Phytochemical Characterization and Scratch Assay of Pergularia daemia for Therapeutic Applications- A Scientific Approach

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Abstract:
The present study evaluates the phytochemical composition and antioxidant potential of the ethanolic extract of Pergularia daemia, a medicinal plant widely used in traditional systems. Pergularia daemia (Forssk.) Chiov. exhibits dual ecological and socio-economic roles, functioning both as a valued ethnomedicinal plant and an invasive weed across tropical and subtropical. Preliminary phytochemical screening confirmed the presence of bioactive constituents such as phenolics, flavonoids, alkaloids, and terpenoids, with quantitative analysis revealing high levels of total phenolics (131.30±6.2) and flavonoids (57.59±0.72), indicating strong antioxidant relevance. Physicochemical and chromatographic analyses further supported the quality and purity of the plant material. Antioxidant activity was assessed using DPPH radical scavenging and superoxide anion scavenging assays, with ascorbic acid as the reference standard. The extract exhibited a concentration-dependent increase in radical scavenging activity in both assays. In the DPPH assay, the extract showed moderate antioxidant activity with an IC_{50} value of approximately 197 µg/mL, compared to the lower IC_{50} of ascorbic acid, indicating higher potency of the standard. However, at higher concentrations, the extract demonstrated substantial inhibition (73.09±1.22%), approaching the activity of the standard, suggesting strong total antioxidant capacity despite lower potency. Similar trends were observed in the superoxide scavenging assay. The observed antioxidant activity can be attributed to the presence of phenolic and flavonoid compounds, known for their electron-donating and free radical neutralizing abilities. The Anti-Inflammatory activity was evaluated by scratch assay (in-vitro). The extract shows 92.15% of wound closure in 48 hours. Overall, the findings suggest that Pergularia daemia possesses significant antioxidant potential and wound healing activity. It may serve as a promising natural source of bioactive compounds for therapeutic applications, although further studies are required to isolate and characterize the active constituents.

Keywords : Pergularia daemia, Phytochemicals, Antioxidant activity, DPPH assay, Superoxide radical scavenging, Scratch Assay

Introduction

Recent attention shows that herbal medicines are seen as safe options with few side effects. A 2002 report from the World Health Organization (WHO) says that 80% of people around the world use plant-based treatments for different health problems. Now, there is a big increase in demand for herbal products globally, which has led major drug companies to do more research on substances from plants for their healing abilities[1]. This means it's very important to make sure these products are safe and work well.

Pergularia daemia, also called Dudhilata, is a type of climbing plant that grows in the Asclepiadaceae family. It is found in tropical Africa and is common in many parts of India. This plant has thin stems, leaves that grow in pairs, and greenish-yellow flowers, and it grows well in dry and semi-dry areas. Traditionally, it has been used to help increase milk production, act as a diuretic, and reduce inflammation. The leaves and roots are used in traditional medicine to treat breathing problems, joint pain, and skin issues. Extracts from this plant are also used in beauty products and animal medicines because they have antioxidant and antibacterial properties. In India, the AYUSH department, which looks after Ayurveda, Yoga, Unani, Siddha, and Homeopathy, helps control the quality of herbal products through the Drugs and Cosmetics Act.

Materials and Methods

1.Plant material

Pergularia daemia is propagated primarily via seeds (soaked 6–12 h, sown at 1–1.5 cm depth; germination in 10–20 days) or semi-hardwood cuttings (15–20 cm, rooted in 3–4 weeks), thriving in well-drained sandy loam/red soil (pH 7.0–8.0) under full sun (25–35°C). Transplant 20–30-day seedlings at 1 × 1 m spacing with pandals for climbing; irrigate every 7–10 days, weed monthly, and mulch for moisture retention. Harvest leaves from 2–3 months (2–3 t fresh/acre/year) or roots after 1 year (~500–700 kg dried/acre), with optimal activity in Jan–Sep cycle. The identity of the collected leaves was identified by Dr.K.N.Sunil Kumar (Research officer- Department of Pharmacognosy, Siddha) and Dr. Elankani (Research officer-Siddha) P10052401D, dated 21.05.2024) of the

test drug has been retained and deposited for the future reference in the department of Pharmacognosy, B.S AbdulRahman University, Crescent college, Vandalur,Chennai.

