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Original Research Article

In Vitro anti-inflammatory and antiviral activities of *Pluchea indica* (L.) Less. extracts on human monocytes

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ABSTRACT

Pluchea indica (L.) Less., a halophyte from the Asteraceae family, thrives in saline soils and has traditional medicinal uses. This study aimed to investigate the *in vitro* antioxidant activity, as well as the anti-inflammatory and antiviral potential of ethanol extracts from *P. indica*. The crude extract was obtained by refluxing *P. indica* with 70% ethanol. Phytochemical analysis was conducted, and antioxidant properties were assessed. Toxicity was evaluated in white blood cells, red blood cells, and the THP-1 cell line. The anti-inflammatory effects were analyzed by measuring TNF-*α* and TGF-β mRNA expression, while antiviral properties were assessed via IFN-β, TLR3, TLR7, TLR8, and TLR9 mRNA expression in THP-1 cells using quantitative RT-PCR. All ethanol extracts contained alkaloids, phenolics, flavonoids, coumarins, tannins, and terpenoids; however, steroids were only present in the flower extract. Higher levels of phenolic and flavonoid compounds were found in the leaf and flower extracts, correlating with antioxidant activity. None of the extracts exhibited cytotoxicity to immune cells. The leaf extract demonstrated anti-inflammatory properties by decreasing TNF-*α* mRNA expression while stimulating TGF-β mRNA expression. The root extract showed antiviral effects by inducing IFN-β mRNA expression. Additionally, the stem extract exhibited both anti-inflammatory and antiviral properties by reducing TNF-*α* mRNA expression and enhancing the expression of TGF-β, IFN-β, TLR3, TLR8, and TLR9 mRNA. This study indicates that ethanol extracts of *P. indica*, prepared via reflux extraction, possess *in vitro* antioxidant, anti-inflammatory, and antiviral properties in human immune cells, suggesting their potential as therapeutic agents for inflammatory and viral diseases.

Keywords: Pluchea indica (L.) Less., Anti-inflammatory, Antiviral, Halophyte

Introduction

Halophytes are plants that thrive in saline soils and have been utilized for medicinal purposes due to their rich phytochemical content.[1] Soils with salinity levels between 0.5% and 0.75% are classified as extremely saline and can be detrimental to plant growth.[2] Despite their traditional medicinal uses, research on halophytes as sources of pharmacological compounds remains limited. Many halophytes are useful for traditional medicines, which are more effective than non-saline-alkali land in using as raw materials for medicines, such as *Mesembryanthemum edule* L., which can be used to treat sinusitis, diarrhea, eczema in children and tuberculosis, etc.[3] Consequently, there is renewed interest in screening halophytes for antimicrobial properties.[1]

Pluchea indica (L.) Less., a member of the Asteraceae family, is a small shrub classified as a halophyte found in the Chi River Basin of northeastern Thailand.[4] This species thrives in saline soils with salt concentrations exceeding 50%, indicating very high salinity at Phonsim in Kalasin Province, Thailand.[2]

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Previous studies have reported various medicinal properties of *P. indica*, including significant inhibition of glioblastoma cell growth by hexane extracts from its roots.[5]

There have been reports on the anti-inflammatory potential of methanol extracts from *P. indica* roots,[6] as well as strong anti-inflammatory effects due to a suppression of nitric oxide (NO) production from essential oils derived from fresh leaf and stem barks.[7] Additionally, ethanol extracts from *P. indica* herbal tea leaves have demonstrated anti-inflammatory activity on endothelial cells.[8] However, the anti-inflammatory and antiviral properties of ethanol extracts of *P. indica* collected from saline soil areas remain unexplored. Therefore, this study aimed to evaluate the preliminary phytochemical composition, antioxidant capacity, cytotoxicity, anti-inflammatory effects, and antiviral activities of the ethanol extract of *P. indica* on THP-1 cells, an immune cell line. Despite its reported anti-inflammatory and anticancer properties, research into the antiviral and anti-inflammatory potential of ethanol extracts derived from *P. indica* grown in saline environments remains limited, warranting further exploration. By elucidating the biological activities associated with this halophyte, the study may contribute to the development of novel strategies for utilizing *P. indica* in medicinal applications.

