ANTI-INFLAMMATION ACTIVITY OF *JATROPHA PODAGRICA* HOOK EXTRACTS COLLECTING IN DONG NAI

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ABSTRACT

Jatropha podagrica Hook. belongs to the Euphorbiaceae family, and has been traditionally used in folk medicine to alleviate constipation, skin infections, jaundice, and fever. This plant exhibits various pharmacological effects, including antibacterial, anticancer, antioxidant, insect growth inhibition, neuromuscular inhibition, and hypotensive properties. However, its anti-inflammatory activity remains unexplored. In this study, extracts from the leaves, stems, and roots of *Jatropha podagrica* were investigated using the RAW264.7 cells model, which was stimulated with lipopolysaccharide (LPS) to induce inflammation. The findings revealed that the ethyl acetate (EtOAc) fractional from *Jatropha podagrica* roots exhibited potent inhibition of nitric oxide (NO) biosynthesis, with an IC_{50} value of 112.6 ± 8.5 μg/mL. Additionally, the EtOAc extract from *Jatropha podagrica* roots reduced the protein expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in protein expression models. These results suggest that extracts and fractions derived from *Jatropha podagrica* possess promising anti-inflammatory properties, highlighting their potential as valuable resources for the development of future anti-inflammatory drugs.

1. INTRODUCTION

Inflammation represents the body's systemic response to local injury, serving to protect against damaging agents. It is a complex biochemical and cellular process that occurs within damaged tissue, giving rise to mediators known as inflammatory cytokines exudates. These substances play a crucial role in defending the body against infection, as well as promoting tissue recovery and wound healing (Miranda et al., 2023).

All agents capable of causing local cell damage and destruction possess the potential to induce inflammation at the affected site. These causal factors can be broadly categorized into two main groups: external and internal. External triggers of inflammation encompass infections by bacteria, viruses, parasites, and fungi, as well as physical factors like foreign

objects, radiation, temperature fluctuations, UV rays, and various chemical substances including acids, alkalis, and other irritants. Mechanical factors such as external impacts, accidents, and injuries also contribute to inflammation (Miranda et al., 2023). On the other hand, internal triggers are linked to the immune system, manifesting as allergies or hypersensitivity reactions to foreign antigens such as pollen, animal dander, weather conditions, certain food substances, and medications. Additionally, autoimmune diseases represent another significant cause of inflammation, wherein the body produces antibodies against its own healthy cells in specific organs, leading to conditions like rheumatoid arthritis and thyroiditis (Tsutsuki et al., 2024).

In the inflammation process, nitric oxide (NO) is generated, with significant increases observed in activated inflammatory cells (Kopydlowski et al., 1999). Nitric oxide is produced by inducible nitric oxide synthases (iNOS) within macrophages, hepatocytes, and renal cells, stimulated by factors such as lipopolysaccharide (LPS), tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), or interferon-gamma (IFN-γ). The excessive production of NO by iNOS is implicated in the pathology of various inflammatory disorders, including septic shock, tissue damage following inflammation, and rheumatoid arthritis (Min et al., 2009; Adebayo et al., 2019). Hence, NO production induced by LPS through iNOS serves as a marker of inflammation severity, and modulation of NO levels through inhibition of iNOS enzyme activity could be a strategy for evaluating anti-inflammatory effects (Yang et al., 2009).

Jatropha podagrica Hook (Dầu lai có củ), belonging to the genus Jatropha, is recognized as a valuable medicinal herb utilized in the treatment of various diseases. Besides its common name, it is known by other names such as Vạn linh, Sen núi, Ngô đồng, Sen lục bình (Đỗ Huy Bích et al., 2006). Jatropha (Euphorbiaceae family) is distributed widely across the tropics, spanning from the Americas to Africa and Asia (Sharma and Singh 2012). *J. podagrica* contains cyclic peptides (Van den Berg et al., 1996; Odebiyi 1982), xanthophyll, fatty oils, and fraxidine, fraxetin, scoparone, 3-acetyl aleuritolic acid, β-sitosterol, and sitosterone (Jang et al., 2023, Chen et al., 2019), flavonoids, and diterpenoids (Chen et al., 2019; Liu et al., 2014). In traditional Chinese medicine, *J. podagrica* is used to possess heat-clearing, detoxifying, draining, and dissolving properties (Đỗ Huy Bích et al., 2006). Folk experience suggests that its leaves can be used to treat scabies, while the crushed leaf stalks are applied for treating uterine prolapse. Furthermore, the leaf stalks and stems are pounded and used to make a decoction for treating hemorrhagic coughs and dysentery (Đỗ Huy Bích et al., 2006). Some compounds isolated from *J. podagrica* exhibit antibacterial activity (Odebiyi 1980), while others show neuromuscular blocking effects and cytotoxicity in humans (Sharma and Singh 2012). The plant also demonstrates other biological activities such as antioxidant properties and insect growth inhibition (Minh et al., 2019; Odebiyi 1980; Jang et al., 2023, Huynh et al., 2024).