P10052401D

Fig No.1 Leaves of Pergularia daemia

2. Phytochemical and Physico-chemical investigations:

The plant material of P. daemia was pulvierised into a coarse powder using a machine grinder and then passed through a sieve with 60 mesh holes to create a fine, uniform powder[2]. This powder was then checked to ensure it met standards for purity and strength. The dried plant was first examined visually, and then key chemical and physical properties were tested. To measure how much extract could be obtained, different methods like cold, hot, and repeated extraction were used by using various solvent from polar to non polar region. Standard procedures were used to find out the levels of total ash, ash that dissolves in water, and ash that does not dissolve in acid. For fluorescence testing, the powder was mixed with different chemicals and then looked at under normal light and under ultraviolet light at 254 nm and 366 nm. A basic check for plant chemicals found the presence of several important compounds like alkaloids, flavonoids, tannins, saponins, and proteins. All the tests followed methods that are recognized in standard drug references and scientific papers. The percentage yield of extract was tabulated in Table no.1. Based on the percentage yield, high yielding extract was selected for phytochemical screening and pharmacognostical evaluation parameters[3].

Test for tannin[4]

(i) Ferric chloride reagent: A 5% w/v arrangement of ferric chloride in 90% liquor is arranged. Few drops of this arrangement is included to the small of the over filtrate. Dim green or profound blue color shows the nearness of tannins.

(ii) Lead acetic acid derivation test : A 10% w/v arrangement of essential lead acetic acid derivation, broken up in refined water, was included to the test filtrate. If accelerate is gotten, tannins are present.

(iii) Potassium dichromate test : If on expansion of a arrangement of potassium dichromate in a test filtrate, dull color is created, tannins are present.

Test for flavanoid

(i) Shinoda test : A little amount to test buildup is broken up in 5 ml ethanol (95%v/v) and responded with few drops of concentrated hydrochloric corrosive and 0.5 gm of magnesium metal. The pink, blood red or maroon color is created inside a miniature or two if flavonoids are present.

Test for protein

(i) Biuret test : A few mg of the buildup is taken in water, and 1 ml of 4% sodium hydroxide arrangement is included in it. A drop of 1% arrangement of copper sulfate is taken after this. Violet or pink color is shaped if proteins are shown.

(ii) Xanthoproteic test: A small buildup is taken with 2 ml of water, and 0.5 ml of concentrated nitric corrosive is included to it. Yellow color is gotten if proteins are present.

Test for alkaloid

(i)Mayer's Reagent (Potassium mercuri-iodide solution) : It gives a pale yellow accelerate with the test residue.

(ii)Wagner's Reagent (Iodine solution) : It gives a brown or ruddy brown accelerate with the test residue.

(iii) Hager's Reagent (a soaked arrangement of picric corrosive in cold water) :It gives characteristic crystalline accelerate with the test residue. The Results were tabulated at Table No.2

Quantitative estimations of add up to phenol, tannins, alkaloids, basic oil, settled oil, and glycoside were carried out as follows:

Total phenol[5]: For the extraction of the phenolic component, the fat-free test was bubbled with 50 ml of ether for 15 min. 5 ml of the extricate was pipette into a 50 ml carafe, at that point 10 ml of refined water was included. 2 ml of ammonium hydroxide arrangement and 5 ml of concentrated amyl liquor were too included.

