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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

Skin repair is a complex, multi-step biological process that requires close contact among several cell types in a well-synchronized system. Recent research has improved our understanding of the particular intervention of distant stem cells originating from distant tissues like bone marrow to enable cutaneous repair. This information's conclusion will focus briefly on three issues of great concern: scarring, tissue engineering for skin wound healing, and plasma application.

Keywords: Wound healing activity, Phytochemical screening and evaluation, • Phragmites karka (Retz.) Trin.exSteud

Introduction

Skin repair is a complex, multi-step biological process that requires close contact among several cell types in a well-synchronized system. Recent research has improved our understanding of the particular intervention of distant stem cells originating from distant tissues like bone marrow to enable cutaneous repair. These mostly lead to poor repair, which in turn causes persistent ulcers. We selected to concentrate on the dynamics at play in a particularly dire situation of sickle cell disease, as well as mineral corticoid activation, both cause wound healing delays. The following update on skin wound healing focuses on the various stages, informing the reader of current information and fresh ideas. This information's conclusion will focus briefly on three issues of great concern: scarring, tissue engineering for skin wound healing, and plasma application

Wounds

Any abnormality in the natural structure of the skin as well as the loss of connection in body tissue that is characterized as a wound or damage.

Wound Healing

The intricate process of restoring the shapes and capabilities of wounded tissues is known as wound healing.

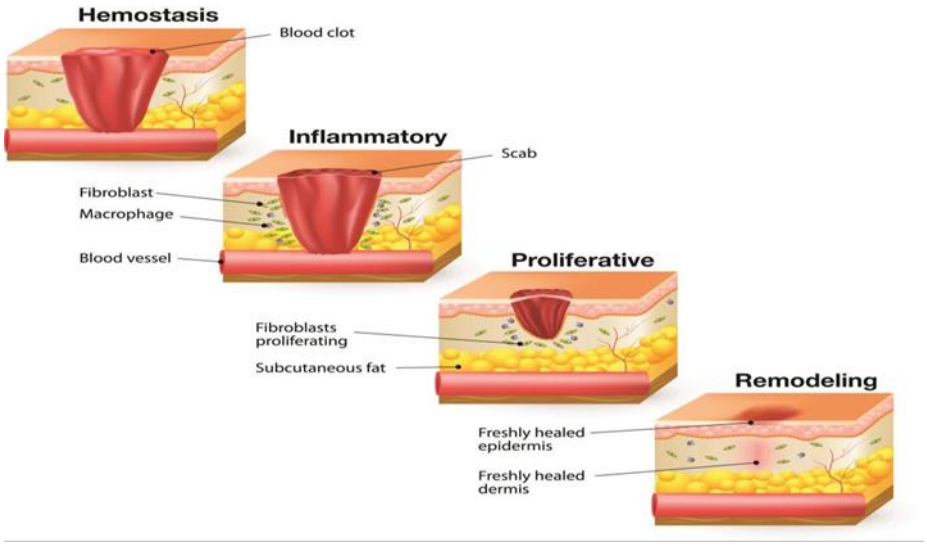

Figure 2: Healing stages for wounds

Generally speaking, the three overlapping phases of remodeling, proliferation, and inflammatory reaction characterize the ordered complex process of wound healing Fig 2.

PLANT PROFILE

Phragmites karka (Retz.)Trin.exSteud

• Synonyms

Phragmites vallatoria, Arundokarka

•Taxonomical classification

Kingdom- Plantae- Plants

Phylum- Angiosperm

Subkingdom-Tracheobionta-Vascular plants Superdivision-Spermatophyta- Seed plants Division - Magnoliophyta -Flowering plants Class-Liliopsida- Monocotyledons

