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PHYTOCHEMICAL SCREENING, CHARACTERIZATION OF BIOACTIVE COMPONENT FROM PLANT PHRAGMITES KARKA(RETZ.) TRIN EX STEUD AND ITS BIOLOGICAL EVALUATION WITH REFERENCE TO WOUND HEALING ACTIVITY

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ABSTRACT
This study investigates the formulation and evaluation of a medicinal herbal extract for its wound healing potential. The extracts of Phragmites karka were subjected to phytochemical screening to identify bioactive compounds, including phenolics and flavonoids, known for their therapeutic properties. The wound healing activity was evaluated using a rat model, where wound closure was monitored on days 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 by tracing the wound margins using transparency paper and a permanent marker. The changes in wound area were measured planimetrically on graph paper, and the reduction in wound size was expressed as a percentage of the original wound area. Results showed that the P. karka extracts possess significant wound healing potential, likely due to their rich phytochemical content. The findings suggest that P. karka could be a promising candidate for developing topical formulations aimed at improving wound healing.

KEYWORDS: Phragmites karka, Wound healing, Phytochemical screening, Bioactive compounds.

INTRODUCTION
Wound healing is a vital biological process that restores the structural and functional integrity of injured tissues. It involves a series of overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Various factors, including infections, oxidative stress, and underlying health conditions, can impede wound healing, necessitating the development of effective therapeutic agents. Traditional medicine, particularly plant-based remedies, has played a significant role in managing wounds due to the natural bioactive compounds present in medicinal plants (Gupta et al., 2018).

Phragmites karka (Retz.) Trin ex Steud, a perennial grass of the Poaceae family, thrives in wetlands and marshy areas across tropical and subtropical regions. It has been traditionally employed for treating various ailments, including skin disorders, due to its pharmacological properties. Ethnobotanical studies have documented the use of *Phragmites karka* in managing wounds, burns, and inflammatory conditions. Its therapeutic potential is attributed to a rich array of secondary metabolites such as flavonoids, phenolics, alkaloids, tannins, and saponins, which possess antimicrobial, antioxidant, anti-inflammatory, and tissue-regenerative properties (Chaudhary et al., 2020; Ahmed et al., 2021).

The bioactive components of *Phragmites karka* have been shown to accelerate wound healing by promoting angiogenesis, enhancing fibroblast proliferation, and stimulating collagen deposition. Studies have highlighted its ability to reduce microbial load and oxidative stress at the wound site, further supporting its traditional use. However, systematic phytochemical screening and characterization of its bioactive constituents, followed by biological evaluation, are essential to establish its efficacy scientifically and explore its potential in modern therapeutic formulations (Singh et al., 2019).

Given the growing interest in plant-based therapeutics, this study focuses on the phytochemical screening, characterization of bioactive components, and evaluation of the wound-healing activity of *Phragmites karka*. By combining advanced analytical techniques and biological assays, the research aims to provide insights into the

plant's medicinal properties and its mechanism of action in wound repair.

KEY OBJECTIVES

1. To identify and characterize the phytochemical constituents of *Phragmites karka*.
2. To evaluate its antioxidant, antimicrobial, and anti-inflammatory properties.
3. To assess its wound-healing potential through biological assays and histopathological studies.

Collection and Authentication of plant material

Phragmites karka was collected locally from Bhopal, M.P., India. The herbarium was prepared and submitted to the Department of Botany, Saifia College of Science, Bhopal, for authentication by Dr. Saba Khan, Head of the Department.

Method

In this study, Phragmites karka was extracted using the continuous hot percolation method with a Soxhlet apparatus. Powdered plant material was initially extracted at 60°C using petroleum ether as a non-polar solvent. The dried marc was subsequently re-extracted with ethyl acetate and methanol. Extraction was deemed complete when no visual color change occurred in the siphon tube, and the absence of residual solvent was confirmed. Extracts were evaporated at 40°C using a rotary vacuum evaporator (Buchi type), observed for organoleptic properties (yield, color, odor), and stored in airtight containers for further use (The Ayurvedic Pharmacopoeia of India).