The tests were made up to stamp and cleared out to respond for 30 min for color improvement. The absorbance of the arrangement was examined utilizing a spectrophotometer at 505 nm wavelengths. **The result was tabulated at Table No.3**

Total tannins: 500 mg of the test was weighed into 100 ml plastic bottle. 50 ml of refined water was included and shaken for 1 h in a mechanical shaker. This was sifted into a 50 ml volumetric carafe and made up to the stamp. At that point, 5 ml of the filtrate was pipette out into a tube and blended with 3 ml of 0.1 M FeCl 3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, inside 10 min. A clear test was arranged, and the color moreover created and examined at the same wavelength. A standard was arranged utilizing tannin corrosive to get 100 ppm and was measured.**The result was tabulated at Table No.3**

Total Alkaloids : 5 g of the powdered test material was accurately weighed into a 250 mL conical flask, and 200 mL of 20% acidic ethanol (ethanolic acid) was added. The mixture was stoppered and allowed to stand for 4 hours with occasional shaking. The solution was then filtered, and the filtrate was concentrated to approximately one-quarter of its original volume on a water bath. Concentrated ammonium hydroxide was added dropwise to the concentrated extract until precipitation was complete. The mixture was allowed to stand for complete settling, and the precipitated alkaloids were collected by filtration, washed, dried, and weighed. **The result was tabulated at Table No.3**

Total Phenolic compounds : Total essential oil was obtained by hydro- distillation of 100 g of the powdered plant material for 3 hours using a Clevenger-type apparatus. The distillate was collected and the essential oil was separated, extracted with ethanol, dried over anhydrous sodium sulfate, and the yield was determined gravimetrically. **The result was tabulated at Table No.3**

3.PHYSIOCHEMICAL EVALUATION[6]

Loss on drying (LOD)

Moisture content of crude drug determines its chemical and microbial stability, as presence of high-water content in crude drugs favors microbial growth and enzymatic destruction of active principles. Therefore, it becomes necessary to set limits for water content of crude drug especially for those materials which absorb moisture easily or deteriorate quickly in the presence of water.

Procedure

Place 2gm of sample drug in a tared China dish. Dry in oven at 105^{o}C for one hour, cool in a desiccators and weigh. Repeat the procedure till the two consecutive readings are same. The moisture content is expressed in percentage with reference to the air-dried drug.

Total ash

Weigh accurately 2-3 gm of the air-dried crude drug in tared platinum or silica dish and incinerate at a temperature not exceeding 450^{o}C until free from carbon, cool and weigh. If carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on ashless filter paper, incinerate the residue and filter paper, until the ash turns white or nearly so, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450^{o}C. Calculate the percentage of the ash with reference to the air-dried drug.

Acid insoluble ash[7]

Boil the total ash with 25ml of 2N HCl for 5min, collect the insoluble matter on an ashless filter paper, wash with hot water, ignite, cool in desiccator and weigh. Calculate the percentage of acid insoluble ash with reference to air dried drug.

Extractive value

Extractive value gives an idea about number of active principles present in plant drug when extracted with solvents. Different solvents like ethyl alcohol, chloroform, water, and diethyl ether are used to determine the extractive value but the amount of phytoconstituents in the solvents depend upon the chemical nature of drug and solvent. This method is employed for those materials for which no chemical or biological assay method exist. It is used for the evaluation of crude drug.

Procedure

To a 5gm of air dried coarsely powdered drug add 100 ml of solvent (90% of alcohol in case alcohol soluble extractive value, similarly ether and chloroform water for ether and water-soluble extractive values respectively) in stoppered flask for 24hrs, shaking frequently for first 6hrs and allowing to stand for 18hrs. After 24 hours, filter rapidly. Take 25ml of filtrate and evaporate to dryness in a tared flat bottomed shallow dish at 100^{o}C and weigh. Calculate the percentage extractive value with reference to the air-dried drug. **The result was tabulated at Table**

4.CHROMATOGRAPHIC STUDIES[8]

THIN LAYER CHROMATOGRAPHY

TLC is an inexpensive technique that has been reported for a wide variety of analytical applications. It is an important analytical tool, not only for the quality control of medicinal plants but also for the analysis of counterfeit

drugs. In the monographies of several pharmacopoeias the identification of great variety of plants is made using TLC methods, furthermore this technique is valuable for the qualitative, semiquantitative and quantitative analysis of this type of products.

Rf values

The behavior of an individual compound in TLC is characterized by a quantity known as Rf and is expressed as a decimal fraction. The Rf is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position.