Subclass- Commelinidae

Order-Cyperales Family- Poaceae

Sub-family- Arundinoideae

Genus-Phragmites Species- karka [2] Vernacular names

English: Tall Reed Reed, tropical reed, flute reed, nodding reed

Assamese: Nal, Nalkhagari

Bengali: Nal

Hindi: Narkul, Nal, Doka-ghas, Kilak Kannada: Hulugilahullu Malayalam: Nain-canna, Nadam Manipuri: Tou

Marathi: Nala

Oriya: Potagala

Sanskrit: Dhamana, Nala

Tamil: Perunanal, Nalam

Telugu: Eelakarra, Nagasaramu-peepalu

Figure -3: Plant Phragmites karka

Uses: as an antiemetic, antipyretic, diuretic, febrifuge, sialogogue, stomachic for abscess, arthritis, cough,earache,fever,hematuria,

Phytochemical Constituents :Pentosans, lignins, flavonoids, saponins, triterpenoids, steroids, terpenoids, tannins, and alkaloids have all been reported to be present [6–8]. The highest polyphenol content is found in its leaf, which may be responsible for its purported anti-oxidant activity.

Materials and Methodology

Glasswares and Chemicals

Glassware of good quality had been used, and before that, each piece had been meticulously cleaned, rinsed with distilled water, and immersed in a chromic acid solution. From SD Fine Chemicals Pvt. Ltd. in Mumbai, India, we obtained petroleum ether, ethyl acetate, methanol, sodium carbonate, potassium ferricyanide, DMSO, NaOH, ferric chloride, and trichloroacetic acid. From Sigma Aldrich chemicals Pvt. Ltd, Hyderabad, India, we obtained monosodium iodoacetate, complete freund's adjuvant (CFA), gallic acid, rutin, folin-ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Nitro blue tetrazolium (NBT), and ascorbic acid. The supplier of the indomethacin was Akums Drugs and Pharmaceuticals in India. Hi Media Laboratories Pvt. Ltd. provided all other substances used in this study. (Mumbai, India), Merck Life Sci. Private Ltd. (Mumbai, India), Lobo Chem, Ltd. (Mumbai, India), and SRL Pvt. Ltd.From Beacon Diagnostics, Pvt. Ltd. in Navsari, Gujarat, India, kits for biochemical estimation were purchased. Triple-distilled water was utilized during the whole experiment and was produced on-site. All other chemicals employed in this investigation were purchased commercially and were of analytical grade.

Collection and Authentication of plant material

Processing of plant

Plant material rinsed under flowing water from the faucet and stored for drying in the shade. The dried plant parts were then ground into a powder using a blender, and the color, smell, and texture were checked before being packaged in a labeled airtight container for future usage.

Extraction

In the current investigation, plant material was extracted utilizing the Soxhlet equipment and the continuous hot percolation method. The % yield for each dried extract was calculated using the formula:

Weight of extract

% Yield ---------- □100

Weight of Plant Material used

According to The Ayurvedic Pharmacopoeia of India, prepared extracts were tested for organoleptic characteristics (percentage yield, color, and odor), then placed in airtight containers and labeled for future use

Table4.1: Plant partused for present investigation

S.No.	Botanical name	Partused
1.	*Phragmiteskarka*	leaf

Percentage yield of different extract

yield expressed as a percentage for various extracts. Phragmites karka was extracted via sloxhlation. Phragmites karka extracts in methanol had the highest yield (6.21%). The Phragmites karka ethyl acetate extract has a yield of 3.74%, but the lowest yield of the Phragmites karka petroleum ether extract is 1.86%.

