their antioxidant, anti-inflammatory, antimicrobial, and tissue-regenerative properties, all of which play a vital role in the wound healing process. Quantitative analysis further supported these findings, with the extract exhibiting a high total phenolic content (77.8 mg gallic acid equivalents/g of extract) and a substantial total flavonoid content (62.5 mg rutin equivalents/g of extract). The abundance of phenolics and flavonoids strongly correlates with enhanced antioxidant activity and improved cellular repair mechanisms.

The antioxidant capacity of the extract was assessed using the DPPH free radical scavenging assay. The extract demonstrated moderate antioxidant activity, with a maximum inhibition of 60.63% and an $IC_{50}$ value of 48.68 µg/mL. These results indicate the extract's ability to neutralize free radicals and reduce oxidative stress, which is a critical factor in accelerating wound healing by preventing cellular damage and promoting collagen synthesis.

Herbal gel formulations containing 1% and 2% *Kyllinga polyphylla* extract were successfully developed and evaluated for physicochemical parameters. Both formulations exhibited good homogeneity, acceptable viscosity, suitable spreadability, and pH values within the skin-compatible range, confirming their appropriateness for topical application. Skin irritation studies further established the safety of the formulations, as no signs of erythema, edema, or adverse reactions were observed during the study period.

The antimicrobial activity of the gel formulations was assessed using the agar well diffusion method. Among the tested formulations, the 2% extract gel exhibited the highest antimicrobial efficacy, producing a zone of inhibition measuring 15 mm. This finding suggests that the extract may effectively prevent microbial colonization at wound sites, thereby reducing the risk of infection and facilitating faster wound repair.

The in vivo wound healing activity was evaluated using the excision wound model in rats. Both the 1% and 2% extract-based gel formulations significantly enhanced

wound contraction and reduced the epithelialization period when compared to the control group. Notably, the 2% formulation demonstrated superior wound healing activity, indicating a dose-dependent effect. Although the standard gentamicin gel produced the fastest healing response, the *Kyllinga polyphylla* formulations showed comparable and promising results, highlighting their therapeutic potential as natural wound healing agents.