Rf = Distance of centre of spot from starting point

Distance of solvent front from starting point

The Rf value is constant for each component only under identical experimental condition. **The result was tabulated at Table No.5**

5. Fluorescence Analysis :

A small quantity (1 gm) of dried and finely powdered leaves was treated with freshly prepared acids, alkaline solutions, and different solvents. The drug powders were treated with acids (10% HCl, Conc. HCl, Conc. H2 SO4, and Conc. HNO3), alkaline solutions (1 N aqueous NaOH, 1 N alcoholic NaOH, and 5% other chemicals (5% iodine, 5% FeCl3, and acetone), and distilled water. They were subjected to study the fluorescence analysis in visible light and in short UV light (254 nm) and day light [9].**The result was tabulated at Table No.6**

6. Spectral Analysis[10]:

LC- HRMS:

Sample preparation:

The ethanolic Crude plant fraction was filtered using 0.22um Nylon membrane filter. The filtered fraction was subjected to LC-HRMS in IIT Madras, Chennai.

Instrumentation and Procedure:

LC- HRMS was used to determine the chemical components (Shimadzu N Series UPLC system coupled with XevoG2 XS QTOF spectrometer). The HPLC was linked to a mass spectrometer equipped with an electro spray ionization (ESI) source via a Q-TOF (quadrapole time of flight) interface. For analysis, C18 column was employed. A total flow rate of 0.3 mL/ min was used to supply the solvents. The extracts were injected into the analytical column in a volume of 20 µL for analysis. Full scan mode was used from 150 to 1000 m/z with a source temperature of 250 -0 C. The MS spectra were obtained in positive ion mode, with the drying gas at 3000 C, a gas flow rate of 13 mL/min, a nebulizing pressure of 35 psi, and a total run period of 30 minutes. The spectrum database for organic molecules was used to identify the mass fragmentation of the ion chromatogram. The identified Compounds were listed as given, constituting relative area, abundance and Retention Time. The report were shown at Fig No.2

7. ANTI-OXIDANT ACTIVITY[11]

DPPH ASSAY

The ability of the plant extract to scavenge 1,1- diphenyl-2-picryhydrazyl (DPPH) free radicals was assessed by the standard method [16]. 0.3mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 1ml of various concentrations of sample and the reference compound (10, 20, 30, 40 and 50 µg/ml), were shaken vigorously and left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm. A control reaction was carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Quercetin was used as standard. Antiradical activity was expressed as inhibition percentage (I %) and calculated using the following equation:

Inhibition percentage (I %) = (Abs control - Abs sample/Abscontrol) X 100

- The results were tabulated at Table no.7 and Fig.no 3

8. Scratch Assay:

The samples were tested for wound-healing activity by using the wound scratch assay [17]. L929 cells were collected from NCTC clone 929 seeded in 12-well plates (4 × 104 cells/well) and cultured until a monolayer confluence was reached. After the adhesion of the cells, the medium was removed from the wells and the cell monolayer was scraped in a straight central line using a p200 micropipette tip, creating a scratch, with reference points being marked in the plates. The wells were washed with PBS to remove floating cells and cell debris. Then, the PBS was removed, the samples were prepared in RPMI-1640 and sonicated, then they were added to the wells. Supplemented RPMI-1640 culture medium was added to the control wells. After this, the plates were placed under a phase-contrast microscope and images were acquired at the initial moment (t = 0 h). Then, the plates were incubated at 37 °C (5% CO2) and examined once again under the microscope after 2, 4, and 24 hr.

The size of the scratch zones was assessed manually using a digital image analysis tool (IC Measure software version 2.0.0.161) (The Imaging Source, Germany) that allowed the estimation of the distance between the injury margins. Using the IC Measure, the distance between the margins of the lesion in the control at 0 h was estimated, which was considered the initial one and was used to scale all other measurements to more easily compare the

estimated distances of the injuries between the samples and the control. The results were tabulated at Table No.8 and Fig. No 4

Results

Table No.1 Percentage Yield of Extract

Extract	% Yield w/w	Extract colour
Petroleum ether	1.11	Weak brownish yellow
Chloroform	4.9	Brownish yellow
Ethyl acetate	3.7	Yellowish brown
Ethanol	10.1	Reddish brown
Water	9.3	Brownish black