Materials and Methods

Plant material

P. indica (L.) Less. (Figure 1A) was collected in December 2019 from saline soil in Ban Phonsim, Hua Na Kham Sub-district, Yang Talat District, Kalasin Province, located at 16°24'01.8"N; 103°16'13.1"E (Supplement 1). The plant was identified by Professor Khwanruan Naksuwankul (Ph.D.) from the Faculty of Science, Mahasarakham

University, following the Flora of Thailand guidebook and reference document.[9] Voucher specimens were deposited in the Natural Medicinal Mushroom Museum at Mahasarakham University under the code number MSUT7447. The plant was separated into root, stem, leaf, flower, and bark components, which were then rinsed and dried at 50°C for three days. All parts were ground and passed through an 80-mesh screen to obtain a fine powder.[10]

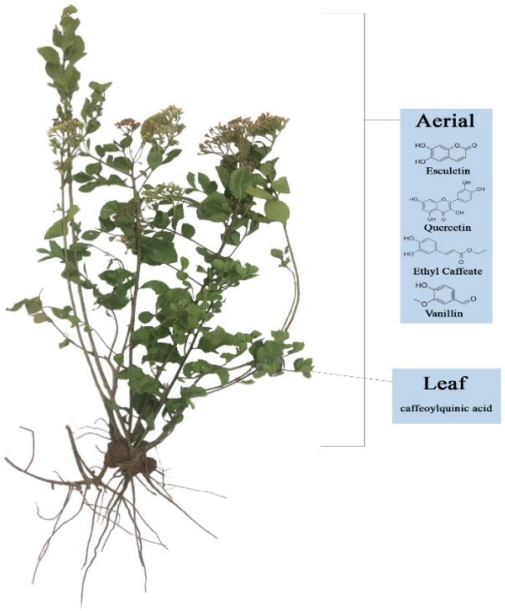


Figure 1: *Pluchea indica* (L.) Less. and reported compounds found in parts of this plant

Crude extract preparation and plant yield
Extraction was performed using the reflux method with a ratio of 1:10 g/mL of the powdered plant material in 70% ethanol for five days. The extracts were filtered using Whatman paper (No. 4), and the solvent was removed using a rotary evaporator. The resulting crude extract was freeze-dried and dissolved in 100% dimethyl sulfoxide (DMSO) before being filtered through 0.45 µm and 0.22 µm filters and stored at -20°C.[11] The yield of the crude extract was calculated using the following equation:[12]

$$\% \text{ Yield} = \frac{weight\ of\ crude\ extract}{weight\ of\ dried\ sample)} \times 100$$

Qualitative phytochemical screening
Phytochemical screening of all crude extracts from *P. indica* was conducted using standardized methods,[13] assessing for alkaloids, phenolics, flavonoids, anthraquinones, coumarins, saponins, tannins, terpenoids, steroids, and glycosides.

Total phenolic content (TPC) and Total flavonoid content (TFC) analysis
The TPC of crude extracts was determined using the Folin–Ciocalteu colorimetric method as previously described, the result was expressed as gallic acid equivalents (mg GAE/g crude extract). The TFC was measured using a colorimetric assay previously described, the result was expressed as quercetin equivalents (mg QE/g crude extract).[10]

Antioxidant assays
In vitro antioxidant activity was evaluated using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and the 2,2'-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assays as previously described.[10]

Thin-layer chromatographic analysis
Crude extracts of *P. indica* were applied to thin-layer chromatography (TLC) aluminum silica gel 60 sheets (Sigma, US). The TLC sheets were developed with two mobile phases: condition 1) absolute ethanol and condition 2) methanol: deionized water (30:70 v/v). After drying, spots on the developed plates were visualized under short UV light (254 nm) and visible light after being sprayed with 5% sulfuric acid in absolute ethanol for color reaction and heating. The retardation factor (Rf) was calculated using the formula:

$$Rf = \frac{distance\ travelled\ by\ compound}{distance\ travelled\ by\ solvent\ front}$$