Qualitative phytochemical estimation of extracts

Qualitative phytochemical estimation of the extracts was conducted to identify the presence of bioactive compounds. Tests were performed for alkaloids, flavonoids, phenolics, saponins, tannins, and other phytoconstituents using standard methods. Results provided an overview of the chemical profile of Phragmites karka extracts.

Quantitative Phytochemical Estimation

Total Phenolic Content Estimation (Ainsworth *et al.*, 2007; Alhakmani, 2013)

To determine total phenolic content, gallic acid solutions (20–100 µg/mL) were prepared in methanol, along with the test sample at 100 µg/mL in methanol or a solvent of similar polarity. A reaction mixture was prepared by adding 0.5 mL of each gallic acid concentration or test sample to 2 mL of diluted Folin-Ciocalteu reagent (1:10 in deionized water), followed by 4 mL of sodium carbonate solution. The mixture was incubated at room temperature for 30 minutes with intermittent shaking. Absorbance was measured at 765 nm using methanol as a blank. A standard curve for gallic acid was plotted, and the regression equation was used to calculate the total phenolic content of the test sample, expressed as mg/g or µg/mg gallic acid equivalents.

Total Flavonoid Content Estimation

To determine the total flavonoid content, rutin solutions (20–100 µg/mL) were prepared in methanol, along with the test sample at 100 µg/mL in methanol or a solvent of similar polarity. A 0.5 mL aliquot of the diluted sample or standard was mixed with 2 mL of distilled water and 0.15 mL of 5% NaNO2 solution. After 6 minutes, 0.15 mL of 10% AlCl3 solution was added, followed by a 6-minute incubation. Then, 2 mL of 4% NaOH solution was added, and water was added to make up the final volume to 5 mL. The mixture was mixed thoroughly and left to stand for 15 minutes. Absorbance was measured at 510 nm against a water blank. A standard curve of rutin was prepared, and the regression equation was used to calculate the total flavonoid content of the test sample, expressed as mg/g or µg/mg rutin equivalents.

Evaluation of in-vitro Antioxidant Activity

Antioxidants help prevent or delay free radical-mediated oxidation, protecting the body from oxidative stress and related degenerative diseases. External antioxidants can strengthen the body's natural defense system by reducing oxidative damage through mechanisms such as decreasing oxygen concentration, scavenging free radicals like hydroxyl radicals, or binding free metal ions to prevent metal-induced oxidative damage. Natural antioxidants, rich in phenolic and polyphenolic compounds, hold significant potential for addressing oxidative stress. This study aimed to evaluate and compare the antioxidant potential and free radical scavenging activity of the extract with the synthetic antioxidant vitamin C (ascorbic acid) against reactive oxygen species.

1,1- diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is based on the reduction of DPPH, a stable free radical that absorbs at 517 nm (purple color). When antioxidants react with DPPH, they donate hydrogen, reducing the radical to DPPHH, resulting in a decrease in absorbance and a shift in color from purple to yellow, indicating the antioxidant's free radical scavenging ability. The degree of decolorization correlates with the antioxidant's reducing power. This assay is widely used for evaluating the free radical scavenging activity of substances (Gulcin et al., 2006; Jain and Jain, 2011).