Table No.2 Phytochemical Investigation of Ethanolic Extract of Pergularia daemia

Test	Leave Extract	Stem Extract	Fruit Extract	Root Extract	Flower Extract	Whole Plant Extract
Phenolics	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Reducing Sugars	+	-	+	-	-	+
Saponin	+	-	+	+	-	+
Steroids	+	+	+	-	+	+
Terpenoids	+	+	+	+	+	+

Table No.3 Quantitative Estimation of Ethanolic extract of Pergularia daemia

S.No	Phytochemicals	Whole Plant
1.	Flavonoids	57.59±0.72
2.	Phenolics	131.30±6.2
3.	Alkaloids	31.03 ±0.61
4.	Terpenoids	73.63 ±0.90

Table No.4 Physicochemical analysis Pergularia daemia

Parameters*	Mean (w/w) ± SEM (n = 3)
Moisture content	5.01 ± 0.15
Total ash	5.01 ± 0.37
Acid insoluble ash	0.996 ± 0.51
Water soluble ash	4.06 ± 0.09

Water soluble extract	9.03 ± 0.04
Alcohol soluble extract	9.52 ± 0.06

Table No.5 Thin Layer Chromatography (TLC) Analysis Report of Ethanolic extract of Pergularia daemia

S.No	Solvent System	Ratio	Long UV 365 nm	Short UV 254 nm	Rf Value
1	Chloroform : Methanol : Benzene	9 : 0.5 : 0.5			0.4
2	Toluene : Ethyl acetate	9 : 1			0.3
3	Benzene : Ethyl acetate	7 : 3			0.8

Table No.6 Fluorescene Analysis Report of Pergularia daemia

Treatment	Under daylight	Under UV light
Whole plant powder	Yellowish brown	Light brown
Powder + 1N NaoH in methanol	Yellowish brown	Pale yellow
Powder + 1N NaoH in water	Yellowish brown	Reddish yellow

Powder + 1 N Hcl	Pale yellow	Pale yellow
Powder + 50% HNO3	Reddish yellow	Red
Powder + 50% H2SO4	Yellowish green	Yellowish green

LC-HRMS - Comprehensive screening of phytochemicals of Ethanolic Extract of Pergularia daemia