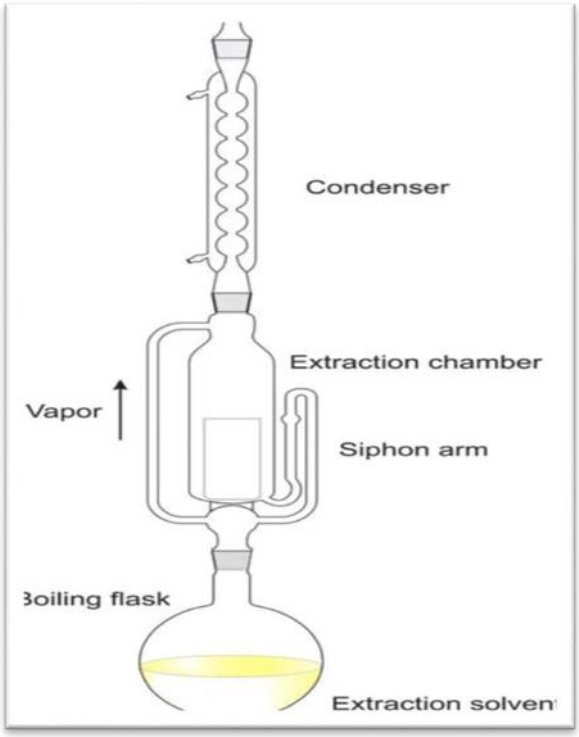

Figure-4: Hot Extraction

Quantitative phytochemical Screening

Phytochemical Quantitative Screening To investigate the presence or absence of different phytochemical elements, extracts were subjected to qualitative phytochemical testing employing protocols. Phragmites karka extracts in petroleum ether, ethyl acetate, and methanol underwent phytochemical testing. Results indicated that extracts included a variety of phytoconstituents. Triterpenoids, steroids, and glycosides were detected in the petroleum ether extract of Phragmites karka according to phytochemical analysis. Triterpenoids, steroids, alkaloids, carbohydrates, reducing sugars, tannins, and phenolic compounds were found in the ethyl acetate extract of Phragmites karka. Phytochemical analysis of the Phragmites karka methanolic extract revealed the presence of proteins, amino acids, alkaloids, reducing sugar, flavonoids, tannins, and phenolic compounds.

screening for phytochemicals in quantity Following a preliminary phytochemical examination of crude extracts, phenolics and flavonoids were discovered in plant material. To determine their concentrations, total phenolic (TPC) and total flavonoid content (TFC) assays were conducted.

Total phenolic contents (TPC)

Using gallic acid as a reference, the total phenolic content of each extract was calculated using the Folin-Ciocalteu method. In terms of gallic acid equivalent weight (GAE), the results were represented in mg. In order to create the Folin-Ciocalteu reagent, phosphotungstic acid and phosphomolybdic acid are combined. When this mixture is used, phenols are oxidized and reduced to a blue tungsten and molybdenum solution that can be detected using a spectrophotometer with an absorption maximum at 750 nm. According to Kamtekar et al. (Block_5) (2014), the blue colour is proportional to the overall amount of phenolics present.It was discovered that the regression coefficient was R2= 0.995. The plot has a 0.002 slope and 0.063 intercept. With gallic acid (20–100 g/ml) used as the standard, the total phenolic content of Phragmites karka extracts was determined using a regression equation based on a standard curve. The maximum phenolic concentration was found in the methanolic extract of at 165.47 0.312 mg GAE/g extract. The lowest amount of GAE/g extract measured was 135.840.113mg for ethyl acetate extract.

Total flavonoid contents (TFC)

Flavonoid content overall (TFC) The majority of plant secondary metabolites come from flavonoids. Better electron-donating characteristics that result in free radical scavenging activity are imparted by the 3, 4'-orthodihydroxy structure in ring B and the carbonyl group at position C4 in ring C. The electron delocalization from ring B caused by the existence of a C2-C3 double bond coupled to the C4 carbonyl in flavonols would tangentially boost the radical-scavenging activity. The quantity and placement of the hydroxyl groups in flavonoids affect their ability to act as antioxidants. (2011) Chua et al. Using quercetin as a reference, the aluminium chloride colorimetric assay was used to calculate the total flavonoid concentration of the extracts. When combined with either the C-3 or C-5 hydroxide group and the C-4 keto group, aluminum chloride generates acid-stable complexes.