Isolation of CD14⁺ monocytes from peripheral blood and cell culture
Peripheral blood was collected from six healthy adult volunteers who provided written consent. The study received approval from the Human Ethics Research Committee of Mahasarakham University (No. 208/2563) on July 23, 2020. Peripheral white blood cells (WBCs) were isolated to obtain $CD14^+$ monocytes following the previously described procedure.[10] The WBCs, along with $CD14^+$ and $CD14^-$ monocytes, were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Thermo Fisher, USA) supplemented with 5% fetal bovine serum (FBS), 5% autologous serum, and 1X antibiotic-antimycotic solution at 37°C with 5% CO2.

Analysis of the survival rate of crude extract on white blood cells and THP-1 cell line
The effects of crude extracts on WBCs, $CD14^+$ monocytes, $CD14^-$ non-monocytes, and THP-1 cell survival were assessed using the MTT assay.[11] Briefly, cells were seeded at a density of $2x10^4$ cells/well into a 96-well culture plate, followed by treatment with crude extract concentrations ranging from 0 to 512 µg/mL for WBCs and from 0 to 400 µg/mL for THP-1 cells. DMSO (0.5%) served as a negative control while propolis (0–10%) acted as a positive control. Following incubation for either 48 hours for WBCs or both 24 and 48 hours for THP-1 cells, MTT solution was added at a concentration of 12 mM (Gibco, USA) in darkness. Formazan crystals formed were dissolved in 100 µL of DMSO. Absorbance was measured at 540 nm to determine cell viability using the formula:

$$\% \text{ Viable cells} = \frac{OD_{sample}}{OD_{control}} \times 100$$

In vitro red blood cell hemolysis assay of P. indica extracts
Peripheral blood from six healthy volunteers was centrifuged at 2,000 rpm for ten minutes to isolate red blood cells (RBCs), which were then processed to achieve a concentration of 2% RBCs in PBS. The hemolysis assay was conducted according to previous described procedure, utilizing crude extracts at concentrations ranging from 0 to 512 µg/mL. [11] Negative controls included PBS and DMSO at a concentration of 0.1%, while Triton X-100 at a concentration of 0.1% served as a positive control. Hemolysis percentage was calculated using the equation:

$$\% \text{ Haemolysis} = \frac{OD_{sample} - OD_{negativecontrol}}{OD_{positivecontrol} - OD_{negativecontrol}} \times 100$$

In vitro Evaluation of the anti-inflammatory and antiviral effects
The anti-inflammatory and antiviral effects of crude extracts on THP-1 cells were analyzed using quantitative reverse transcription polymerase chain reaction (RT-qPCR) as previously described. [11] Briefly, THP-1 cells were seeded at a density of $1x10^6$ cells per well in a six-well culture plate and pre-treated for two hours with either LPS-EK or Poly(I:C) HMW at concentrations of 100 ng/mL each as TLR4 and TLR3 agonists respectively. After pre-treatment, crude extracts were added at concentrations of 25 and 50 µg/mL and incubated for an additional twenty-four hours. Total RNA was extracted from treated THP-1 cells using the Trizol method. mRNA expression levels of TNF-α and TGF-β were measured for anti-inflammatory activity while IFN-β, TLR3, TLR7, TLR8, and TLR9 expression levels were assessed for antiviral activity using specific primers listed in Table 1. Experimental groups included extract-treated group (1), pre-treated with LPS group (2), pre-treated with Poly(I:C) group (3), LPS-treated positive control group (4), Poly(I:C)-treated positive control group (5), and negative control group (6).