A 0.1 mM DPPH solution (4 mg/100 mL) in methanol was mixed with different concentrations of the plant extracts. The reaction mixture was vortexed and incubated in the dark at room temperature for 30 minutes. Ascorbic acid served as the reference standard, and methanol was used as the control. The change in color was measured at 517 nm using a spectrophotometer, with methanol as the reference solution. The percentage of DPPH radical scavenged was used to calculate the IC50 value, with lower IC50 values indicating higher antioxidant activity. All tests were conducted in triplicates. The percentage inhibition of free

radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control- absorbance of sample)/absorbance of control] × 100%

HPTLC Analysis

For the analysis, an automatic CAMAG TLC Sampler with WinCATS software was used. Silica gel 60 F 254 pre-coated plates (20 cm x 10 cm, 0.2 mm thickness) were employed as the stationary phase, with 8 tracks and a band length of 8 mm. The mobile phase consisted of toluene, ethyl acetate, and formic acid in a ratio of 8:2:0.5. The standard, a light yellow powder, was dissolved in methanol at a concentration of 50 µg/mL, while the sample, a brown powder, was dissolved at 1 mg/mL. For the standard, injection volumes of 2, 4, 6, 8, and 10 µL were applied, while 2 µL was used for the sample. The chromatographic separation was conducted in a twin trough chamber (20 x 10 cm) in ascending mode, running the distance of 75 mm. After development for 15 minutes, the plates were dried at room temperature and scanned at 260 nm. The regression equation was derived using the WinCATS software, based on the calibration curve plotting the standard drug concentration (X-axis) against the average peak area (Y-axis).

***In vivo* wound healing activity**

Formulation of ointment (British pharmacopoeia, 1996)

For the preparation of the ointment base, 1g of wool fat, 1g of hard paraffin, 1g of cetostearyl alcohol, and 17g of white soft paraffin were mixed and gently heated with continuous stirring. Once the components were thoroughly combined, the mixture was allowed to cool. This resulted in a simple ointment base weighing 20g.

To prepare a 5% ointment, 1g of the 50% methanolic extract of *Phragmites karka* was added to 20g of the prepared ointment base. For a 10% ointment, 2g of the same extract was added to the 20g of base. These preparations were thoroughly mixed to ensure uniform distribution of the plant extract within the base.

The animals used in the study were Wistar rats, each weighing between 100 and 150g, with either sex being used. They were housed in groups of six in separate cages under controlled conditions at a temperature of 22 ± 2°C. All animals were provided with a standard diet (Golden Feed, New Delhi) and water ad libitum throughout the study period.

WOUND HEALING ACTIVITY

Excision wound model

The surgical materials were sterilized, and the dorsal fur of the rats was shaved using an electric clipper. Anesthesia was administered using 2% Xylocaine® Jelly (Cadila, Zydus Healthcare India Ltd). The anticipated wound area was marked on the back of the rats with methylene blue and a circular stainless-steel stencil. A full-thickness excision wound, measuring 500mm² in area and 2mm in depth, was created using toothed forceps, a scalpel, and pointed scissors. Wound contraction was monitored by measuring the wound area planimetrically on alternate days until the wound was fully healed.

A total of 24 animals were divided into four groups for treatment. Group 1 served as the vehicle control, where a simple ointment base was applied once daily. Group 2 received the standard drug nitrofurazone ointment (0.2% w/w) once daily as the positive control. Group 3 was treated with 5% w/w *Phragmites karka* 50% methanolic extract ointment, while Group 4 received 10% w/w *Phragmites karka* 50% methanolic extract ointment, both applied once daily. The treatments began on the day of wound creation and continued until the 20th day of healing.

Wound closure was assessed by tracing the wound margins on days 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 using transparency paper and a permanent marker. The changes in the wound area were measured planimetrically by tracing the wound margin on graph paper. The reduction in wound size was expressed as a percentage of the original wound area (Mustafa et al., 2005).

% Wound contraction= Healeda area/ Total area × 100

RESULTS AND DISCUSSIONS

Collection and Authentication of plant material

The leaves of Phragmites karka were collected from the local area of Bhopal (M.P.), India. A herbarium of the plant was prepared and submitted to the Department of Botany, Saifia College of Science, Bhopal, for authentication. The plant was authenticated by Dr. Saba Khan, Head of the Department of Botany, Saifia College of Science, Bhopal, India.

Table: Authentication of plant material.