In-vitro antioxidant activity

antioxidant activity in vitro Activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in scavenging free radicals An antioxidant assay based on electron transfer, the DPPH (1,1- Diphenyl-2-picrylhydrazyl) free radical approach generates a violet solution in ethanol. In the absence of an antioxidant molecule, this free radical becomes less stable at ambient temperature and produces colorless ethanol solution. The IC50 values for methanol, ethyl acetate, and ascorbic acid, respectively, for the scavenging activity of extracts and the standard on the DPPH radical were 20.15, 114.3, and 13.5. The IC50 value of the methanolic extract was efficient and close to that of the well-known antioxidant ascorbic acid.

RESULTS AND DISCUSSIONS

Collection and Authentication of plant material

Phragmites karka leaves were gathered from the neighborhood in Bhopal, Madhya Pradesh, India.

Table 5.1: Plant material authentication

S.No.	**Plant**	**Part**	**Family**	**Voucher Specimen**
1	*Phragmiteskarka*	Leaves	Poaceae	

yield in percentages for various extracts

Table 5.2: Yield percentages (%) and organoleptic properties of various extracts Extract%Yield of Characters the color odor

Characters	**Extract**	**%Yield**	**Colour**	**Odour**
Phragmiteskarka	Pet. ether	1.86	GreenishBrown	Characteristic
	Ethylacetate	3.74	GreenishBrown	Characteristic
	Methanol	6.21	GreenishBrown	Characteristic

Phytochemical Quantitative Screening

Table 5.3: Phragmites karka ethyl acetate extract phytochemical screening

S.No.	**Experiment**	**Result**
		Pet. etherextract
1.Alkaloids		
1.1	Mayer'stest	-ve
1.2	Wagner'stest	-ve
2.Carbohydrates		
2.1	Molish'stest	-ve
2.2	Barfoed'stest	+ve
3. TestforReducingSugar's		
3.1	Fehling'stest	-ve
3.2	Benedict'stest	-ve
4. Flavonoids		
4.1	Alkalinereagent test	-ve
4.2	Shinodatest	-ve
5. Glycoside		
5.1	Borntragertest	+ve
5.2	Killer-Killianitest	+ve
6. TanninandPhenoliccompound		
6.1	Ferricchloridetest	-ve
6.2	LeadAcetatetest	-ve
7.Saponin		
7.1	FaomTest	-ve

Table 5.4: Phragmites karka ethyl acetate extract phytochemical screening

S.No.	Experiment	Result
		Ethylacetateextract
1.Alkaloids		
1.1	Mayer'sreagenttest	+ve
1.2	Wagner'sreagenttest	+ve
1.3	Hager'sreagenttest	+ve
2.Carbohydrates		
2.1	Molish'stest	+ve
2.2	Barfoed'stest	+ve
3. TestforReducingSugar's		
3.1	Fehling'stest	+ve
3.2	Benedict'stest	+ve
4. Flavonoids		
4.1	Alkalinereagent test	-ve
4.2	Shinodatest	-ve
4.3	Leadacetatetest	-ve
5. Glycoside		
5.1	Borntragertest	-ve
5.2	Legal'stest	-ve
5.3	Killer-Killianitest	-ve
6. TanninandPhenoliccompound		
6.1	Ferricchloridetest	+ve
6.2	LeadAcetatetest	-ve
6.3	DiluteIodinesolution	+ve
7.Saponin		
7.1	FaomTest	-ve
8.Test forProteinsandamino acid		

8.1	Ninhydrintest	-ve
9.Test forTriterpenoidsandSteroids		
9.1	SalwonskiTest	+ve
9.2	Libberman-Burchard'stest	+ve

+ve:Present;**–ve**:Absent

Quantitative phytochemical screening

I Total phenolic contents (TPC)