In our screening program, the ethanolic extract of *J. podagrica* exhibited significant inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 cells *in vitro*. However, despite its traditional use and documented pharmacological activities, there are currently no studies on the anti-inflammatory effects of *J. podagrica* in Vietnam. Therefore, this study aims to investigate the anti-inflammatory effect by assessing its ability to inhibit NO formation in RAW264.7 cells stimulated by LPS-induced inflammation.

2. METHODOLOGY

2.1. Plant collection

Jatropha podagrica (DLCC) plant were collected in October 2022 from Phu Lap commune, Tan Phu district, Dong Nai province. The specimen is currently stored at the Laboratory of the Department of Pharmacognosy and Drug Control, School of Medicine and Pharmacy, The University Da Nang.

2.2. Extraction

The fresh leaves, stems, and roots of *J. podagrica* were dried in the shade and then ground into powder form. Powder samples of leaf (50 g), stem (50 g), and root (50 g) were subjected to reflect extraction with 70% ethanol (250 mL × 3 times). The resulting extract solution was recovered and filtered through filter paper. Subsequently, the filtered solution underwent solvent evaporation under reduced pressure using a rotary vacuum evaporator, yielding a 70% ethanol extraction residue of leaves, stems, and roots. This extraction residue was then dissolved in warm water (200 mL) and fractionated using *n*-hexane and ethyl acetate to obtain *n*-hexane (Hex) and ethyl acetate (EtOAc) fractions, along with the remaining water fraction (W). These fractionated samples were stored at 4°C in a refrigerator until use. Detailed information regarding the extract samples and fractions is presented in Table 1.

Table 1. Sample codes

Samples	Extracts/ Fractions	Code	Weight (g)
Leaves			
1	70%EtOH	L-EtOH	3.82
2	*n*-hexane fraction	L-Hex	0.85
3	EtOAc fraction	L-EA	1.12
Stems			
4	70%EtOH	S-EtOH	4.15
5	*n*-hexane fraction	S-Hex	1.21
6	EtOAc fraction	S-EA	1.23
Roots			
7	70%EtOH	R-EtOH	2.74
8	*n*-hexane fraction	R-Hex	0.85
9	EtOAc fraction	R-EA	0.91

2.3. Cell culture and cell viability assay

The RAW 264.7 cells (American Type Culture Collection, Rockville, MD, USA) were maintained at sub-confluence in a humidified 5% CO_2 atmosphere at 37 °C condition. The Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY, USA) with supplementation of 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 µg/mL) was used to cell culture. After 24 and 48 hours, the cells were collected and counted by a hemocytometer, the number of viable cells was determined by trypan blue dye exclusion. The viability assay was evaluated by MTS [3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] method with a slight modification (Dewi et al., 2015; Cuong et al., 2015).

2.4. NO production inhibition

The determination of NO concentration relies on the conversion of nitrate to nitrite catalyzed by the enzyme nitrate reductase. The resulting nitrite is measured, as it is a product of the Griess reaction (Dewi et al., 2015; Cuong et al., 2015). This reaction involves the diazotization of NO_2, which produces a nitrifying agent that reacts with sulfanilic acid to form a diazonium ion. Subsequently, this ion combines with ethylenediamine N-(1-naphthyl) to generate a chromophore azo derivative that absorbs light at wavelengths between 540 and 570 nm. The RAW264.7 macrophage anti-inflammatory cell line (ATCC, Rockville, MD, USA) was cultured in Petri dishes using

