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Bioactivities of *Psychotria* sp. ethanol extract from Gia Lai, Vietnam: Antioxidant, antibacterial, anti-inflammatory, and wound-healing activities

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Abstract. A newly discovered species of the genus *Psychotria*, recently identified in Vietnam, is a traditional medicinal herb widely used in the country. However, the biological activity of this species has not been specifically investigated. This study analysed the compounds, evaluated the antioxidant, antibacterial, anti-inflammatory, and wound-healing potential, and cytotoxic activities of *Psychotria* sp. extract (PRE). The compound analysis shows that PRE has 7 compounds with antibacterial properties, 5 compounds with anti-inflammatory properties, and 6 compounds with antioxidant properties. The antioxidant capacity is powerful with IC_{50} of 19.32 μg/mL and 48.11 μg/mL for ABTS and DPPH, comparable with vitamin C (15 μg/mL and 41.07 μg/mL). The average antibacterial activity, as indicated by MIC concentration, is 12.5 mg/mL in *E. coli* and *S. pyogenes*. The cytotoxicity survey shows that PRE does not cause significant toxicity on the 3T3 cell line, confirming the initial biological safety of the extract. PRE shows a significant inhibitory effect on TNF-α production, especially at a concentration of 100 μg/mL. Notably, the extract also exhibits a positive effect in promoting wound healing, as evidenced from the "wound healing" model on 3T3 cells. These results show the potent application of *Psychotria* sp. in research and development of bioactive pharmaceutical products. At the same time, this is also the first study to comprehensively evaluate the biological activities of *Psychotria* sp. in Vietnam.

Keywords: *Psychotria rubra*, antibacterial, anti-inflammatory, antioxidant, wound-healing

1 Introduction

The global rise of antibiotic-resistant bacteria and the increasing incidence of cancer present major challenges to the healthcare sector, complicating disease treatment and control. These issues have intensified the urgent need for identifying alternative medicinal sources that are safe, effective, and compatible with human health. According to the World Health Organisation, up to 80% of the population in developing countries continues to rely on traditional medicine and herbal products for primary healthcare [1].

Plants are widely recognised as reservoirs of bioactive compounds with diverse therapeutic properties, including anti-cancer, anti-inflammatory, antibacterial, and antioxidant effects. These benefits often arise from the synergistic interactions of phytochemicals acting on multiple biological targets.

Psychotria L. is the largest genus in the *Rubiaceae* and the third largest among

angiosperms. Its native distribution spans tropical and subtropical regions [2]. According to the International Plant Names Index and the World Checklist of Vascular Plants, the genus currently includes 1,642 accepted species [3]. Pitard [4] documented 26 species of *Psychotria* from the Indo-China region, of which 17 were recorded in Vietnam. Later, Pham [5] illustrated 30 species and one variety in Vietnam, while Tran [6] listed 26 species and one variety. Members of *Psychotria* are predominantly shrubs, though some species occur as small trees or subshrubs. The leaves are opposite, petiolate, and stipules. The flowers are typically small, sessile or pedicellate, 4–5-merous, hermaphroditic, borne in terminal capitate or paniculate inflorescences with bracts and bracteoles that may be small or large. The ovary is usually 2-locular (rarely 3–4-locular), with each locule containing a single erect ovule. The fruits are fleshy, with 2–5 pyrenes having smooth or ribbed hemispherical cross-sections. Additionally, *Psychotria* is characterised with strongly ruminate endosperm, seeds with a hard red-brown or purplish testa, and distinctive stipules, which are leaf-like appendages at the base of the petiole serving a protective role for developing leaves at the shoot apex [2, 4–6].