S. No.	Plant	Part	Family
1	*Phragmites karka*	Leaves	Poaceae

Percentage yield of different extract

The Soxhlet extraction of Phragmites karka yielded the highest amount in methanol (6.21%), followed by ethyl acetate (3.74%), and the lowest in petroleum ether (1.86%). All extracts exhibited a greenish-brown color and characteristic odor.

Table 2: Percentage yield (%) and organoleptic characteristic of different extracts.

Characters	Extract	% Yield	Colour	Odour
Phragmites karka	Pet. ether	1.86	Greenish Brown	Characteristic
	Ethyl acetate	3.74	Greenish Brown	Characteristic
	Methanol	6.21	Greenish Brown	Characteristic

Quantitative phytochemical Screening

The results of qualitative phytochemical estimation are shown in the table 3.

Table 3: Phytochemical screening of petroleum ether extract of *Phragmites karka*.

S. No.	Experiment	Result
		Pet. ether extract
1. Alkaloids		
1.1	Mayer's reagent test	-ve
1.2	Wagner's reagent test	-ve
1.3	Hager's reagent test	-ve
2. Carbohydrates		
2.1	Molish's test	-ve
2.2	Barfoed's test	+ve
3. Test for Reducing Sugar's		
3.1	Fehling's test	-ve
3.2	Benedict's test	-ve
4. Flavonoids		
4.1	Alkaline reagent test	-ve
4.2	Shinoda test	-ve
4.3	Lead acetate test	-ve
5. Glycoside		
5.1	Borntrager test	+ve
5.2	Legal's test	+ve
5.3	Killer- Killiani test	+ve
6. Tannin and Phenolic compound		
6.1	Ferric chloride test	-ve
6.2	Lead Acetate test	-ve
6.3	Dilute Iodine solution	-ve
7. Saponin		
7.1	Foam Test	-ve
8. Test for Proteins and amino acid		
8.1	Ninhydrin test	-ve
9. Test for Triterpenoids and Steroids		
9.1	Salwonski Test	+ve
9.2	Libberman-Burchard's test	+ve

+ve: Present; –ve: Absent

Ethyl acetate extract of Phragmites karka

Phytochemical estimation of ethyl acetate extract of Phragmites karka showed the presence of alkaloids, carbohydrates, reducing sugars, tannins, phenolic compounds and triterpenoids and steroids. The results of qualitative phytochemical estimation are shown in the table 4.

Table 4: Phytochemical screening of ethyl acetate extract of Phragmites karka.

S. No.	Experiment	Result
		Ethyl acetate extract
1. Alkaloids		
1.1	Mayer's reagent test	+ve
1.2	Wagner's reagent test	+ve
1.3	Hager's reagent test	+ve
2. Carbohydrates		
2.1	Molish's test	+ve
2.2	Barfoed's test	+ve
3. Test for Reducing Sugar's		
3.1	Fehling's test	+ve
3.2	Benedict's test	+ve
4. Flavonoids		
4.1	Alkaline reagent test	-ve
4.2	Shinoda test	-ve

4.3	Lead acetate test	-ve
5. Glycoside		
5.1	Borntrager test	-ve
5.2	Legal's test	-ve
5.3	Killer- Killiani test	-ve
6. Tannin and Phenolic compound		
6.1	Ferric chloride test	+ve
6.2	Lead Acetate test	-ve
6.3	Dilute Iodine solution	+ve
7. Saponin		
7.1	Faom Test	-ve
8. Test for Proteins and amino acid		
8.1	Ninhydrin test	-ve
9. Test for Triterpenoids and Steroids		
9.1	Salwonski Test	+ve
9.2	Libberman-Burchard's test	+ve

+ve: Present; –ve: Absent

Methanolic extract of Phragmites karka

Phytochemical estimation of methanolic extract of *Phragmites karka* showed the presence of alkaloids, reducing sugar, flavonoids, tannins and phenolic compounds, proteins and amino acid. The results of qualitative phytochemical estimation are shown in the table 5.

