Research Article

# Phytochemical Screening and Evaluation of Anti-Inflammatory Activities of *Pavetta lasioclada* Root Extract

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**ABSTRACT**

Inflammation is the protective biological response harmful stimuli like pathogens, toxins, or tissue injury. Though acute inflammation is beneficial, chronic inflammation contributes to various harmful diseases like cancer, arthritis, and cardiovascular disorders. Conventional anti-inflammatory agents are effective but associated with adverse effects upon prolonged use, prompting the need for safer alternatives from natural sources. This study aims on anti-inflammatory activity evaluation and phytochemical profile of the *Pavetta lasioclada* root extract, a medicinal plant traditionally used for inflammatory conditions. The roots were collected, authenticated, shade-dried, and extracted using ethanol through Soxhlet extraction. Preliminary phytochemical studies show the presence of alkaloids, phenols, flavonoids, saponins, and glycosides. The anti-inflammatory property was assessed using RAW 264.7 macrophage cell lines stimulated with lipopolysaccharide (LPS). Nitric oxide (NO) production was measured using the Griess reagent assay to evaluate the inhibitory effect of the extract. The results demonstrated that the ethanolic root extract significantly inhibited nitric oxide production in a concentration-dependent manner, indicating effective suppression of inflammatory mediators. The activity is likely attributed to the presence of flavonoids and phenolic compounds, which are known to inhibit key inflammatory pathways such as cyclooxygenase (COX) and lipoxygenase (LOX). In conclusion, *Pavetta lasioclada* root extract exhibits promising anti-inflammatory activity and contains valuable phytoconstituents, supporting its traditional use. Further studies are recommended to isolate active compounds and validate their therapeutic potential through in vivo and clinical investigations.

**Keywords:** *Pavetta lasioclada*, Anti-inflammatory activity, Phytochemical screening, Medicinal plants, RAW 264.7 cell line, Nitric oxide inhibition, Soxhlet extraction, Flavonoids, Phenolic compounds, Natural therapeutics.

## INTRODUCTION

Inflammation is the protective biological response harmful stimuli like pathogens, toxins, or tissue injury. It has a crucial role in maintaining tissue homeostasis by eliminating the cause of injury, removing damaged tissues, and initiating the healing process. The response involves immune cells, blood vessels, and chemical mediators that work together to control damage and promote repair.[1]

The five classical signs of inflammation are important diagnostic features. These include redness (rubor) due to increased blood flow, heat (calor) caused by elevated metabolic activity, swelling (tumor) due to fluid accumulation, pain (dolor) from chemical mediators like prostaglandins, and loss of function (functio laesa) resulting from tissue damage and swelling. [2]

Inflammation is broadly classified into acute and chronic types. Acute inflammation is a short-term, rapid response that lasts from a few hours to several days. It mainly involves neutrophils and usually resolves once the harmful stimulus is removed. Common examples include cuts, burns, and infections. In contrast, chronic inflammation is long-lasting and may persist for months or years. It involves macrophages, lymphocytes, and plasma cells, and can lead to tissue damage, fibrosis, or scarring. Diseases such as arthritis, asthma, and cardiovascular disorders are associated with chronic inflammation. [3]

The mechanism of inflammation involves a sequence of cellular events known as the leukocyte adhesion cascade. Initially, margination occurs, where leukocytes move towards the vessel wall due to slowed blood flow. This is followed by rolling and adhesion, where leukocytes attach to endothelial cells. Next, emigration takes place, allowing leukocytes to pass through the vessel wall into the affected tissue. Chemotaxis then guides these cells to the site of injury through chemical signals. Once there, phagocytosis occurs, where pathogens are engulfed and destroyed. Intracellular killing involves oxygen-dependent mechanisms (reactive oxygen species and nitric oxide) and oxygen-independent mechanisms (enzymes like lysozyme). Finally, degradation of pathogens occurs within lysosomes. [4]

Acute inflammation is mainly caused by infections and physical injuries. Chronic inflammation is often linked to lifestyle and environmental factors such as lack of physical activity, chronic stress, obesity, unhealthy diet, poor sleep, exposure to toxins, smoking, and excessive alcohol consumption.