The genus *Psychotria* is quite common in Vietnam, but *Psychotria* sp. is a new species that has never been studied before. Some *Psychotria* species have excellent anti-inflammatory effects, which help prevent and relieve conditions such as colds, fevers, and sore throats, but the specific mechanism in treating inflammatory conditions caused by respiratory infections, such as sore throats, remains unclear [2]. Currently, there is no work focusing on clarifying the antioxidant, antibacterial, and anticancer activities of this new *Psychotria* sp. During a study of medicinal plants at the An Toan Nature Reserve in Gia Lai Province (formerly part of Binh Dinh Province), we documented a species of *Psychotria* (Rubiaceae) (Fig. 1)

Fig. 1. Photos of *Psychotria* sp.

While a formal botanical description is in progress, this study was undertaken to preliminarily assess the plant's biological activities, with the goal of establishing a scientific foundation for future research and potential therapeutic development.

2 Material and methods

Plant and bacterial samples

Psychotria sp. samples were collected from natural populations in An Lao, Gia Lai province, Vietnam, with vouchered specimens deposited at the herbarium SGN. It was identified at the Institute of Advanced Technology, Vietnam Academy of Science and Technology.

The bacterial strains used to evaluate the antibacterial activity of the extract included *Escherichia coli* (ATCC 85922) and *Streptococcus pyogenes* (ATCC 25023).

Preparation of plant extract

Plant samples were collected, cleaned, and cut into small pieces before being ground into fine powder. It was dried at 45 °C for 24 hours. The powder was then extracted with a 70% ethanol

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under reflux for 4 hours at 70–75 °C with a powder-to-solvent ratio of 1:10. The extract was cooled and filtered to remove residue, first through a cotton layer and later through a 20 µL filter paper. The filtrate was concentrated with a vacuum rotary evaporator (Stuart RE 400, USA) at 90 bar and 55 °C for 1 hour and 50 min. The concentrated extract was dried at 50 °C for 72 hours and stored at –4 °C. In the subsequent experiments, the extracts were dissolved in distilled water and diluted to different concentrations [7].

Analysing of compounds from extracts

The extract was processed with a gas chromatograph-mass spectrometer (GC-MS) at the Quality Assurance and Testing Centre 2 to identify the compounds in it. Gas chromatography-mass spectrometry (GC-MS) analysis of the P70 extract was performed on a gas chromatograph (Model QP2020NX, Shimadzu, Japan) equipped with a splitless injector, a Shimadzu AOC 20i Plus autosampler, and a Shimadzu Rxi 5MS fused silica column (5% phenyl-methylpolysiloxane, 30 m long, 0.25 mm in diameter, and 0.25 mm film thickness). The injection volume was 1 mL, and the GC conditions included programmed heating from 50 to 300 °C at 10 °C/min, followed by 10 min at 300 °C. The injector was maintained at 280 °C. Helium was used as a carrier gas at a 1.0 mL/min flow rate. The MS conditions were as follows: ionisation energy was 70 eV; electron impact ion source temperature was 230 °C; the quadrupole temperature was 150 °C; the scan rate was 3.2 scans/s; the mass range was 50–1000 m/z. Compounds were identified on the basis of their mass spectra and retention indices with the NIST/Wiley 275 library (Wiley, New York, USA). The relative abundance of each feature was calculated from the Total Ion Chromatography (TIC) plot [8].

ABTS Free radical scavenging activity assay

$ABTS^+$ [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)] is a stable free radical and exhibits blue fluoresce with an absorbance at 734 nm. Upon addition of antioxidants to a solution containing $ABTS^+$, the ion is reduced to ABTS, resulting in a decrease of absorbance. This decrease was measured to assess the antioxidant activity relative to a standard. In a potassium persulfate medium, the $ABTS^+$ radical remains stable for up to 2 days at ambient temperature in the dark [9]. To generate the $ABTS^+$ radical, 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) were mixed at the 3:1 ratio, incubated in the dark at ambient temperature for 12–16 hours. The resulting $ABTS^+$ solution was diluted with water to obtain an absorbance of 1.00 ± 0.02 at 734 nm. Subsequently, 750 µL of $ABTS^+$ was combined with 150 µL of samples with different concentrations. The absorbance at 734 nm was measured after 5 min. All experiments were performed in triplicate. The ABTS free radical scavenging capacity (H%) was calculated as follows:

$$\%H = [(A_{Control} - A_{Sample})/A_{Control}] \times 100\% \quad (1)$$

where $A_{Control}$ is the maximum absorbance of blank sample without extract, and A_{Sample} is the maximum absorbance of sample to be measured.