Table 5.6 Standard curve of gallic acid

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

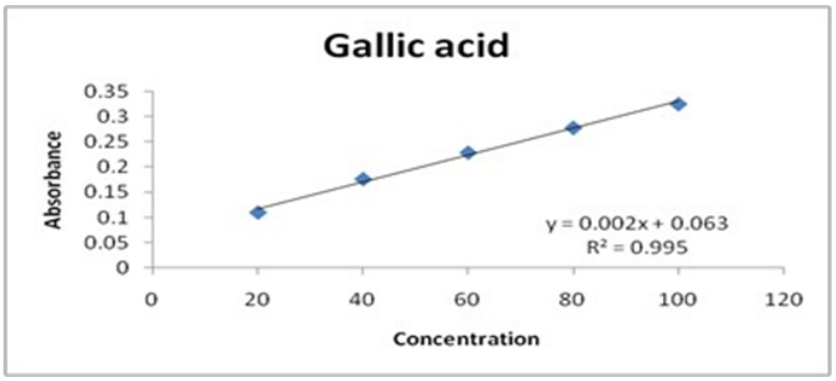

Figure:6 standard curve of Gallic acid

Table 5.7: Total phenolic content in ethyl acetate extract of Phragmites karka

S.No.	**Absorbance**	**Concentration**	**Totalphenoliccontentinmg/g equivalent ofgallicacid**
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 5.8: Total phenolic content in methanolic extract of Phragmites karka

S. No.	Absorbance	Concentration	Totalphenoliccontentinmg/g equivalent ofgallicacid
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**

II. Total flavonoid contents (TFC)

Table 5.9: 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

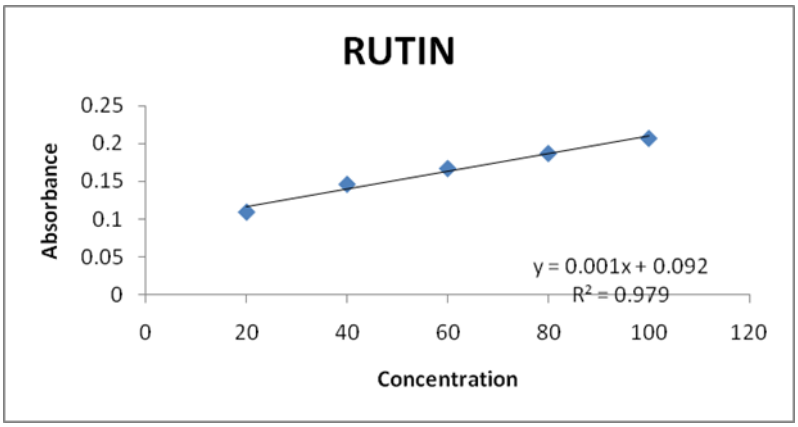

Figure:7 standard curve of rutin

Table 5.10: Total flavonoid content in ethyl acetate extract of Phragmites karka

S.No.	Absorbance	Concentration	Totalflavonoid content inmg/g equivalentofrutin
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 5.11: Total flavonoid content in methanolic extract of Phragmites karka

S.No.	**Absorbance**	**Concentration**	**Totalflavonoid content inmg/g equivalentofrutin**
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

Table 5.12: DPPH radical scavenging activity of standard ascorbic acid, ethyl acetate and methanolic extract

		Ascorbicacid (Std.)		**Methanolicextract**		**Ethylacetate 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
IC50		**13.5**		**20.15**		**114.3**	