Table 1: Primers used for quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Gene	Forward primer 5´-3´	Reverse primer 5´-3´
GAPDH	GGACCTGACCTGCCGTCTAG	TAGCCCAGGATGCCCTTGAG
TNF	CATACTGACCCACGGCTTCAC	CGTCCCGGATCATGCTTTC
TGFB1	CCGCGTGCTAATGGTGGAAAC	GAGGTATCGCCAGGAATTGTTGC
IFNB1	TGCTCTGGCACAACAGGTAGTAG	GGAGGAGACACTTGTTGGTCATG
TLR3	CAGCCGCCAACTTCACAAGG	CAGTCAAATTCGTGCAGAAGGCA
TLR7	ACTCTGCCCTGTGATGTCACTC	GTGAGGGTGAGGTTCGTGGT
TLR8	CCACCCAAACTGCCAAGCTC	CACCATCACAAGGCACGCATG
TLR9	GGGATGTAGGCTGTCTGAGAGG	CAGCAGCGGCTCAGAGAATAGA

Statistical analysis

All experiments were performed in triplicates. Data analysis was conducted using analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test for one-way ANOVA utilizing Prism version 10 software (GraphPad Software Inc., La Jolla, CA, USA). Results are expressed as mean ± standard deviation (SD), with statistical significance determined at p-value ≤ 0.05.

Results and Discussion

Crude extraction of P. indica (L.) Less.

To determine the effects of distinct plant parts, we separated P. indica into five components (leaf, root, flower, stem, and bark) and extracted them using a 70% ethanol reflux method. The crude extract from the five parts of *P. indica* (L.) Less. was obtained using a reflux method with 70% ethanol, and the yield was expressed as a percentage. The highest yield was recorded for the leaf (12.92%), followed by the root (10.32%), flower (9.02%), stem (8.81%), and bark (7.02%) (Figure 2). Among the parts, the leaf exhibited the highest extraction yield, followed by root, flower, stem, and bark.

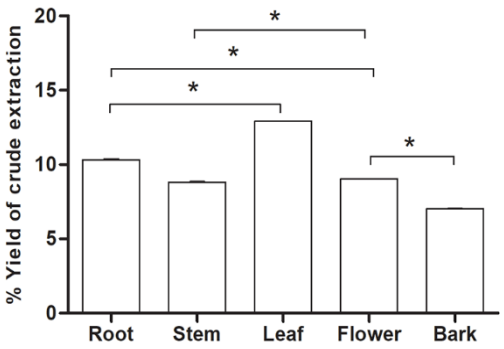


Figure 2: The %yield of crude extracts of *Pluchea indica* (L.) Less. by reflux method. The values are expressed as mean ± SD of data in three times independently. (*) *p*-value < 0.05 compared between groups

Phytochemical screening and antioxidant activity

Preliminary phytochemical screening of the crude extracts from *P. indica* revealed the presence of alkaloids, phenolics, flavonoids, coumarins, tannins, and terpenoids, with steroids detected exclusively in the flower extract (Table 2). This aligns with previous findings reporting phenolic acids and flavonoids in *P. indica*,[14] compounds widely known for their antioxidant and anti-inflammatory properties.[15] The total phenolic content (TPC) and total flavonoid content (TFC) were quantified using standard curves for gallic acid (Y=0.0112X-0.0676, R^2=0.9883) and quercetin (Y=0.001X-0.001735, R^2=0.9733), respectively. The leaf and flower extracts exhibited significantly higher TPC and TFC values compared to the other three parts. The TPC values for the crude extracts were as follows: leaf (703.04±21.43 mg GAE/g), flower (514.64±2.68 mg GAE/g), bark (155.39±3.48 mg GAE/g), root (107.54±1.67 mg GAE/g), and stem (62.63±1.43 mg GAE/g) (Figure 3A). The TFC values were also highest in the leaf and flower extracts: leaf (1919.00±100.00 mg QE/g), flower (1679.00±20.00 mg QE/g), root (359.80±14.00 mg QE/g), stem (327.80±98.00 mg QE/g), and bark (243.80±24.00 mg QE/g) (Figure 3B). Quantitative analysis showed high total phenolic content (TPC) and total flavonoid content (TFC), particularly in the leaf extract, followed by flower, bark, root, and stem. These results are consistent with reports of high phenolic acid and flavonoid levels in *P. indica* leaves.[16]