Table 5: Phytochemical screening of methanolic extract of *Phragmites karka.*

S. No.	Experiment	Result
		Methanolic extract
1. Alkaloids		
1.1	Mayer's reagent test	+ve
1.2	Wagner's reagent test	+ve
1.3	Hager's reagent test	+ve
2. Carbohydrates		
2.1	Molish's test	-ve
2.2	Barfoed's test	-ve
3. Test for Reducing Sugar's		
3.1	Fehling's test	+ve
3.2	Benedict's test	+ve
4. Flavonoids		
4.1	Alkaline reagent test	+ve
4.2	Shinoda test	+ve
4.3	Lead acetate test	+ve
5. Glycoside		
5.1	Borntrager test	-ve
5.2	Legal's test	-ve
5.3	Killer- Killiani test	-ve
6. Tannin and Phenolic compound		
6.1	Ferric chloride test	+ve
6.2	Lead Acetate test	+ve
6.3	Dilute Iodine solution	+ve
7. Saponin		
7.1	Faom Test	-ve
8. Test for Proteins and amino acid		
8.1	Ninhydrin test	-ve
9. Test for Triterpenoids and Steroids		
9.1	Salwonski Test	+ve
9.2	Libberman-Burchard's test	+ve

+ve: Present; –ve: Absent

Quantitative phytochemical screening

Preliminary phytochemical testing of crude extracts confirmed the presence of Phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total phenolic contents (TPC)

Phenolics are key secondary metabolites in plants, contributing to antioxidant activity due to their redox properties, hydrogen-donating, reducing, and oxygen-scavenging abilities. This helps prevent oxidative stress-related diseases like cardiovascular, neurodegenerative diseases, and cancer (Harikumar et al., 2008; Wang et al., 2006; Dimitrios, 2006). Total phenolic content of Phragmites karka extracts was determined using the Folin-Ciocalteu method, with gallic acid as the standard. The results, expressed as mg of gallic acid equivalent (GAE), showed that the methanolic extract had the highest phenolic content (165.47 ± 0.312 mg GAE/g), while the ethyl acetate extract had the lowest (135.84 ± 0.113 mg GAE/g). The regression coefficient was R^2 = 0.995, with a slope of 0.002 and intercept of 0.063.

Table 6 Standard curve of gallic aci.

S. No.	Concentration (µg/ml)	Absorbance
1	20	0.1086
2	40	0.1678
3	60	0.1959
4	80	0.2862
5	100	0.3124

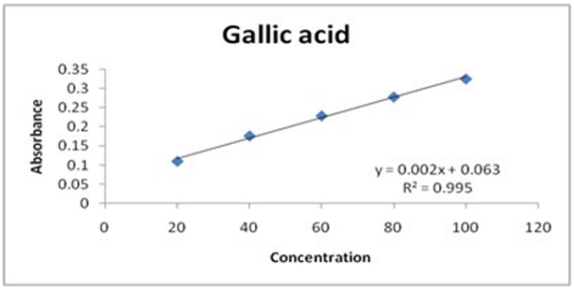

Figure 1: Standard curve of Gallic acid.

Table 7: Total phenolic content in ethyl acetate extract *Phragmites karka.*

S. No.	Absorbance	Concentration	Total phenolic content in mg/g equivalent of gallic acid
1	0.742	1mg/ml	136.94
2	0.743	1mg/ml	135.30
3	0.743	1mg/ml	135.30
MEAN±SD			**135.84±0.113**

Table 8: Total phenolic content in methanolic extract of *Phragmites karka.*

S. No.	Absorbance	Concentration	Total phenolic content in mg/g equivalent of gallic acid
1	0.885	1mg/ml	165.31
2	0.884	1mg/ml	165.21
3	0.888	1mg/ml	165.91
MEAN±SD			**165.47±0.312**

Total flavonoid contents (TFC)