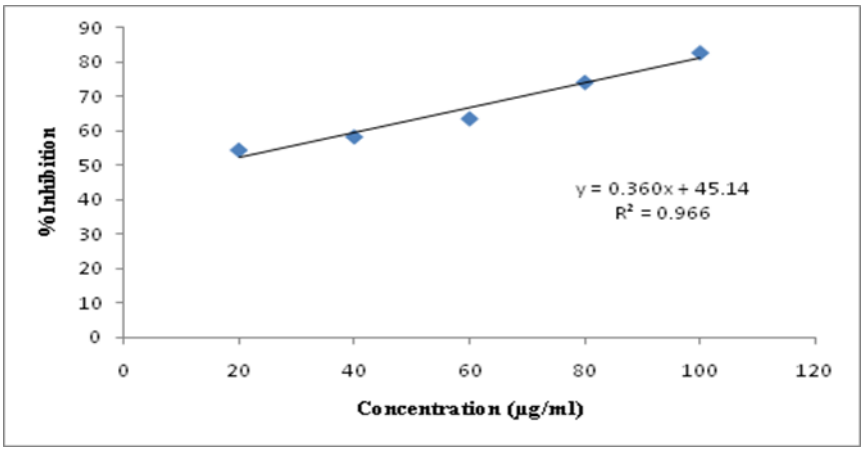

Figure:8 DPPH assay of ascorbic acid

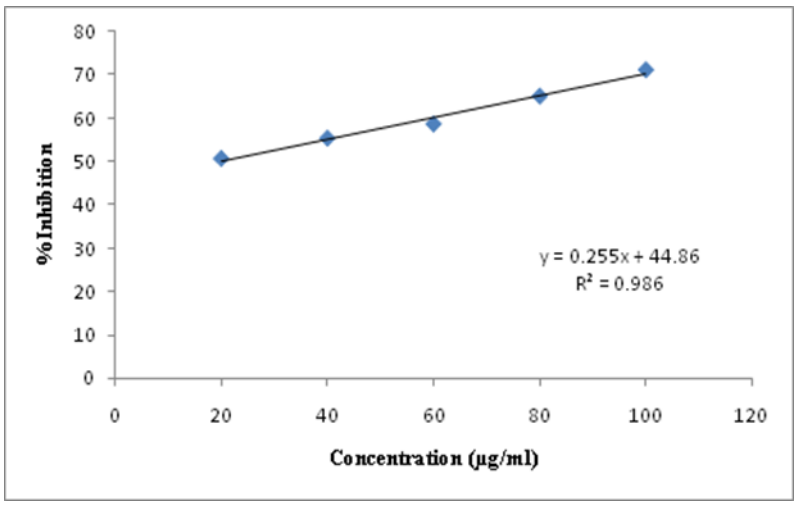

Figure:9 DPPH assay of methanolic extract

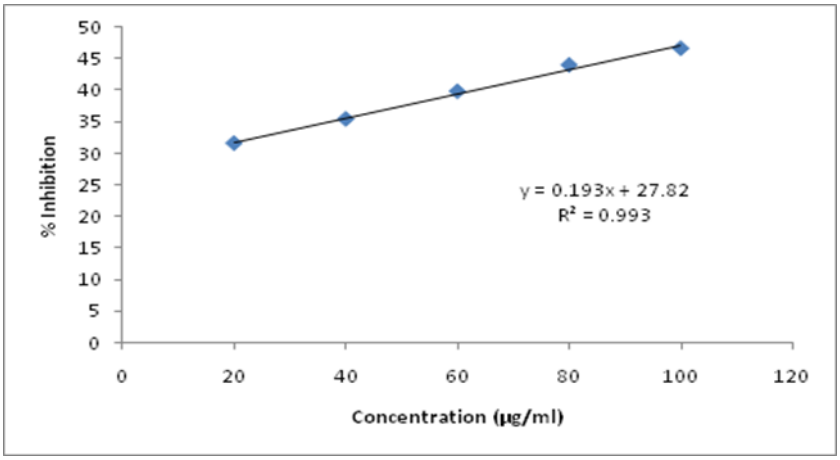

Figure 10 DPPH assay of ethyl acetate

Wound healing activity

Table 5.13: Nitrofurazone (0.2%w/w) and Phragmites karka extract ointment (5% and 10% w/w) are evaluated. Ointment used in rats for excision wound healing Post wounding (days) Wound area (mm2) (mean±S.E.) and percentage of wound contraction.