Table 2: Phytochemical screening of *P. indica* (L.) Less. extracts by reflux extraction with 70% ethanol

Phytochemical compounds	Root extract	Stem extract	Leaf extract	Flower extract	Bark extract
Alkaloids	+	+	+	+	+
Phenolic	+	+	+	+	+
Flavonoids	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Coumarin	+	+	+	+	+
Saponins	-	-	-	-	-
Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Steroids	-	-	-	+	-
Glycosides	-	-	-	-	-

Note: + : presence (detected); − : absent (Non detected).

Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays, with ascorbic acid as the control; both leaf and flower extracts demonstrated significantly higher antioxidant activity than the other parts and were comparable to ascorbic acid in efficacy. The IC50 values for the crude extracts in both DPPH (Figure 3C) and ABTS assays (Figure 3D) were ranked in the following order: flower, leaf, bark, stem, and root, with the leaf extract's IC50 in the ABTS assay not differing from that of ascorbic acid. This result demonstrated strong antioxidant potential in leaf and flower extracts, comparable to ascorbic acid. This corroborates previous findings where methanol leaf extracts exhibited potent antioxidant activity with low IC50 values.[17]

Thin-layer chromatography (TLC) confirmed the presence of vanillin and esculetin in the crude extracts of *P. indica*, particularly in conditions involving methanol:water mixtures, with notable concentrations in the leaf, flower, and bark extracts (Figures 3E and 3F), consistent with earlier reports.[14]

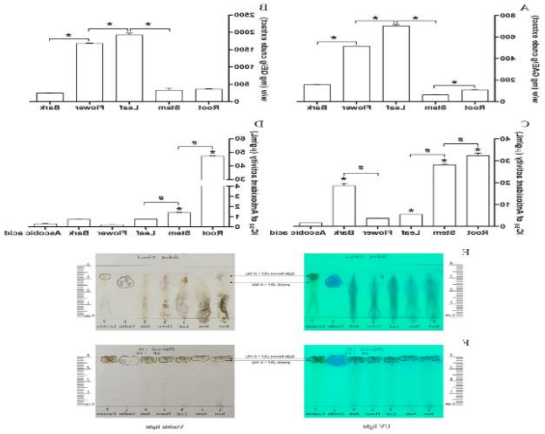


Figure 3: (A) Total Phenolic Content (TPC) of crude extracts and (B) Total Flavonoid Content (TFC) of crude extracts of *Pluchea indica* (L.) Less. (*) *p*-value < 0.05 compared between groups. (C) DPPH scavenging activities of crude extracts and (D) ABTS scavenging activities of crude extracts. (*) *p*-value < 0.05 compared to ascorbic acid, (#) *p*-value < 0.05 compared between groups. The values were presented as mean ± SD of data in three times independently. TLC separation of crude extract in conditions A (E) and conditions B (F)

Cytotoxicity of P. indica (L.) Less. extracts on White Blood Cells by MTT Assay and Red Blood Cells by Hemolysis Assay

The cytotoxicity of crude extracts was evaluated in total white blood cells, $CD14^+$ monocytes, and $CD14^-$ non-monocytes at concentrations ranging from 0 to 510 µg/mL, revealing no cytotoxic effects on these cell types (Figures 4A–4C). Two patterns emerged: one showed no cytotoxicity or enhancement of cell proliferation, observed with root, stem, and bark extracts on total WBCs; leaf, flower, and bark extracts on $CD14^+$ monocytes; and root, flower, and bark extracts on $CD14^-$ monocytes, while another indicated no cytotoxicity but enhanced cell proliferation at higher concentrations for leaf and flower extracts on total WBCs, stem extract on $CD14^+$ monocytes, and stem and leaf extracts on $CD14^-$ monocytes.