Dulbecco's Modified Eagle Medium (DMEM; Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma, USA), 100 units/mL penicillin, and 100 µg/mL streptomycin. The cells were maintained in an incubator at 37°C with 5% CO_2. After incubation, the cells were harvested by centrifugation at 1000 rpm for 3 minutes. The supernatant was removed, and the cell pellet was resuspended in 10 mL of 10% DMEM-FBS medium. The cell density was adjusted to the desired level for further experimentation. The NO concentration in the culture medium was determined by reacting NO with the Griess reagent. Specifically, 100 µL of culture medium was mixed with an equal volume of Griess reagent (consisting of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water). The mixture was incubated for 10 minutes at room temperature in the dark. The absorbance was then measured at 540 nm using a 96-well reader. The percentage inhibition concentration and IC_{50} inhibition concentration were calculated using the formula:

Inhibition concentration (%) = [1 − (B − C)/(A – C)] × 100

Where:

A: Absorbance for LPS (+), test sample (−)

B: Absorbance for LPS (+), test sample (+)

C: Absorbance for LPS (−), test sample (−)

A standard curve of NO was constructed using sodium nitrite at various concentrations (0, 12.5, 25.0, 50.0, and 100.0 µM), and the optical absorbance was measured at 540 nm wavelength.

2.5. Evaluation of iNOS and COX-2 by Western Blot analysis

RAW264.7 cells were washed with phosphate buffered saline (PBS) and subsequently incubated and lysed using a buffer containing 10% glycerol, 1% Triton X-100, 1 mM Na_3PO_4, 1 mM egtazic acid (EGTA), 10 mM NaF, 1 mM $Na_4P_2O_7$, 20 mM Tris buffer (pH 7.9), 100 mM β-glycerophosphate, 137 mM NaCl, and 5 mM ethylene diamine tetraacetic acid (EDTA) on ice for approximately 1 hour. The lysed samples were then centrifuged at 12000 rpm for 30 minutes at 4°C, and the protein pellets were washed several times with PBS. Subsequently, the proteins were dissolved in cold PBS in preparation for gel electrophoresis. Approximately 20 - 30 µg of protein was loaded onto a 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel for separation of the proteins by electrophoresis. Following electrophoresis, the separated proteins were transferred from the gel to a polyvinylidene difluoride membrane. The membrane-bound proteins were blocked with 5% skim milk at room temperature in Tris-buffered saline with Tween (TBST; 20 mM Tris, 500 mM NaCl, pH 7.5, 0.1% Tween 20). The membrane was then incubated with monoclonal anti-iNOS or anti-COX-2 antibodies (diluted at 1:1000) in 5% nonfat dry milk/TBST for 2 hours at room temperature. After incubation with the primary antibodies, the membrane was washed three times with TBST at room temperature and then incubated with an IgG secondary antibody (anti-mouse IgG secondary antibody, Sigma, St. Louis, MO, USA) diluted at a ratio of 1:2000 in 2.5% nonfat dry milk/TBST for 1 hour at room temperature. Following incubation with the secondary antibody, the protein-containing membrane was washed three times with TBST, and immunoreactive proteins were detected using chemiluminescence (ECL, Amersham International PLC, Buckinghamshire, UK) and visualized using hyperfilm and photochemical reagents. The Western blot results were quantified by measuring the optical density correlation compared to control samples using Fujifilm Image Reader Las-4000 software

(FujiFilm Corp., Tokyo, Japan) (Dewi et al., 2015; Cuong et al., 2015).

3. FINDINGS AND DISCUSSION

To assess the cytotoxic effects of the extracts and fractions on RAW 264.7 cells, we employed the MTS assay on both LPS-stimulated and unstimulated cell groups. The findings indicated that the extracts and fractions from the leaves (L-EtOH, L-Hex, L-EA), stems (S-EtOH, S-Hex, S-EA), and roots (R-EtOH, R-Hex, R-EA) of DLCC, did not impact cell viability, even at high concentrations of 300 µg/mL, following 24 hours of incubation. This demonstrates that the extracts derived from all three sources as leaves, stems, and roots are non-toxic to RAW 264.7 cells (Figure 1).

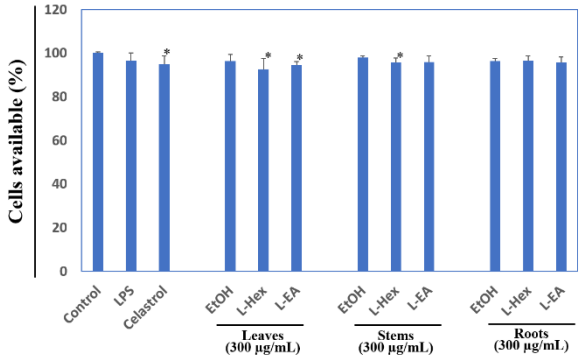

Figure 1. Effect on cell viability by LPS stimulation in the presence of DLCC. Cell viability was determined by MTS assay and expressed as a percentage of the control without sample addition. Data were expressed as mean ± SD (n = 3). *$p < 0.01$ compared to negative control. Celastrol (1.0 µM) was used as a positive control.