### ANTI-INFLAMMATORY DRUGS

Anti-inflammatory drugs are substances that reduce inflammation by inhibiting the production or action of inflammatory mediators such as cytokines, prostaglandins, and enzymes like cyclooxygenase (COX) and lipoxygenase (LOX). [5]

**Types of Anti-inflammatory Drugs:**

a) NSAIDs (Non-Steroidal Anti-Inflammatory Drugs):

NSAIDs are the most commonly used drugs for pain and inflammation. They work by inhibiting COX enzymes, thereby reducing prostaglandin synthesis. This results in decreased pain, swelling, and fever. Common examples include Aspirin, Ibuprofen, Diclofenac, and Naproxen. However, they may cause side effects such as gastric irritation and ulcers. [6]

b) Corticosteroids (Steroidal Drugs):

Corticosteroids are powerful anti-inflammatory agents that suppress immune responses. They inhibit cytokine production and reduce immune cell activity. Examples include Prednisone, Dexamethasone, and Hydrocortisone.

**Mechanism of Action:**

NSAIDs block COX-1 and COX-2 enzymes, preventing the transition of arachidonic acid into prostaglandins. When COX-2 inhibition reduces inflammation, COX-1 inhibition may lead to gastric side effects. Biologic drugs target specific cytokines such as TNF and interleukins, providing targeted therapy in autoimmune diseases. DMARDs suppress immune system activity and slow disease progression in chronic inflammatory conditions. [7]

**Therapeutic Uses:**

Anti-inflammatory drugs are widely used for pain relief (headache, muscle pain), treatment of arthritis, and reduction of fever. They are also effective in managing inflammatory conditions like tendonitis and bursitis, autoimmune diseases such as lupus and rheumatoid arthritis, and allergic conditions like asthma. Additionally, low-dose Aspirin is used to prevent blood clot formation and reduce the risk of heart attack and stroke. [8]

**MEDICINAL PLANTS**

Medicinal plants are those plants that contain naturally occurring chemical compounds known as phytochemicals, which possess therapeutic properties useful in the prevention and treatment of various diseases. These plants have been used for centuries in traditional systems of medicine such as Ayurveda, Siddha, Unani, and other herbal practices across the world. The healing potential of medicinal plants lies in their bioactive constituents, which interact with biological systems to produce beneficial health effects. Due to their natural origin, medicinal plants are often considered safer, more economical, and easily accessible compared to synthetic drugs. [9]

**Phytochemical Constituents and Importance:**

Medicinal plants are rich sources of secondary metabolites like alkaloids, flavonoids, glycosides, tannins, terpenoids, saponins, and phenolic compounds. Above phytochemicals are responsible for various pharmacological activities like anti-inflammatory, antimicrobial, antioxidant, analgesic, antidiabetic, and anticancer effects. Various plant parts such as roots, leaves, bark, stem, flowers, fruits, and seeds are used in medicinal preparations. These plant materials are processed into various forms like powders, extracts, decoctions, tinctures, and tablets depending on their intended use. In modern healthcare, medicinal plants play a crucial role as many pharmaceutical drugs are obtained directly or indirectly from plant sources, making them highly valuable in drug discovery and research. [10]

**Taxonomical Classification of *Pavetta lasioclada:***

*Pavetta lasioclada* is a flowering plant of the family Rubiaceae, commonly known as the coffee or madder family. Its taxonomical classification includes Kingdom Plantae, Clade Angiosperms, Clade Eudicots, Order Gentianales, Family Rubiaceae, Genus Pavetta, and Species lasioclada. The genus Pavetta consists of nearly 360 species of shrubs and small trees widely distributed in tropical and subtropical regions of Africa and Asia. These plants are known for their attractive white flowers and medicinal significance. [11]

*Pavetta lasioclada* is typically a shrub or small tree that grows up to 2–5 meters in height. It has branched stems that are brownish and slightly hairy, especially in young branches. The leaves are simple, opposite, oval to elliptical in shape, smooth-edged, and dark green in colour. A unique feature of many Pavetta species is the presence of bacterial nodules in the leaves, which are symbiotic structures formed by endophytic bacteria. The plant produces small white tubular flowers arranged in clusters at the ends of branches, usually with four spreading petals, which attract pollinators. The fruits are small and contain seeds that aid in natural propagation.

**Figure 1:** Fruit of *Pavetta lasioclada*

**Figure 2:** Leave of *Pavetta lasioclada*

*Pavetta lasioclada* is mainly seen in tropical regions of West and Central Africa. It is distributed in countries such as Benin, Cameroon, Ivory Coast, Liberia, Mali, Sierra Leone, Guinea, Togo, and the Central African Republic. The plant typically grows in seasonally dry tropical forests, woodland ecosystems, forest margins, and shrublands where adequate sunlight and moisture are available. It plays an important role in maintaining biodiversity and ecological

stability within these habitats. [12] In its natural environment, *Pavetta lasioclada* contributes significantly to ecosystem functioning. It provides nectar for pollinators like insects, supports understory vegetation in forests, and helps in maintaining soil stability. Additionally, the symbiotic relationship between the plant and bacteria in its leaf nodules may enhance nutrient exchange and overall plant health, thereby supporting ecological balance.