Evaluation of antioxidant activity with DPPH radical scavenging method

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical compound, purple in color and has a maximum absorption at 517 nm. In the presence of antioxidants, it is reduced to yellow 2,2-diphenyl-1-picrylhydrazine (DPPH-H). The decrease in absorbance at 517 nm was measured to determine the DPPH radical scavenging ability of the antioxidant [9]. 0.3 mM DPPH solution and extracts were prepared in different concentrations. The reaction mixture consists of

100 μL of sample (blank sample with water added) and 100 μL of 0.3 mM DPPH solution. The suspension was incubated at 37 °C for 30 min and the absorbance was measured at 517 nm. Each experiment was performed in triplicate. The DPPH free radical scavenging ability (%*I*) was calculated as follows:

$$\%I = [(A_{Contro} - A_{Sample})/A_{Control}] \times 100\% \quad (2)$$

where $A_{Control}$ is the maximum absorbance of blank sample without extract, and A_{Sample} is the maximum absorbance of sample to be measured.

Minimum inhibitory concentration (MIC) and Minimum bactericide concentration (MBC)

A 96-well plate was prepared by adding 50 μL of bacteria at a concentration of 10^6 CFU/mL in Mueller-Hinton (MH) liquid medium to each well. Then, 50 μL of *Psychotria* sp. extract (PRE) at various diluted concentrations (3.125 to 100 mg/mL) was added. The positive control consisted of bacteria and MH, while the negative control included PRE and MH. The mixture was incubated overnight at 37 °C. After 24 hours, 30 μL of 0.015% diluted resazurin (SBC Scientific, Vietnam) was added to each well. The plate was incubated again at 37 °C, and the color change in each well was observed. MIC was determined as the concentration of PRE at which no resazurin color change occurred. For MBC, 100 μL from wells without resazurin color change was transferred onto agar plates, incubated at 37 °C for 24 hours, and colony formation was assessed. MBC was recorded at the lowest PRE concentration with no colonies [10]. Each experiment was performed in triplicate.

Cytotoxic activity determination

The cell line used in the study is HEK-293A, which is a human embryonic kidney stem cell. The *in-vitro* cytotoxicity test method is recognised by the National Cancer Institute as a standard cytotoxicity test to screen and detect substances that can inhibit the growth or kill cells *in-vitro* [11]. The test determines the total cell protein content on the basis of the optical density (OD) when the cell protein component is stained with sulforhodamine B (SRB). The measured OD value is proportional to the amount of SRB attached to the protein molecule, thus the more cells (the more protein) are attached, the larger is the OD value. The test was performed under the following specific conditions.

The sample was dissolved in DMSO (100%) at an initial concentration (stock) of 20 mM (with the concentrate) or 20 mg/mL. Before use, the stock was diluted with the culture medium without fetal bovine serum (FBS) to obtain 4 serial concentrations, ensuring that the final DMSO concentration in wells does not exceed 0.5–1%.

Trypsin was added to the experimental cells to loosen the cells and adjust the density to suit the experiment. Then, 190 μL of cells (grown in the culture medium supplemented with 5% FBS) was transferred to the 96-well plate, and it was placed in a CO_2 incubator for stable growth for 18–20 hours.

After this stable growth period, the cells were incubated with the test sample as follows: 10 μL of diluted sample with the concentrations prepared above were placed into the wells of the 96-well plate where the cells have been prepared. The well without reagent but with cells (190 μL) + 10% DMSO (10 μL) was used as the control for day 0. After 1 hour, the control well with cells on day 0 was fixed with trichloracetic acid – TCA 20%.