Based on the initial preliminary results, samples extracted from the leaves, stems, and roots of DLCC exhibited non-cytotoxic effects on RAW264.7 cells even at high concentrations. Consequently, these samples were further investigated for their potential to inhibit NO production. In this experiment, RAW264.7 cells were treated with extracts ranging from 0 to 300 µg/mL, and the level of NO production, indicative of inflammation induced by LPS, was measured by assessing nitrite concentration in the cell culture supernatant. According to the findings presented in Table 2, the 70% EtOH extracts from leaves, stems, and roots exerted weak inhibitory effects on NO production by RAW264.7 cells during inflammation, with respective IC_{50} values of 255.6 ± 10.5, 284.3 ± 10.7, and 265.0 ± 12.0 µg/mL. Notably, the extract obtained from the DLCC stem, S-EtOH, demonstrated a weak or possibly ineffective inhibitory effect, with an IC_{50} value of 284.3 ± 10.7 µg/mL. Meanwhile, during the evaluation of RAW264.7 cells with three samples of *n*-hexane fraction extract as L-Hex, S-Hex, and R-Hex, it was observed that all samples exhibited inhibition values of NO production with relatively high IC_{50} values exceeding 300 µg/mL. This suggests that oily compounds, long-chain molecules, steroids, or non-polar compounds present in the *n*-hexane fractions derived from leaf, stem, and root samples lack significant anti-inflammatory effects.

Interestingly, the EtOAc fractions derived from leaves, stems, and roots exhibited significant inhibition of NO production. The leaf extract, L-EA, demonstrated a notable effect with an IC_{50} value of 140.5 ± 7.8 µg/mL, while the stem extract, S-EA, displayed an inhibitory effect with an IC_{50} value of 162.6 ± 7.3 µg/mL. Particularly noteworthy was the tuber extract, R-EA, which exerted the strongest inhibitory effect on NO production, boasting an IC_{50} value of 112.6 ± 8.5 µg/mL. In this experiment, celastrol, a natural secondary metabolite, served as a positive control, demonstrating potent inhibition of LPS-induced NO production with an IC_{50} value of 1.0 µM. This indicates that the majority of polar compounds present in the EtOAc fraction of DLCC plants possess notable anti-inflammatory effects against LPS-induced inflammation in the RAW264.7 cell line. Based on these results, the R-EA root extract exhibited a robust inhibitory effect on LPS-induced NO production in RAW264.7 cells during inflammation.

Table 2. NO production inhibitions in RAW264.7

Samples	Extracts Fractions	Code	IC_{50} value (μg/mL)[a)]
Leaves			
1	70%EtOH	L-EtOH	255.6 ± 10.5
2	*n*-hexane fraction	L-Hex	> 300
3	EtOAc fraction	L-EA	140.5 ± 7.8
Stems			
4	70%EtOH	S-EtOH	284.3 ± 10.7
5	*n*-hexane fraction	S-Hex	> 300
6	EtOAc fraction	S-EA	162.6 ± 7.3
Roots			
7	70%EtOH	R-EtOH	265.0 ± 12.0
8	*n*-hexane fraction	R-Hex	> 300
9	EtOAc fraction	R-EA	112.6 ± 8.5
10	Celastrol[c)]		1.0 ± 0.1[c)]

[a)] The 50% inhibitory concentration (IC_{50}) value was determined in comparison with the control batch; [b)] The inhibition value of the extract was measured at a concentration of μg/mL; [c)] The positive control was in μM. S.D. standard deviation, n=3.