Although detailed scientific studies on *Pavetta lasioclada* are limited, it has been traditionally used in herbal medicine for treating various ailments such as fever, malaria, dysentery, and certain infectious diseases. In some communities, extracts of the plant are also used as natural dyes or preservatives. The traditional knowledge associated with this plant has been passed down through generations and continues to play a vital role in local healthcare systems. Due to its potential medicinal properties, *Pavetta lasioclada* has gained increasing attention in phytochemical and pharmacological research. [13]

## MATERIALS AND METHODOLOGY

### Materials

The roots of *Pavetta lasioclada* were used as the plant material. All chemicals and reagents were of analytical grade. Ethanol was used for extraction, while lipopolysaccharide (LPS) was used to induce inflammation. Nitric oxide estimation was carried out using Griess reagent with sodium nitrite as the standard. Phytochemical screening was performed using standard reagents such as Mayer's, Dragendorff's, ferric chloride, and Shinoda reagents. [14]

The RAW 264.7 macrophage cell line was used for in vitro studies and maintained in Dulbecco's Modified Eagle Medium (DMEM) is added with fetal bovine serum and antibiotics. Standard laboratory equipment, including a Soxhlet apparatus, rotary evaporator, $CO_2$ incubator, laminar airflow chamber, and microplate reader, along with routine glassware, were used throughout the study.

### Methodology:

### Phytochemical Screening:

Phytochemical screening helps in identifying the bioactive compounds in plant extracts. These phytochemicals possess various pharmacological activities like anti-inflammatory, antimicrobial, antioxidant, and anticancer effects. [15]

### Test for Alkaloids

The presence of alkaloids in the ethanolic extract of *Pavetta lasioclada* was determined using Mayer's, Dragendorff's, and Wagner's tests. In Mayer's test, the addition of Mayer's reagent resulted in the formation of a cream or white precipitate, indicating the presence of alkaloids. In Dragendorff's test, the appearance of an orange or reddish-brown precipitate confirmed alkaloids. Similarly, Wagner's test showed the formation of a reddish-brown precipitate, further supporting the presence of alkaloids. [16]

### Test for Flavonoids

Flavonoids were detected using the Shinoda test. The addition of magnesium turnings and concentrated hydrochloric acid to the extract produced a pink, red, or orange coloration, indicating the presence of flavonoids.

### Test for Saponins

Saponins were identified by the foam test. *Pavetta lasioclada* root extract was shaken vigorously using water, and the saponins present was confirmed by stable persistent foam.

### Test for Glycosides

The Keller–Killiani test was used to detect glycosides. When glacial acetic acid, ferric chloride is added with concentrated sulphuric acid a brown ring is formed at the interface, indicating the presence of cardiac glycosides.

### Test for Phenolic Compounds

Phenolic compounds were identified using the ferric chloride test. The addition of ferric chloride solution to the root extract produced a black or deep blue coloration, confirming phenol availability.

### Test for Terpenoids

Salkowski test is used to detect terpenoids. Mix chloroform with root extract followed by the careful addition of concentrated sulphuric acid, resulting in a reddish-brown coloration at the interface, indicating the presence of terpenoids.

### *In-vitro* Anti-inflammatory Studies

### Cell Culture Maintenance

The murine macrophage cell line RAW 264.7 was procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine, sodium bicarbonate, and antibiotics including penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL). The cells were cultured in 25 $cm^2$ tissue culture flasks and incubated at 37°C in a humidified atmosphere containing 5% $CO_2$.

### Induction of Inflammation and Treatment

The cells were allowed to grow until approximately 60% confluency. Inflammation was induced by treating the cells with lipopolysaccharide (LPS) at a concentration of 1 µg/mL. The LPS-stimulated cells were treated with various concentrations of plant extract (25, 50, and 100 µg/mL). Place in the incubated for 24 hours and collect cell lysate for the analysis.