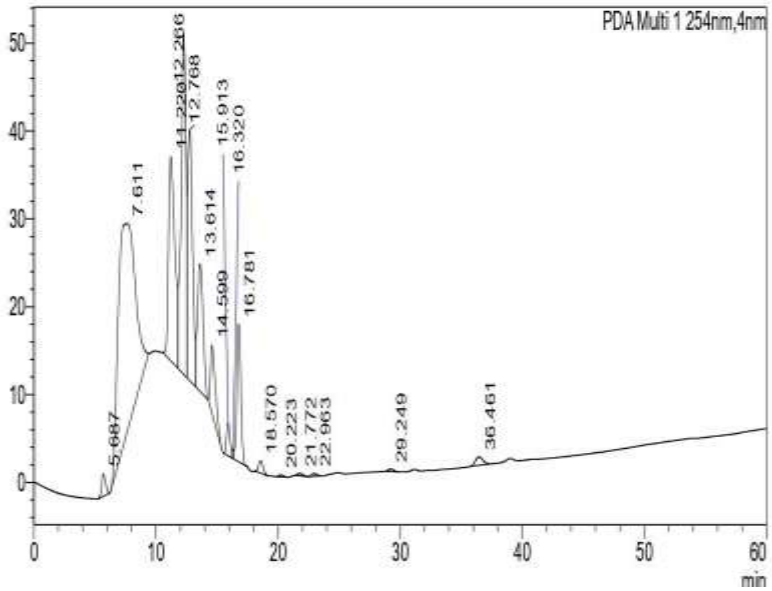


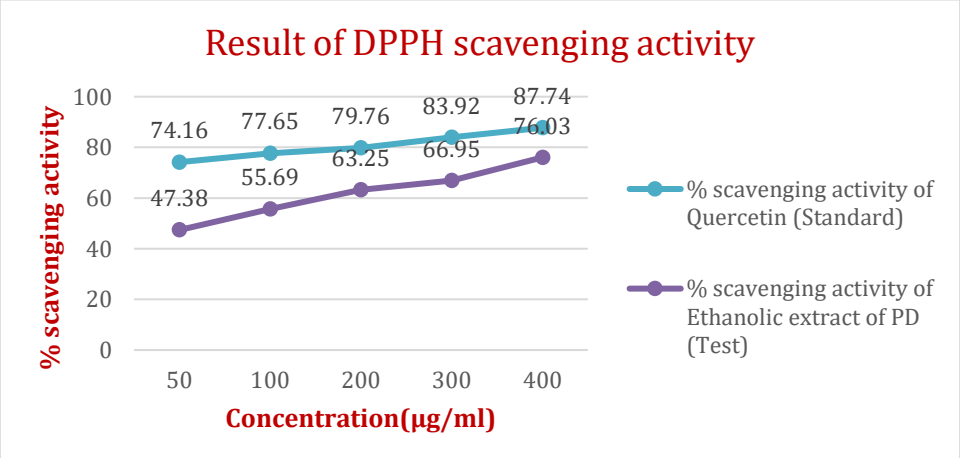
Fig No.2 LC-HRMS Ethanolic Extract of Pergularia daemia

Table No.7 Effect of Anti-oxidant Activity of Ethanolic extract of Pergularia daemia by DPPH method:

S.No	Concentration (µg/ml)	% scavenging activity of Quercetin (Standard)	% scavenging activity of Ethanolic extract of Pergularia daemia (PD)
1.	50	74..16± 0.005	47.38± 0.037
2.	100	77.65± 0.051	55.69± 0.752
3.	200	79.76± 0.057	63.25± 0.275
4.	300	83.92± 0.016	66.95± 0.101
5.	400	87.74± 0.064	76.03± 0.220

IC_{50} (Pergularia daemia extract) ≈ 65.8 µg/mL
IC_{50} (Quercetin) ≈ 33.7 µg/mL

Fig No3. Anti-oxidant Activity of Ethanolic extract of Pergularia daemia by DPPH method comparison with Quercetin