Based on the initial preliminary results, samples extracted from the leaves, stems, and roots of DLCC exhibited non-cytotoxic effects on RAW264.7 cells even at high concentrations. Consequently, these samples were further investigated for their potential to inhibit NO production. In this experiment, RAW264.7 cells were treated with extracts ranging from 0 to 300 μg/mL, and the level of NO production, indicative of inflammation induced by LPS, was measured by assessing nitrite concentration in the cell culture supernatant. According to the findings presented in Table 2, the 70% EtOH extracts from leaves, stems, and roots exerted weak inhibitory effects on NO production by RAW264.7 cells during inflammation, with respective IC_{50} values of 255.6 ± 10.5, 284.3 ± 10.7, and 265.0 ± 12.0 μg/mL. Notably, the extract obtained from the DLCC stem, designated as S-EtOH, demonstrated a notably weak or possibly ineffective inhibitory effect, with an IC_{50} value of 284.3 ± 10.7 μg/mL.

Meanwhile, during the evaluation of RAW264.7 cells with three samples of *n*-hexane fraction extract as L-Hex, T-Hex, and R-Hex, it was observed that all samples exhibited inhibition values of NO production with relatively high IC_{50} values exceeding 300 μg/mL. This suggests that oily compounds, long-chain molecules, steroids, or non-polar compounds present in the *n*-hexane fractions derived from leaf, stem, and root samples lack significant anti-inflammatory effects.

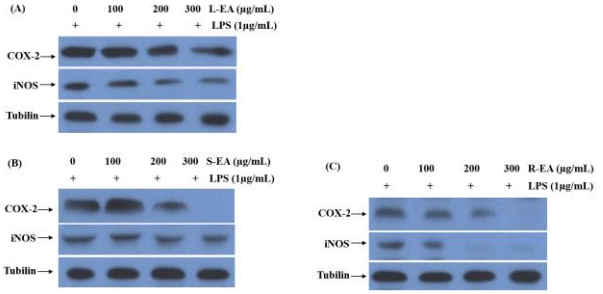

Figure 2. LPS-induced inhibition of iNOS protein expression in RAW264.7 cells treated with EtOAc fractions from DLCC leaves, stems, and roots. RAW264.7 cells were pretreated for 30 minutes with L-EA (A), S-EA (B), and R-EA (C) at concentrations ranging from 0 to 300 μg/mL, followed by stimulation with LPS (1 μg/mL) for 24 hours. Protein localization was performed using specific antibodies as indicated. The expression level of α-tubulin served as a control. The expressions of iNOS and COX-2 were assessed through immunoblot analysis of protein detection via SDS-PAGE gel electrophoresis.

Interestingly, the ethyl acetate fractions derived from leaves, stems, and roots exhibited significant inhibition of NO production. The leaf extract, L-EA, demonstrated a notable effect with an IC_{50} value of 140.5 ± 7.8 μg/mL, while the stem extract, S-EA, displayed an inhibitory effect with an IC_{50} value of 162.6 ± 7.3 μg/mL. Particularly noteworthy was the tuber extract, R-EA, which exerted the strongest inhibitory effect on NO production, boasting an IC_{50} value of 112.6 ± 8.5 μg/mL. In this experiment, celastrol, a natural secondary metabolite, served as a positive control, demonstrating potent inhibition of LPS-induced NO production with an IC_{50} value of 1.0 μM. This indicates that the majority of polar compounds present in the EtOAc fraction of DLCC plants possess notable anti-inflammatory effects against LPS-induced inflammation in the RAW264.7 cell line. Based on these results, the R-EA root extract exhibited a robust inhibitory effect on LPS-induced NO production in RAW264.7 cells during inflammation.

In the subsequent experiment, three extract samples of ethyl acetate fractions derived from DLCC leaves, stems, and roots were selected to assess their interaction abilities and potential inhibitory effects on iNOS and COX-2 (Miranda et al., 2023). iNOS is one of the primary enzymes responsible for synthesizing NO from the amino acid L-arginine. NO produced by iNOS plays a pivotal role in various pathophysiological processes, including regulation of blood pressure, inflammation, infection, and oncogenesis. iNOS has been extensively investigated as a marker and therapeutic target for the aforementioned conditions, particularly inflammation. On the other hand, COX-2 is an enzyme responsible for generating inflammatory mediators such as prostaglandins (PG) and their metabolites, including PGE2, PGF2α, and PGD2. Protein levels of both iNOS and COX-2 enzymes were undetectable in RAW 264.7 cells under normal conditions without LPS stimulation (Min et al., 2009). However, upon inflammatory stimulation by LPS, the expression of these cytokines was significantly upregulated at the protein level (Figure 2).