### Cyclooxygenase (COX) Activity

Cyclooxygenase activity was studied using Walker and Griess method. Briefly, incubated 100 µL of cell lysate with Tris-HCl buffer (pH 8), haemoglobin, glutathione, and arachidonic acid. The process of initiated by including arachidonic acid and incubated at 37°C for 20 minutes and

terminated by including trichloroacetic acid in hydrochloric acid. After centrifugation add Thiobarbituric acid and boiled for 20 minutes, cooled, and centrifuged. [17]

**Lipoxygenase (LOX) Activity**

Lipoxygenase activity performed using the method of Axelrod et al. The reaction mixture consisted of Tris-HCl buffer (pH 7.4), sodium linoleate, cell lysate. The activity was verified by measuring the increase in absorbance at 234 nm, indicating conjugated dienes the formation.

**Myeloperoxidase (MPO) Activity**

For MPO activity, the cell lysate was homogenized in potassium phosphate buffer containing hexadecyl trimethyl ammonium bromide (HTAB). After centrifugation, the supernatant was used for analysis. The reaction mixture contained phosphate buffer, guaiacol, and hydrogen peroxide, and the change in absorbance was measured at 460 nm. MPO activity was expressed as units per mL of cell lysate.

**Inducible Nitric Oxide Synthase (iNOS) Activity**

The activity of inducible nitric oxide synthase was determined using the method of Salter et al. The reaction mixture included L-arginine, manganese chloride, dithiothreitol (DTT), NADPH, tetrahydropterin, oxygenated haemoglobin, and cell lysate, and the reaction was carried out under controlled conditions.

**Estimation of Cellular Nitrite Levels**

Nitrite levels were calculated using Lepoivre et al method. The cell lysate was treated with sulphosalicylic acid and centrifuged to obtain a protein-free supernatant. Mix supernatant with Tris-HCl buffer and sodium hydroxide and add Griess reagent. Incubate in the dark for 10–15 minutes and measure the absorbance at 540 nm. Use Sodium nitrite as standard to determine the nitrite concentration.

## RESULTS

The phytochemical screening of the root extract of *Pavetta lasioclada* was performed to determine the availability of various bioactive compounds. The qualitative analysis revealed the availability of several important secondary metabolites that may be liable for the medicinal properties of the plant. [18]

**Table 1:** Phytochemical screening result

| Chemical Test | | Results |
|---|---|---|
| Alkaloids | Mayer's test | Positive |
| | Wagner's testd | |
| | Dragendroff's test | |
| Phenols | Ferric chloride test | Positive |
| Saponins | Foam test | Positive |
| Flavonoids | Alkaline test | Positive |
| Glycosides | Keller–Killiani test | Positive |
| Terpenoids | Salkowski's test | Positive |

**Cyclooxygenase (COX) activity:**

**Calculation:**

calculated percentage inhibition of the enzyme using the formula,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

**Table 2:** Result of COX activity

| Concentration (µg/ml) | OD at 632nm | Percentage inhibition |
|---|---|---|
| Sample Code-ARN | | |
| LPS | 0.217 | 0.0000 |
| 25 | 0.182 | 16.1290 |
| 50 | 0.164 | 24.4240 |
| 100 | 0.145 | 33.1797 |

$IC_{50}$ Value- ARN-173.9521µg/mL (Calculated using ED50 PLUS V1.0 Software)

**Lipoxygenase (LOX) activity:**

**Calculation:**

Percentage inhibition of the enzyme was calculated as,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

**Table 3:** Result of LOX activity

| Concentration (µg/ml) | OD at 234nm | Percentage inhibition |
|---|---|---|
| Sample Code-ARN | | |
| LPS | 0.117 | 0.0000 |
| 25 | 0.097 | 17.0940 |
| 50 | 0.082 | 29.9145 |
| 100 | 0.069 | 41.0256 |

$IC_{50}$ Value- ARN- 126.0000µg/mL (Calculated using ED50 PLUS V1.0 Software)

**Myeloperoxidase (MPO) activity:**

U = (ΔOD · 4 · Vt · dilution factor) / (L · £470 ·Δt · Vs)

ΔOD = Optical density change

Vt = total volume (mL) (1.1 mL) & L=light path (1 cm)

£470 = extinction coefficient for tetra guaiacol (26.6 mM-1·cm$^{-1}$)

**Table 4:** Result of LOX activity

| Concentration (µg/ml) | ΔOD | Enzyme Activity (U/ml) |
|---|---|---|
| **Sample Code-ARN** | | |
| LPS | 0.0693 | 0.0229 |
| 25 | 0.0603 | 0.0199 |
| 50 | 0.0487 | 0.0161 |
| 100 | 0.0440 | 0.0145 |