The plate was then placed in the incubator for 48 hours, and the cells were fixed with cold TCA 20% (v/v) for 1 hour. TCA was removed, and the cell plate was gently washed with dH_2O and dried at ambient temperature. Subsequently, the cells were stained with SRB 0.4% (w/v in 1% acetic

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acid) for 30 minutes at 37 °C, washed 3 times with 1% acetic acid, and dried at ambient temperature again.

200 μL unbuffered Tris base 10 mM was added to dissolve SRB, and the cells were shaken gently for 10 minutes. Finally, the OD was read at the 540 nm wavelength on an ELISA Plate Reader (BioTek).

– The percentage of cell growth inhibition in the presence of the test substance was determined according to formula (3)

$$\% \text{ inhibition} = 100 - [(OD_{sample} - OD_{day0})/-[(OD_{DMSO} - OD_{day0})] \times 100 \quad (3)$$

– The test was repeated 3 times to ensure accuracy. Ellipticine at concentrations of 10, 2, 0.4, and 0.08 g/mL was used as a control.

– DMSO 10% was always used as a negative control (final concentration in the test well was 0.5%). The IC_{50} value (concentration that inhibits 50% of growth) was determined by the computer software TableCurve 2Dv4.

In-vitro cytokine production inhibitory activity study

RAW264.7 cells are macrophage cell lines grown as monolayers in the DMEM culture medium supplemented with 10% fetal FBS, 1% PSF antibiotics at 37 °C, and 5% CO_2. The cells were subcultured with EDTA (0.05%) after 2 days of culture.

RAW 264.7 cells were seeded into the experimental wells of 96-well plates with appropriate cell numbers (2×10^6 cells/mL) and incubated at 37 °C overnight to be stabilised. The test samples (10 μL) dissolved in 10% DMSO were added to the wells at different concentrations. Dexamethasone was used as a control with a final concentration of 10 M in the wells. Some wells without reagent but with cells (190 μL) and 10 μL of 10% DMSO were used as controls with LPS (+LPS). The wells containing only culture medium were blank wells. After 2h, LPS at a concentration of 1 g/mL was added to all experimental wells. Some wells containing only cells and no LPS were used as physiological controls (–LPS). After 24 h, the supernatants from the wells of the experimental plate were collected for testing and determining the cytokine content immediately or stored at –80 °C for subsequent experiments.

The supernatant obtained above was used immediately to quantify TNF-alpha after diluting 10 times with the buffer solution (included in the kit). The presence of TNF-alpha in the cell culture was determined by using the TNF mouse ELISA Kit, following the instructions of the kit manufacturer (R&D Systems, Minneapolis, US). The kit contained the corresponding cytokine able to construct a standard curve, and the corresponding cytokine content was determined according to this curve [12–14].

In-vitro cell scratch test

3T3 cells are mouse embryonic fibroblasts cultured as monolayers in the DMEM culture medium with the accompanying ingredients of 2 mM L-glutamine, 10 mM HEPES, and 1.0 mM sodium pyruvate, in addition to 10% calf serum. The cells were subcultured after 3 days at a ratio of 1:3 and cultured in a CO_2 incubator at 37 °C and 5% CO_2.

The cells were cultured on 24-well plates at a cell concentration of 2×10^5 cells/mL. After 24 hours, when the cells were stably adhered to the bottom of the well, the surface of the monolayer was scratched with the sharp tip of a 200 μL micropipette. The medium was discarded, and the cells was washed with PBS to remove non-attached cells. Then, the samples were added to each culture well with different concentrations. 1% DMSO was used as a negative control. The cells were further cultured in a CO_2 incubator for 24 h. Cell images at 200× magnification were taken

under an inverted microscope after 0 and 24 h of incubation. The images were analysed with the ImageJ software to determine the ability of cells to narrow the scratches under the influence of the sample. The scratch narrowing/healing ability of the sample was determined as the percentage of the scratch width in the sample incubation well compared with that at the initial time. The results were expressed as the percentage of the narrowed scratch area and calculated according to formula (4)

Scratch reduction area (%) =
[A(0h) – A(24h)]/A(0h) × 100% (4)

where *A* is the scratch area measured at 0 h and 24 h after incubation [15, 16].