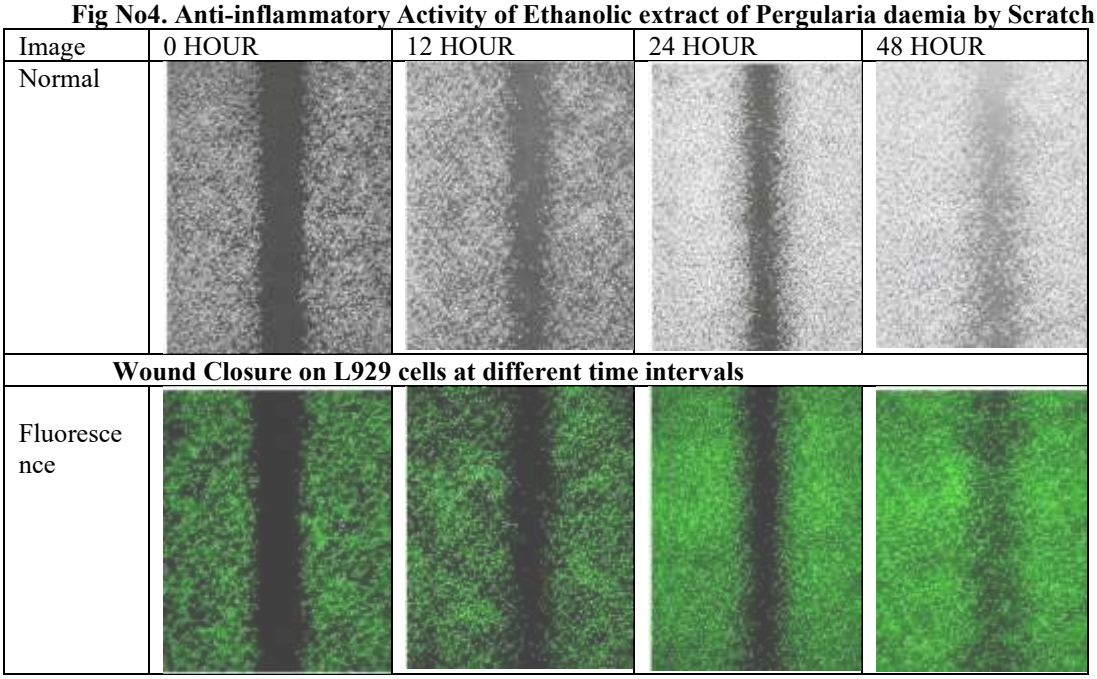


One-way ANOVA revealed that increasing concentrations significantly enhanced DPPH scavenging activity in both quercetin and Pergularia daemia extract ($P < 0.05$). The effect was concentration-dependent, with maximum activity observed at 400 µg/mL.

Table No.8 Effect of Anti-inflammatory Activity of Ethanolic extract of Pergularia daemia by Scratch Assay:

S.No.	Time in hour(s)	% of Wound Closure
1	0	0
2	12	62.36
3	24	75.14
4	48	92.15

Fig No4. Anti-inflammatory Activity of Ethanolic extract of Pergularia daemia by Scratch Assay



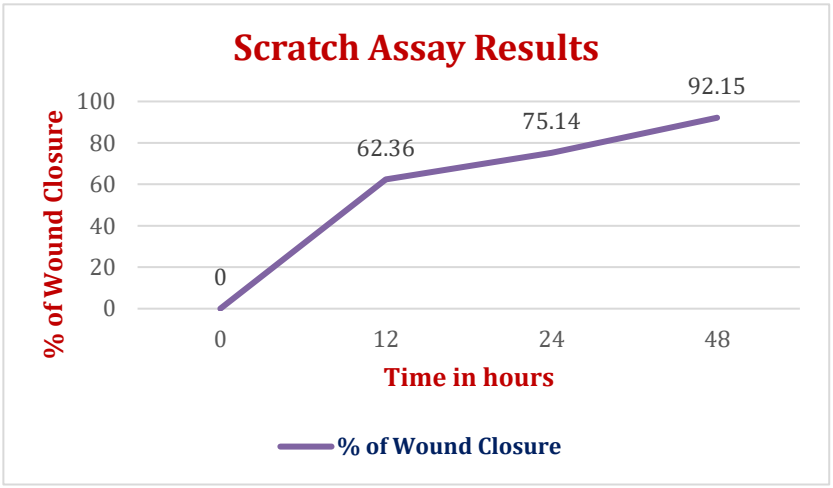


Fig No5. Anti-inflammatory Activity of Ethanolic extract of Pergularia daemia by Scratch Assay

Discussion

The present investigation demonstrates that the ethanolic extract of Pergularia daemia possesses significant phytochemical constituents and notable antioxidant activity[13]. Preliminary phytochemical screening confirmed the presence of phenolics, flavonoids, alkaloids, and terpenoids, which are well-known for their therapeutic and free radical scavenging properties. Quantitative estimation revealed a high content of phenolic (131.30±6.2) and flavonoid compounds (57.59±0.72), which strongly correlates with the observed antioxidant potential. These compounds are recognized for their ability to donate hydrogen atoms or electrons, thereby stabilizing free radicals and preventing oxidative damage[14].

The physicochemical parameters such as moisture content, total ash, and extractive values were within acceptable limits, indicating good quality, purity, and stability of the crude drug[15]. Chromatographic analysis further supported the presence of multiple phytoconstituents, confirming the chemical complexity of the extract.