Flavonoids, major secondary metabolites in plants, contribute to free radical scavenging due to their electron-donating properties. The 3, 4'-orthodihydroxy configuration in ring B and a carbonyl group at C4 in ring C enhance electron donation, increasing radical-scavenging activity. The antioxidant potential also depends on the number and position of hydroxyl groups (Chua et al., 2011). Total flavonoid content of Phragmites karka extracts was measured using the aluminium chloride colorimetric assay, with rutin as the standard. The absorbance was measured at 510 nm using UV Spectrophotometry. The total flavonoid contents were calculated with a regression equation (y = 0.001x + 0.092, R^2 = 0.979). The methanolic extract had the highest flavonoid content (89.67 ± 2.516 mg RTE/g), while the ethyl acetate extract had the lowest (22.67 ± 3.055 mg RE/g).

Table 9: Standard curve of Rutin.

S. No.	Concentration (µg/ml)	Absorbance
1	20	0.136
2	40	0.152
3	60	0.163
4	80	0.177
5	100	0.198

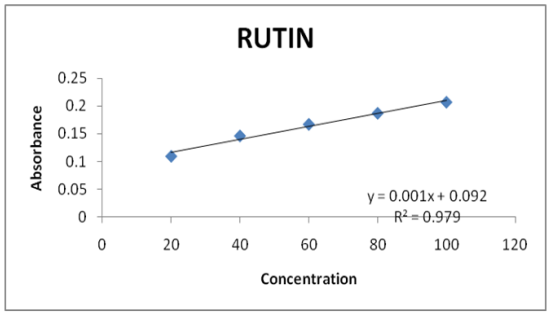

Figure 2 Standard curve of rutin.

Table 10: Total flavonoid content in ethyl acetate extract of *Phragmites karka.*

S. No.	Absorbance	Concentration	Total flavonoid content in mg/g equivalent of rutin
1	0.141	1mg/ml	22
2	0.145	1mg/ml	26
3	0.139	1mg/ml	20
MEAN±SD			**22.67±3.055**

Table 11: Total flavonoid content in methanolic extract of *Phragmites karka.*

S. No.	Absorbance	Concentration	Total flavonoid content in mg/g equivalent of rutin
1	0.209	1mg/ml	90
2	0.211	1mg/ml	92
3	0.206	1mg/ml	87
MEAN±SD			**89.67±2.516**

***In-vitro* antioxidant activity**

1,1- Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical method is an antioxidant assay based on electron transfer, where the violet DPPH solution becomes colorless in the presence of antioxidants. The scavenging activity of Phragmites karka extracts and ascorbic acid on the DPPH radical was expressed as IC50 values: methanol (20.15), ethyl acetate (114.3), and ascorbic acid (13.5). The methanolic extract showed an IC50 value similar to ascorbic acid, indicating its effectiveness as an antioxidant.

Table 12: DPPH radical scavenging activity of standard ascorbic acid, ethyl acetate and methanolic extract.

		Ascorbic acid (Std.)		Methanolic extract		Ethyl acetate extract	
S. No.	**Conc. µg/ml**	**Abs**	**% Inhibition**	**Abs**	**% Inhibition**	**Abs**	**% Inhibition**
1.	**20**	0.259	54.64	0.278	50.61	0.391	31.52
2.	**40**	0.237	58.49	0.252	55.34	0.369	35.37
3.	**60**	0.207	63.74	0.235	58.66	0.344	39.75
4.	**80**	0.147	74.25	0.220	65.14	0.320	43.95
5.	**100**	0.098	82.83	0.185	71.27	0.305	46.58
IC_{50}		**13.5**		**20.15**		**114.3**	

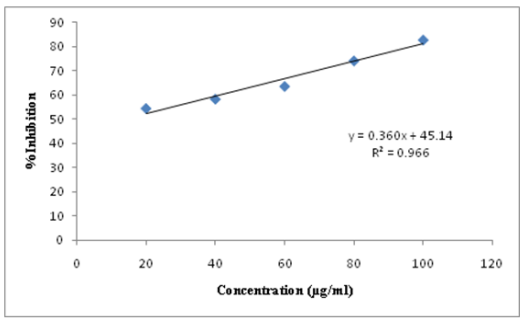

Figure 3: DPPH assay of ascorbic acid.