**Figure 1: Yield of plant material *Kyllinga polyphylla* by methanol.**

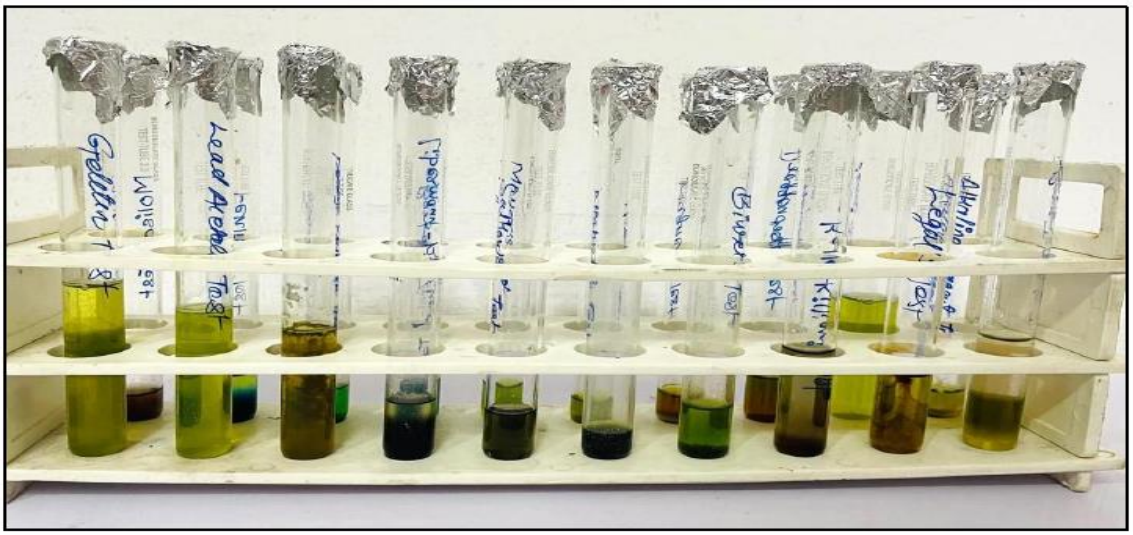


**Figure 2: Phytochemical Testing of *Kyllinga polyphylla* by Methanol.**

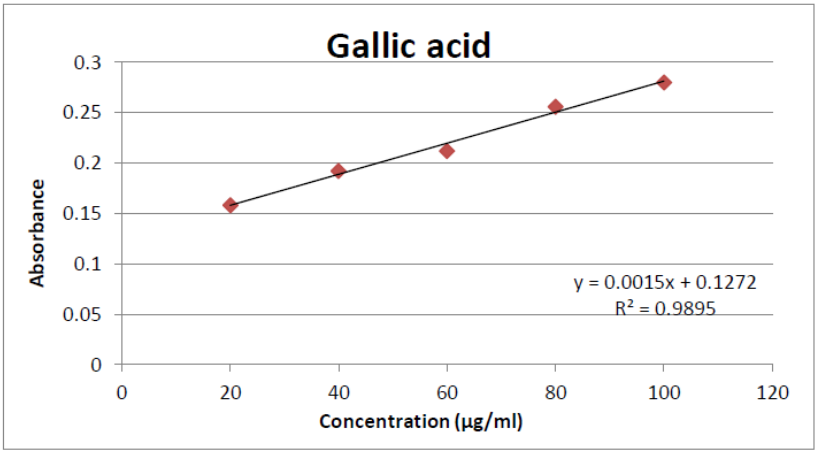



**Figure 3: Represent standard curve of Gallic acid.**

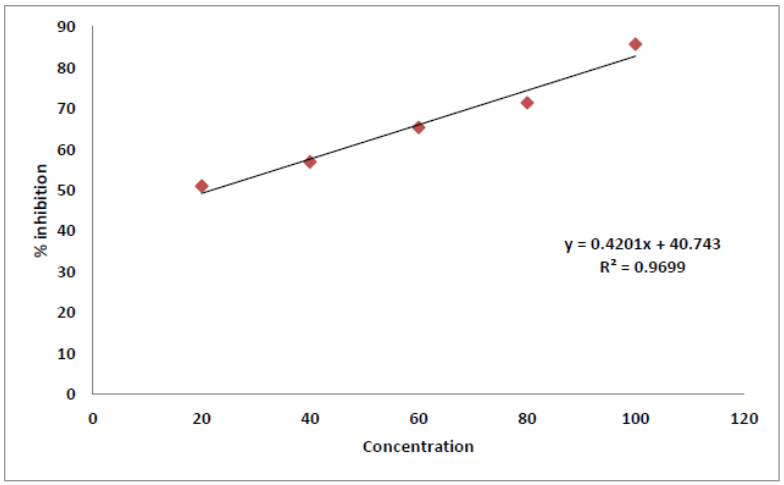



**Figure 4: DPPH radical scavenging activity of Std. Ascorbic acid.**

**Figure 5: Antimicrobial Activity of Herbal gel.**

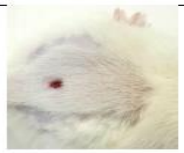
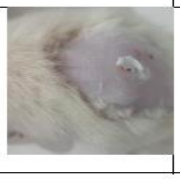
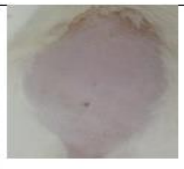
| Group | 4 Day | 8 Day | 12 Day | 16 Day | 21 Day |
|---|---|---|---|---|---|
| Control group by formulation I |  |  |  |  |  |
| Formulation II *Kyllinga polyphylla* 1% gel. |  |  |  |  |  |
| Formulation III *Kyllinga polyphylla* 2% gel. |  |  |  |  |  |
| Reference Standard (Gentamicin gel) |  |  |  |  |  |

**Figure 6: Excision Wound Healing from Herbal gel.**

## CONCLUSION AND FUTURE PROSPECTS

The results of the present study clearly demonstrate that the methanolic extract of *Kyllinga polyphylla* possesses significant wound healing, antioxidant, and antimicrobial activities. These effects can be attributed to the presence of diverse bioactive phytochemicals, particularly phenolic compounds and flavonoids. The formulated herbal gels, especially at a 2% concentration, effectively promoted wound contraction, exhibited favorable physicochemical characteristics, and showed excellent safety profiles upon topical application.

Overall, *Kyllinga polyphylla* emerges as a promising natural candidate for topical wound management, supporting its traditional medicinal use and offering potential for development as a phytopharmaceutical product. Future research should focus on the isolation and characterization of the specific bioactive compounds responsible for the observed wound healing effects. In-depth mechanistic studies are required to elucidate the molecular pathways involved in tissue regeneration and antimicrobial action. Furthermore, formulation optimization, stability studies, and well-designed clinical trials will be essential to confirm its efficacy and safety in human subjects. Exploring synergistic combinations with other herbal extracts or conventional drugs may also open new avenues for the development of more effective wound care therapies.

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