For the leaf samples, the L-EA extract exhibited a weak effect, as evidenced by the prominent, intense display of iNOS and COX-2 protein fragments (Figure 2A). This indicates that compounds within the L-EA extract may alter the protein content of these two enzymes, albeit weakly, necessitating further investigation into other mechanisms. Significantly, upon increasing the concentration of S-EA extracts from DLCC stem samples from 100 to 300 μg/mL, the protein levels of both iNOS and COX-2 enzymes gradually decreased in LPS-stimulated cells (Figure 2B). This suggests that all three EA fraction extracts have a concentration-dependent effect on inhibiting the expression of inflammatory enzymes. Particularly noteworthy in R-EA root extracts, within the concentration range of 0-300 μg/mL, the protein fragments of COX-2 became faint and were expressed quite weakly, especially at the concentration of 300 μg/mL. With iNOS, protein fragments were also gradually expressed, albeit with a lesser effect. This indicates that compounds within the S-EA extract may interact more strongly with COX-2 than with iNOS. However, in the case of the R-EA extract sample, the expression of both COX-2 and iNOS enzymes was notably reduced (Figure 2C). At a concentration of 300 μg/mL, the protein content of COX-2 was completely reduced. Particularly at concentrations of 200 and 300 μg/mL, the R-EA extract completely reduced iNOS protein fragments. This suggests that the R-EA sample exerts a strong and direct effect on iNOS, leading to its inactivation and consequently reducing the NO concentration in RAW264.7 cell cultures when stimulated by LPS. In this western blotting experiment, the expression of α-tubulin protein remained unchanged in all three experimental batches.

Anti-inflammatory drugs can interfere with the pathophysiology of inflammation to minimize tissue damage and provide greater patient comfort. Therefore, given the large number of species available for research, the successful development of new naturally occurring anti-inflammatory drugs largely depends on multidisciplinary efforts to discover new molecules (Lima et al., 2018).

The main groups of anti-inflammatory drugs are glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs). Glucocorticoids work by inhibiting prostaglandins and proteins involved in inflammation, such as corticosteroids, and are also used in the treatment of asthma and autoimmune inflammatory responses. On the other hand, NSAIDs inhibit the COX enzyme and are used to relieve mild to moderate pain and control body temperature (Virshette et al., 2019). The primary mechanism action of NSAIDs is COX inhibition, both centrally and peripherally, interfering with the conversion of arachidonic acid to PGE2, prostacyclins, and thromboxane. The enzymes involved in NSAID action are COX-1 and COX-2, which function in different regions. COX-1 is found in most cells, including amniotic and fetal fluids, and is involved in regulatory and protective effects. Conversely, COX-2 is activated by inflammatory and proinflammatory cytokines (Bassiouni et al., 2019).

Plants are a rich source of natural compounds for the development of new drugs, which has increased the interest of the pharmaceutical industry in exploring plant-derived substances. This interest is particularly strong because most species have not been studied chemically or biologically, especially in terms of their anti-inflammatory properties (Newman and Cragg, 2016). Therefore, screening and developing anti-inflammatory drugs from medicinal plants is crucial, and plants have become a primary source of raw materials for developing new drugs, with many prescribed drugs worldwide originating from them (Shah et al., 2011; Li et al., 2020).

In recent decades, hundreds of research articles have been published on the anti-inflammatory activity of plants. Phytochemical studies of plants in the Anacardiaceae family have identified phenols, triterpenes, flavonoids, and cinnamic acid as compounds with anti-inflammatory effects (Shah et al., 2011; Cabral et al., 2016). The Euphorbiaceae family is primarily rich in phenolic compounds, saponins, tannins, and triterpenes, which are responsible for their anti-inflammatory effects. Research on plants from the Apocynaceae, Boraginaceae, Rubiaceae, Solanaceae, and Zingiberaceae families also shows the presence of compounds with anti-inflammatory activity Shah et al., 2011; Zahidin et al., 2017).

It is important to note that the extraction of plant material is a crucial first step in testing for biological activities. Previous articles have highlighted that research on plant extracts has several advantages and some disadvantages compared to isolating pure active compounds. When whole extracts are used, the synergy between the active ingredients may be diminished compared to extracting individual pure compounds (Azab et al., 2016). However, there are many instances where compounds within the same extract have complementary effects on each other, creating stronger activity. This phenomenon is known as a synergistic effect. This synergistic effect has been observed in several clinical trials, including those for prophylactic and anti-inflammatory activities. Conversely, mixing different compounds can also result in an inhibitory effect, where one ingredient reduces the biological activity of another. Supporting this assumption, several studies have shown that the anti-inflammatory activity of pure compounds is higher than that of extracts (Maione et al., 2015; Azab et al., 2016).