Data processing

Data were processed on Excel, presented as Mean ± standard error (SD). Statistical algorithms, t-test, F-test, and one-way ANOVA were used to test the significant differences relative to the negative control, where $p < 0.05$, considered as a statistically significant difference.

3 Resutl and discussion

3.1 Identification of compounds in the leaf extract of *Psychotria* sp.

PRE was found to contain various compounds, including resorcinol, tetradecanoic acid, neophytadiene, palmitoleic acid, n-hexadecanoic acid, ethyl hexadecanoate, phytol, 9,12-octadecadienoic acid (Z,Z), 9,12,15-octadecatrienoic acid (Z,Z,Z), oleic acid, octadecanoic acid, cis-10-nonadecenoic acid, eicosanoic acid, squalene, γ-tocopherol (vitamin E), campesterol, stigmasterol, and β-sitosterol. Among them, resorcinol, neophytadiene, phytol, squalene, γ-tocopherol, β-sitosterol, and oleic acid have reported antibacterial activity; phytol, squalene, γ-tocopherol, β-sitosterol, and oleic acid possess anti-inflammatory potential; resorcinol, phytol, squalene, γ-tocopherol, β-sitosterol, and oleic acid exhibit antioxidant properties (Table 1) [17].

Table 1. Chemical composition in extract

No.	Retention time (min)	Peak area (%)	Compound name	Molecular formula
1	10.908	1.99	Resorcinol	$C_6H_6O_2$
2	19.223	0.87	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$
3	19.787	1.80	Neophytadiene	$C_{20}H_{38}$
4	20.317	0.52	Palmitoleic acid	$C_{16}H_{30}O_2$
5	21.201	17.4	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
6	21.493	1.16	Hexadecanoic acid, Ethyl este	$C_{18}H_{36}O_2$
7	21.754	0.76	Phytol	$C_{20}H_{40}O$
8	22.816	17.0	9,12-Otadecatrienoic acid (Z,Z)	$C_{18}H_{32}O_2$
9	22.957	5.02	9,12,15- Otadecatrienoic acid (Z,Z)	$C_{18}H_{30}O_2$
10	22.989	9.02	Oleic acid	$C_{18}H_{34}O_2$
11	23.023	5.76	Octadecanoid acid	$C_{18}H_{36}O_2$
12	23.210	1.83	cis-10-Nonadecenoic Acid	$C_{19}H_{36}O_2$
13	23.832	0.68	Eicosanoic acid	$C_{20}H_{40}O_2$
14	24.681	3.28	Squalene	$C_{30}H_{50}$

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No.	Retention time (min)	Peak area (%)	Compound name	Molecular formula
15	27.671	0.59	Gamma-Tocopherol	$C_{28}H_{48}O_2$
16	29.078	9.54	Vitamin E	$C_{29}H_{50}O_2$
17	29.759	2.19	Campesterol	$C_{28}H_{48}O$
18	30.704	3.32	Stigmasterol	$C_{29}H_{48}O$
19	31.030	10.2	beta-Sitoterol	$C_{29}H_{50}O$

3.2 ABTS free radical scavenging ability

Fig. 2 presents the ABTS free radical inhibition efficiency of the extract at different concentrations, demonstrating a clear dose-dependent antioxidant activity. At low concentrations (6.25 and 12.5 μg/mL), the inhibition efficiency is limited, fluctuating between 10–20%, reflecting a weak free radical scavenging ability. However, its IC_{50} corresponds to a concentration of 19.32 μg/mL or higher, slightly higher than IC_{50} of vitamin C (15 μg/mL), indicating strong antioxidant activity. Compared with white turmeric extract (IC_{50} = 25.19 μg/mL), PRE demonstrates a stronger antioxidant capacity, as evidenced by its lower IC_{50} value [18]. Therefore, it can be affirmed that PRE is a potential source of raw materials for applications in the pharmaceutical and functional food fields with the goal of preventing oxidative stress and related diseases.