Post-wounding(days)	Woundarea(mm^2)(mean±S.E.)andpercentageofwoundcontraction			
	Simple ointment(control)	Standard ointment(0.2%,w/w)	Extract ointment(5%,w/w)	Extractointment(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 were statistically significant compared with the corresponding control values(simpleointment)*P<0.01,**P<0.001

HPTLC analysis

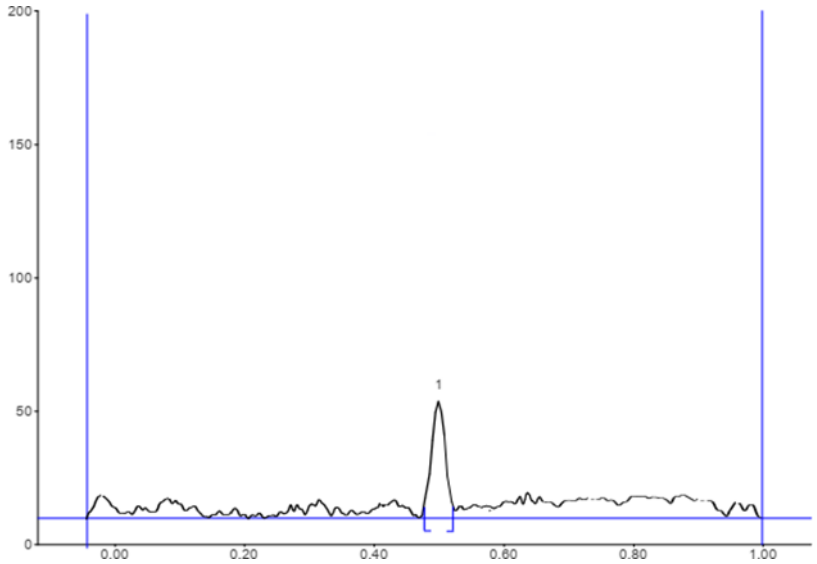

Figure:11 HPTLC of pure p-Coumaric acid

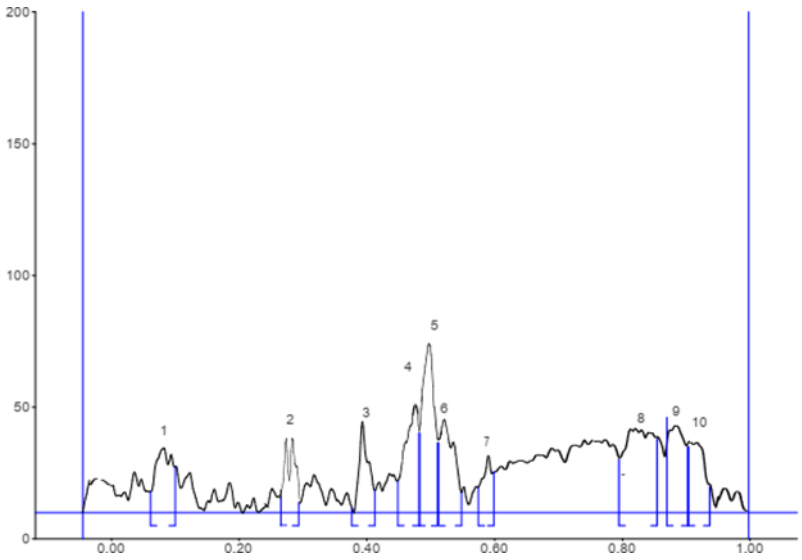

Figure:12 HPTCL of Methanolic extract of Phragmites karka

Conclusion

The Phragmites karka plants have undergone phytochemical analyses that have revealed a wide range of biomolecules that may be the reason for its usage in traditional medicine. Different biologically active chemicals were discovered using various bioassays. This demonstrates that bioassays can be utilized as a guide during isolation because bioautography was employed to track the extract's capacity to speed up the healing of wounds.

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