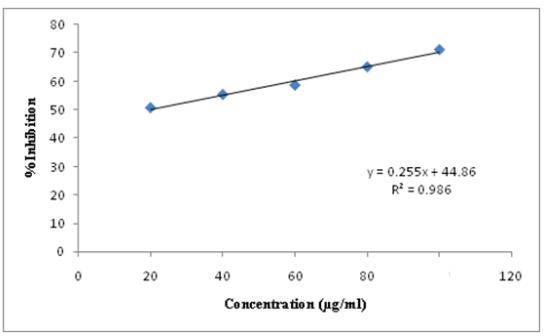

Figure 4: DPPH assay of methanolic extract.

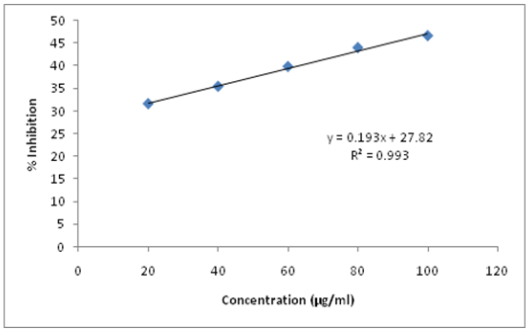

Figure 5 DPPH assay of ethyl acetate.

Wound healing activity

Excision wound model

Wound healing is a complex process involving inflammation, granulation tissue formation, and extracellular matrix remodeling, with cellular activities such as migration, proliferation, and differentiation. Proteoglycans and glycosaminoglycans (GAGs) play crucial roles in wound healing events, including inflammation and tissue repair. The Phragmites karka leaf extract ointment (5% and 10% w/w) demonstrated significantly better wound contraction compared to the simple ointment control group. The 10% extract ointment showed substantial healing from day 4, with 100% wound contraction by day 18, similar to the nitrofurazone standard group. The 5% extract ointment achieved 100% wound contraction by day 20.

Table 13: Evaluation of *Phragmites karka* extract ointment (5% and 10% w/w) and Nitrofurazone (0.2%w/w) Ointment in wound healing by excision wound method in rats.

Post- wounding (days)	Wound area (mm2) (mean±S.E.) and percentage of wound contraction			
	Simple ointment (control)	Standard ointment (0.2%, w/w)	Extract ointment (5%, w/w)	Extract ointment (10%, w/w)
0	521±2.1	507±2.6	526±2.8	518±3.2
2	433±2.1 (16.33%)	409±1.5 (19.96%)	421±1.7 (20.94%)	402±2.1 (24.85%)
4	387±2.3 (25.5%)	301±1.6** (40.20%)	347±1.2 (33.70%)	326±4.1* (36.60%)
6	309±3.2 (35.2%)	228±2.6** (54.50%)	288±2.3*(44.80%)	241±2.6*(52.9%)
8	301±3.9 (41.8%)	184±1.6** (63.10%)	223±4.3 (57.1%)	171±5.2** (66.3%)
10	284±0.6 (45.1%)	103±1.8** (63.10%)	160±1.2** (68.9%)	112±2.8** (77.60%)
12	263±2.1 (49.0%)	59±1.2** (87.50%)	123±2.4** (75.9%)	68±1.1** (86.00%)
14	237±1.2 (54.0%)	25±1.2** (94.10%)	77±1.1** (84.50%)	39±1.1** (93.50%)
16	213±0.5 (58.5%)	3±0.1** (98.40%)	31±0.3** (93.20%)	06±0.1** (97.90%)
18	191±1.4 (62.7%)	00±00** (100%)	07±0.8** (97.7%)	00±00** (100%)
20	182±3.6 (64.4%)	00±00** (100%)	00±00** (100%)	00±00** (100%)