In this experiment, we used the extract of DLCC to examine the anti-inflammation. In folk medicine, DLCC is commonly used to

relieve constipation, skin infections, jaundice, and fever. Additionally, DLCC has various pharmacological effects, including antibacterial, anticancer, antioxidant, insect growth inhibition, neuromuscular inhibition, and hypotensive properties (Huynh et al., 2024). However, the anti-inflammatory activity of DLCC remains unexplored. In this study, extracts from the leaves, stems, and roots of DLCC were studied using the RAW264.7 cell model, which was stimulated with LPS to induce inflammation. Findings showed that the EtOAc fraction from DLCC roots exhibited strong inhibition of NO biosynthesis with an IC_{50} value of 112.6 ± 8.5 µg/mL. Additionally, the EtOAc extract from DLCC roots reduced the protein expression of COX-2 and iNOS. Our previous research demonstrated that DLCC contains many primary chemical components, including diterpenoids, coumarin, aliphatic acids, ferulic acid esters, alkaloids, flavonoids, volatile oil compounds, and various other important compounds. These exhibit a diverse range of pharmacological properties, including antibacterial, anti-inflammatory, antioxidant, insect growth inhibitory, anti-tumor, and antiviral activities (Huynh et al., 2024). Based on the experimental results, we assert that the extracts and fractions of DLCC have promising anti-inflammatory properties, highlighting their potential as a valuable resource for the development of future anti-inflammatory drugs. The findings presented in this review underscore the scientific basis for further exploration and research on DLCC.

4. CONCLUSION

Jatropha podagrica were evaluated for their anti-inflammatory potential in a model assessing their ability to inhibit LPS-induced NO production in RAW264.7 macrophage cells. The study revealed that the ethyl acetate fraction from *Jatropha podagrica* roots exhibited significant inhibition of nitric oxide biosynthesis, with an IC_{50} value of 112.6 ± 8.5 µg/mL. Furthermore, the EtOAc extract *Jatropha podagrica* roots reduced the protein expression of COX-2 and iNOS in protein expression models. These findings suggest that extracts and fractions derived from *Jatropha podagrica* hold promise as anti-inflammatory agents, primarily due to their ability to inhibit NO production.

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NGHIÊN CỨU HOẠT TÍNH KHÁNG VIÊM CỦA CHIẾT XUẤT *JATROPHA PODAGRICA* HOOK THU HÁI TẠI ĐỒNG NAI

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TÓM TẮT

Cây dầu lai có củ (DLCC), *Jatropha podagrica* Hook. thuộc họ Euphorbiaceae thường được sử dụng trong y học dân gian để điều

Ngày duyệt đăng: 13/06/2024

TỪ KHOÁ

Euphorbiaceae;

Viêm;

Jatropha podagrica Hook;

RAW264.7

Ức chế sản sinh NO.

trị táo bón, nhiễm trùng da, vàng da và sốt. Loại cây này có nhiều tác dụng dược lý khác nhau, bao gồm kháng khuẩn, chống ung thư, chống oxy hóa, ức chế sự phát triển của côn trùng, ức chế thần kinh cơ và hạ huyết áp. Tuy nhiên, hoạt tính chống viêm của nó vẫn chưa được khám phá. Trong nghiên cứu này, chiết xuất từ lá, thân và rễ của cây DLCC đã được nghiên cứu trên mô hình tế bào RAW264.7 được kích thích gây viêm bởi lipopolysaccharide (LPS). Kết quả cho thấy phân đoạn etyl axetat (EtOAc) từ rễ cây DLCC ức chế mạnh quá trình sinh tổng hợp oxit nitric (NO), với giá trị IC_{50} là 112,6 ± 8,5 µg/mL. Ngoài ra, chiết xuất EtOAc từ rễ cây DLCC làm giảm sự biểu hiện protein cyclooxygenase-2 (COX-2) và nitric oxit synthase cảm ứng (iNOS) bằng phương pháp Western Blot. Những kết quả này cho thấy các chất chiết xuất và các phân đoạn từ DLCC có đặc tính chống viêm đầy hứa hẹn, và có thể là nguồn tài nguyên quý giá để phát triển thành thuốc kháng viêm trong tương lai.