3.3 DPPH free radical scavenging ability

Fig. 3 shows the DPPH free radical inhibition efficiency of the extract at different concentrations, indicating that the antioxidant activity is clearly dose-dependent. At low concentrations (6.25 and 12.5 μg/mL), the inhibition efficiency is concentration of 48.11 μg/mL or higher, slightly higher than IC_{50} of vitamin C (41.07 μg/mL), indicating strong antioxidant activity. Compared with white turmeric extract (IC_{50} = 129 μg/mL), PRE exhibits a clear antioxidant advantage, as evidenced by its lower IC_{50} value [15]. Therefore, it can be affirmed that PRE has very strong antioxidant capacity. The analysis results show the presence of many compounds, such as resorcinol, phytol, squalene, γ-tocopherol, β-sitosterol, and oleic acid, in the PRE extract. This explains the extremely strong antioxidant properties of PRE.

3.4 Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an extract that inhibits bacterial growth by more than 80%, with no color change of the resazurin indicator. The minimum bactericidal concentration (MBC) refers to the lowest concentration at which the extract completely kills the bacteria (100%). According to Table 2, PRE inhibits the growth of both tested strains: *E. coli* (Gram-negative) and *S. pyogenes* (Gram-positive), with an identical MIC value of 12.5 mg/mL. However, the MBC values differ: 50 mg/mL for *E. coli* and 100 mg/mL for *S. pyogenes*. This suggests that while the initial inhibitory effect of the extract is comparable for both strains, a higher concentration is required to achieve complete bactericidal activity against *S. pyogenes*. The difference may result from intrinsic resistance mechanisms or structural differences in the cell walls of Gram-positive and Gram-negative bacteria. Overall, PRE demonstrates antibacterial potential, particularly through its ability to inhibit bacterial growth at relatively low concentrations, and shows stronger bactericidal activity against *E. coli* than *S. pyogenes*. With this result, PRE is considered to have antibacterial activity at a fairly good level compared with other plant extracts [19].