Result was statistically significant compared with the corresponding control values (simple ointment) *P<0.01, **P<0.001

HPTLC analysis

HPTLC analysis of Phragmites karka methanol leaf extract, using p-Coumaric acid as a standard and Toluene: Ethyl acetate: Formic acid (8:2:0.5) as the mobile phase, revealed well-resolved peaks with an Rf of 0.42. The percentage of p-Coumaric acid in the extract was 0.69. Peak purity was assessed, confirming the presence of p-Coumaric acid. This HPTLC fingerprinting method is reliable for herbal identification and can aid in the authentication and characterization of medicinal plants.

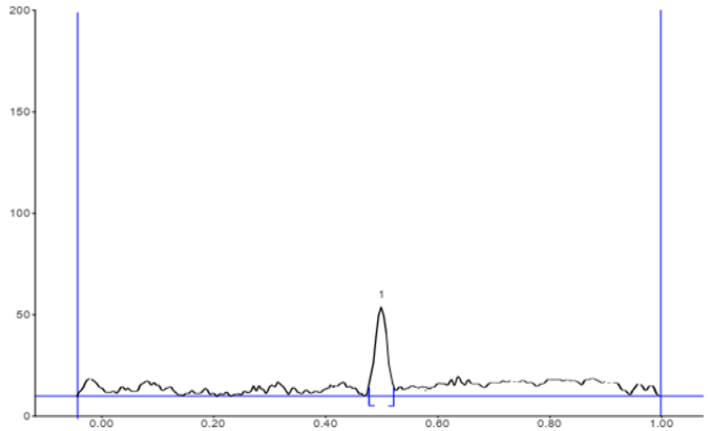

Figure 6: HPTCL of pure p-Coumaric acid.

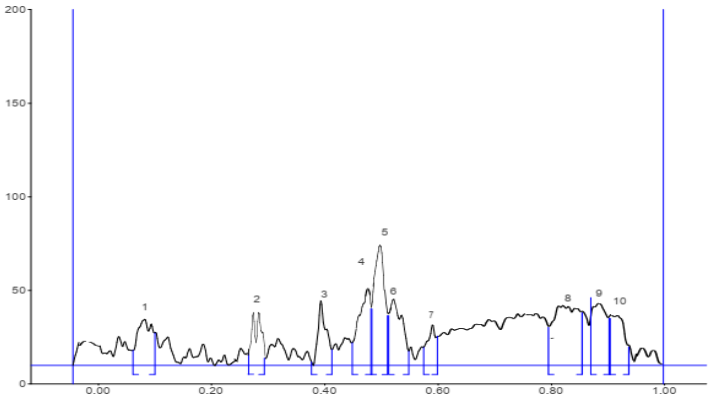

Figure 7: HPTCL of Methanolic extract of Phragmites karka.

CONCLUSION

Phytochemical investigations of *Phragmites karka* have revealed a diverse spectrum of biomolecules, which may contribute to its traditional medicinal applications. Various bioassays conducted during the study have identified several biologically active compounds, suggesting the plant's potential therapeutic properties. Bioautography, which was specifically employed to monitorw the wound healing activity of the extract, demonstrated that bioassays can be valuable tools for guiding the isolation of active compounds. Although p-coumaric acid, a compound isolated during the study, could not be definitively identified due to a contaminant, the results indicate the presence of a phenolic derivative with significant biological activity.

This finding is particularly intriguing, as the phenolic compound could potentially represent a novel and unique therapeutic agent, especially for wound healing. Given the promising bioactivity observed, further investigation is needed to accurately identify the specific phenolic compound responsible for the observed effects. Additionally, it is crucial to explore its full range of biological activities, assess its potential uniqueness, and evaluate its safety and efficacy for treating infected wounds. Such efforts could pave the way for the development of new plant-based treatments, contributing to the advancement of wound care therapies.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research.

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