The chromatogram exhibits multiple well-resolved peaks, indicating the presence of several bioactive compounds. Major peaks are observed around at Rt ~7.6 min,Rt ~12–13 min (cluster of intense peaks) ,Rt ~15–17 min .These peaks with higher intensity suggest the presence of abundant phytoconstituents. The clustering of peaks between 10–17 minutes typically indicates compounds of moderate polarity, commonly might be Phenolic compounds and Flavonoids[16]. The LC–HRMS chromatogram confirms that Pergularia daemia contains a complex mixture of bioactive phytochemicals ,The dominant peaks correspond to compounds likely responsible for antioxidant

activity . Moderate IC_{50} value and Strong activity at higher concentrations. The LC–HRMS analysis of Pergularia daemia ethanolic extract revealed a diverse array of phytoconstituents with distinct retention times, indicating the presence of multiple bioactive compounds. The prominent peaks observed in the mid-retention region suggest the abundance of phenolic and flavonoid compounds, which are known contributors to antioxidant activity. This chromatographic profile supports the phytochemical findings and correlates well with the observed free radical scavenging potential, highlighting the therapeutic significance of the plant[17].

Ethanolic extract of Pergularia daemia (PD) significantly scavenged the DPPH radical and the result is given in Table-1. The 1, 1- diphenyl -2-picryl hydroxyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as extract of plants in a relatively short time. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of the extract and the standard quercetin as a reference compound. The radical scavenging activity of Ethanolic extract of PD was evident at all the concentrations but only at moderate level not as significant as that of standard quercetin. The scavenging activity of the PD was increased with increase in concentration of extract and that of the standard. PD showed the % scavenging activity by 76.03 at 400 µg/ml when compared to quercetin showed 87.74 at 400 µg/ml.[18]

The wound healing (scratch) assay performed on L929 cells demonstrated a clear time-dependent increase in wound closure. At 0 h, an initial scratch with 0% closure was observed, indicating a complete cell-free gap. Progressive cell migration into the wound area was evident at subsequent time points. At 12 h, partial closure of the wound was observed, with cells beginning to migrate toward the scratched region. By 24 h, a substantial increase in cell density within the wound area indicated active migration and proliferation. At 48 h, near-complete wound closure (92.15%) was achieved, suggesting efficient cellular repair and coverage of the scratched region. Both phase-contrast and fluorescence images corroborated these findings, showing increased cell presence and density within the wound gap over time. Fluorescence imaging further confirmed enhanced cellular activity and distribution across the wound area. Statistical analysis using one-way ANOVA (assuming $n = 3$) indicated that the increase in wound closure over time was statistically significant ($P < 0.05$), confirming a strong time-dependent effect on cell migration.

The comparatively lower potency of the extract may be attributed to the complex mixture of phytochemicals present, as opposed to the pure compound nature of ascorbic acid. Nevertheless, the synergistic effect of multiple bioactive compounds may contribute to its overall efficacy[19].

Summary

The study successfully established the phytochemical profile and antioxidant potential of Pergularia daemia ethanolic extract. The presence of bioactive compounds such as phenolics and flavonoids were confirmed through qualitative and quantitative analyses. Physicochemical and chromatographic evaluations indicated the quality and authenticity of the plant material. The LC–HRMS chromatogram of Pergularia daemia reveals multiple well-resolved peaks, indicating a complex mixture of bioactive compounds. Prominent peaks in the mid-retention region (10–17 min) suggest the presence of phenolics and flavonoids. These compounds are likely responsible for the observed antioxidant activity. The results support phytochemical findings and confirm the plant's potential as a natural antioxidant source. The extract exhibited significant antioxidant activity in both DPPH and superoxide radical scavenging assays, with a concentration-dependent increase in inhibition. Although the IC_{50} value was higher than that of ascorbic acid, the extract demonstrated considerable radical scavenging activity at higher concentrations, supporting its potential as a natural antioxidant source. At 48 h, near-complete wound closure (92.15%) was achieved, suggesting efficient cellular repair and coverage of the scratched region.

Conclusion

In conclusion, the ethanolic extract of Pergularia daemia exhibits appreciable antioxidant activity, which can be attributed to its rich phytochemical composition, particularly phenolic and flavonoid compounds. Despite showing lower potency compared to the standard ascorbic acid, the extract demonstrated strong free radical scavenging capacity at elevated concentrations. These findings highlight the potential of Pergularia daemia as a promising natural antioxidant and anti-inflammatory for therapeutic applications and possess wound healing activity which was confirmed by scratch assay. Further studies involving isolation, characterization, and in vivo evaluation of active constituents are recommended to fully explore its pharmacological potential.

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