**Inducible Nitric Oxide Synthase:**

**Calculation**

Percentage inhibition of the enzyme was calculated as,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

**Table 5:** Result of INOS assay

| Concentration (µg/ml) | ΔOD | Percentage inhibition |
|---|---|---|
| **Sample Code-ARN** | | |
| LPS | 0.0445 | 0 |
| 25 | 0.0370 | 16.8539 |
| 50 | 0.0273 | 38.6517 |
| 100 | 0.0242 | 45.6180 |

$IC_{50}$ Value- ARN-105.064µg/mL (Calculated using ED50 PLUS V1.0 Software)

**Estimation of Cellular Nitrite Levels**

**Table 6:** Result of Cellular Nitrite Levels

| Concentration (µg/ml) | OD | Concentration of Nitrite (µg) |
|---|---|---|
| **Sample Code-ARN** | | |
| LPS | 0.1025 | 507.375 |
| 25 | 0.0954 | 472.23 |
| 50 | 0.0867 | 429.165 |
| 100 | 0.0730 | 361.35 |

## DISCUSSION

The phytochemical screening of *Pavetta lasioclada* root extract was confirmed the presence of alkaloids, tannins, flavonoids, phenolics, saponins, terpenoids, and glycosides. These compounds are well known for their diverse pharmacological properties. Alkaloids contribute to analgesic and antimicrobial effects, while phenolic compounds and flavonoids provide strong anti-inflammatory and antioxidant activities. Tannins exhibit astringent properties that help in tissue protection and inflammation control. The presence of these metabolites source that the plant possesses significant therapeutic effect. Phytochemicals such as flavonoids, phenolics, and terpenoids play a key role in reducing inflammation. Flavonoids inhibit enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing the formation of inflammatory mediators. Phenolic compounds act as antioxidants and help in minimizing oxidative stress, which is closely associated with inflammatory processes. The root extract showed moderate inhibition of protein denaturation, indicating its anti-inflammatory potential. This activity is mainly attributed to the presence of flavonoids, phenolics, and tannins, which stabilize proteins and prevent inflammatory damage.

The results are consistent with earlier studies on *Pavetta* species, particularly *Pavetta indica*, which also reported similar biological activities due to comparable phytochemical profiles.

The findings support the traditional use of *Pavetta lasioclada* as a medicinal plant. However, further studies such as compound isolation, in-vivo analysis, toxicity evaluation, and clinical trials are required to confirm its therapeutic potential and ensure safe drug development.

## CONCLUSION

In this study the root extract of Pavetta lasioclada was evaluate for finding the phytochemical constituents and anti-inflammatory potential. The plant material was successfully collected, processed, and extracted by Soxhlet extraction method using ethanol as the solvent, ensuring efficient extraction of bioactive compounds. The obtained extract was subjected to preliminary phytochemical screening and in-vitro anti-inflammatory evaluation, which provided strong support to the medicinal potential of the plant.

The phytochemical analysis confirmed the presence of several important secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, and glycosides. These compounds are widely recognized for their diverse pharmacological activities like anti-inflammatory, antimicrobial, antioxidant, and protective effects. The presence of these phytochemicals suggests that the root extract possesses a strong biochemical foundation for therapeutic applications.

The anti-inflammatory activity of the extract was assessed using the protein denaturation method, a widely accepted in-vitro model for evaluating anti-inflammatory potential. The results demonstrated a moderate level of inhibition of protein denaturation, indicating that the extract has the ability to prevent inflammatory processes. This activity may be primarily attributed to flavonoids and phenolic compounds, which are known to inhibit the release of inflammatory mediators and reduce oxidative stress. Additionally, tannins and saponins may also contribute to stabilizing proteins and protecting tissues from damage.

Overall, the findings of this study support the traditional medicinal use of *Pavetta lasioclada* and highlight its potential as a natural source of anti-inflammatory agents. The study emphasizes the importance of medicinal plants in the development of safer and more effective therapeutic alternatives to synthetic drugs, which often produce undesirable side effects.

However, despite the promising results, future research is essential to explore the medicinal potential of this plant. Future studies should look into the isolation, characterization of active compounds, detailed in-vivo pharmacological investigations, toxicity and safety assessments, and clinical trials. Such studies will help in validating the efficacy and safety of *Pavetta lasioclada* and may contribute in the processing of novel plant-based anti-inflammatory drugs.

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