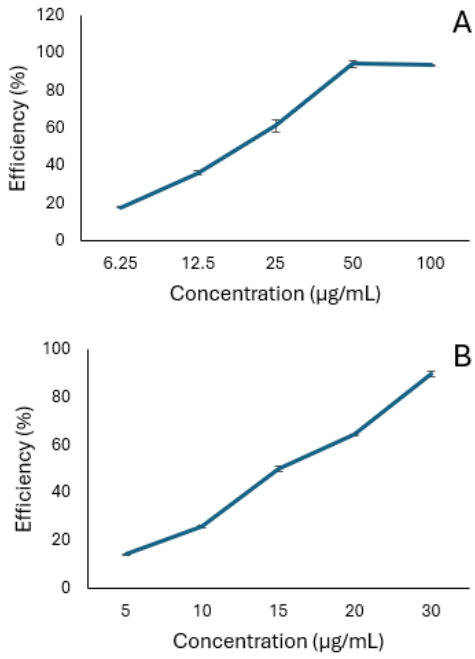

Fig. 2. ABTS free radical scavenging efficiency of PRE: (A) PRE; (B) Vitamin C

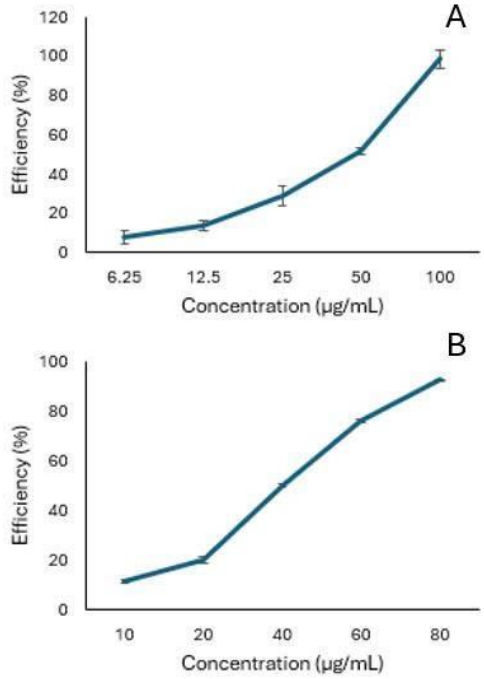

Fig. 3. DPPH antioxidant efficiency of PRE: (A) PRE; (B) Vitamin C

Table 2. MIC and MBC values of PRE on bacterial strains

Bacterial strains	MIC (mg/mL)	MBC (mg/mL)
E. coli	12.5	50
S. pyogenes	50	100

3.5 Evaluation of cytotoxic activity

The cytotoxicity assay results indicate that PRE exhibits weak cytotoxic activity, with an IC_{50} value greater than 100 µg/mL. Even at the highest tested concentration (100 µg/mL), the inhibition rate is only 34.44 ± 1.82%, suggesting limited cell growth suppression. In contrast, the positive control, ellipticine, exhibits strong cytotoxicity with an IC_{50} of 0.33 ± 0.02 µg/mL. These findings suggest that PRE has low cytotoxic potential under the tested conditions (Table 3, $p < 5\%$).

Table 3. Cytotoxic effects of PRE

Sample		Ellipticine	
Concentration (µg/mL)	% inhibition	Concentration (µg/mL)	% inhibition
100	34.44 ± 1.82	10	98.64 ± 2.05
20	13.19 ± 1.01	2	81.95 ± 1.53
4	8.14 ± 0.65	0.4	51.30 ± 1.14
0.8	–0.57 ± 0.07	0.08	23.16 ± 1.02
IC_{50}	>100	IC_{50}	0.33 ± 0.02

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3.6 Evaluation of anti-inflammatory activity

The anti-inflammatory activity of the PRE was assessed by measuring TNF-α levels in the LPS-stimulated macrophages. At a concentration of 100 μg/mL, the extract significantly reduces TNF-α secretion to 3919.05 ± 145.30 pg/mL, which is lower than that of the positive control, dexamethasone, at 10 μM (4577.78 ± 106.51 pg/mL). In contrast, lower concentrations (20 and 4 μg/mL) indicate minimal inhibition, with the TNF-α levels close to those of the LPS-treated control (9176.19 and 9592.06 pg/mL, respectively). The negative control (–LPS) shows the lowest TNF-α level at 2255.56 ± 199.13 pg/mL. These results suggest that the extract exhibits dose-dependent anti-inflammatory activity, with strong TNF-α inhibition at high concentration (100 μg/mL), comparable or even superior to dexamethasone. Furthermore, cell viability remains above 100% across all tested concentrations, indicating that the extract is not cytotoxic under the experimental conditions (Table 4).

Table 4. Effect of PRE on TNF-α expression

Concentration (μg/mL)	**Sample**	
	TNF- α (pg/ml)	**% living cells**
100	3919.05** ± 145.30	100.75 ± 1.28
20	9176.19 ± 284.08	101.22 ± 1.01
4	9592.06 ± 251.09	103.03 ± 1.28
Concentration (μM)	**Dexamethasone (Reference)**	
	TNF- α (pg/mL)	**% living cells**
10	4577.78** ± 106.51	99.65 ± 0.81
Sample concentration (μM)	**Negative control**	
	TNF- α (pg/mL)	**% living cells**
+LPS (control)	9711.11 ± 240.48	101.79 ± 2.53
–LPS (control)	2255.56** ± 199.13	103.39 ± 2.65

**p < 0.05; **p < 0.01 compared with negative control containing LPS.*

3.7 Evaluation of wound healing potential

PRE exhibits a concentration-dependent wound healing effect on 3T3 mouse embryonic fibroblast cells. At the highest concentration (100 μg/mL), the wound closure area is only 8.32%, the lowest among all tested groups, suggesting potential cytotoxicity or inhibition of tissue regeneration. As the concentration decreases to 20 μg/mL and 4 μg/mL, wound closure improves to 24.09 and 41.68%, respectively, with the result at 4 μg/mL being comparable with the untreated control (41.64%). Notably, at the lowest concentration (0.8 μg/mL), PRE demonstrates the highest healing activity, with a wound closure area of 47.32%, exceeding that of the control. This suggests a stimulatory effect on fibroblast proliferation and migration. These findings indicate that PRE has potential to promote wound healing at low concentrations, whereas higher concentrations may exert adverse effects on cell viability (Table 5 and Fig. 4).

Table 5. Evaluation of scratch area after PRE treatment

Concentration (μg/mL)	**Scratch area was reduced compared to the control**
100	8.32 ± 2.32
20	24.09 ± 2.12
4	41.68 ± 2.50
0.8	47.32 ± 2.10
Control	41.64 ± 2.21

4 Conclusion

The leaf extract of *Psychotria sp.* contains a variety of bioactive compounds with demonstrated antioxidant, antibacterial, anti-inflammatory, and wound healing properties. This is the first comprehensive study on the bioactivities of *Psychotria sp.* from Vietnam. The extract exhibits strong antioxidant capacity, with IC_{50} values comparable with or better than standard compounds (IC_{50} of 19.32 μg/mL for ABTS and

48.11 μg/mL for DPPH). It manifests moderate antibacterial activity against both Gram-positive and Gram-negative bacteria. It also demonstrates significant anti-inflammatory activity by reducing TNF-α production at high concentrations without cytotoxic effects. Furthermore, PRE promotes wound healing in a dose-dependent manner, with the highest activity observed at low concentrations. These findings suggest that PRE is a promising natural source for the development of therapeutic agents and functional food products targeting oxidative stress, inflammation, infection, and tissue repair.

Acknowledgement

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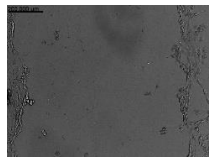
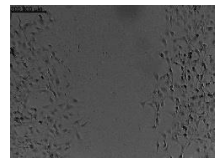
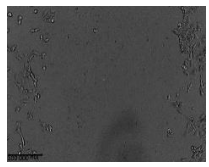
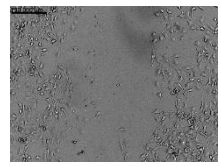
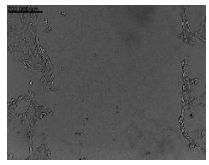
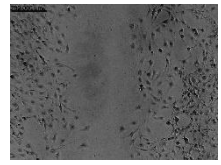
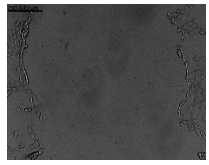
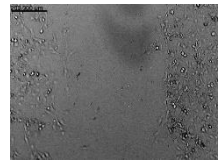
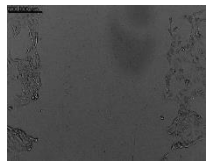
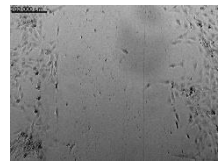
Concentration (μg/mL)	0 h	24 h
Control		
0.8		
4		
20		
100		

Fig. 4. Scratch area change at various concentrations of PRE

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