The hemolytic activity of crude extracts on red blood cells was assessed at concentrations ranging from 0 to 512 µg/mL; all extracts exhibited no hemolytic activity compared to controls with 0.5% DMSO as a negative control and 0.1% Triton X-100 as a positive control (Figure 4D). Based on these findings, concentrations of 50 µg/mL and 500 µg/mL were selected for subsequent experiments.

Interestingly, in addition to nontoxicity on red blood cells and white blood cell subtypes (total WBC, $CD14^+$ monocytes, and $CD14^-$ non-monocytes) of *P. indica* extracts across concentrations (0–512 µg/mL), extracts promoted cell proliferation in total WBC (leaf and flower), $CD14^+$ monocytes (stem), and $CD14^-$ non-monocytes (stem and leaf) at higher concentrations, comparable to propolis, a known natural immune stimulator and macrophage activation (Supplement 2).[18]

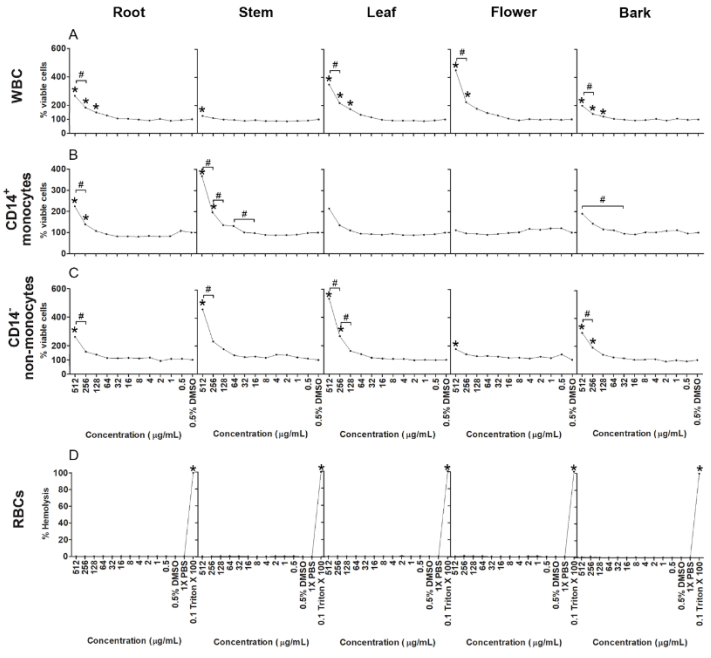
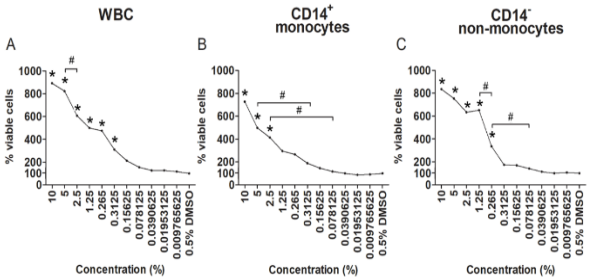


Figure 4: The cytotoxicity of crude extract from *Pluchea indica* (L.) Less. treating for 48 h by MTT assay on white blood cells including (A) total white blood cells, (B) $CD14^+$ monocytes, and (C) $CD14^-$ non-monocytes. (D) The cytotoxicity on red blood cells by hemolysis assay. The values were presented as mean ± SD of data in three times independently. (*) *p*-value < 0.05 compared to 0.5% DMSO, (#) *p*-value < 0.05 compared between groups

Supplement 1: (A) Saline soil at Ban Phonsim, Hua Na Kham Sub-district, Yang Talat District, Kalasin Province (at 16°24'01.8"N; 103°16'13.1"E), Thailand. (B and C) Soil characteristics with *Pluchea indica* (L.) Less. that are covered with salt



Supplement 2: The cytotoxicity of propolis treating for 48 h by MTT assay on white blood cells including total (A) white blood cells, (B) $CD14^+$ monocytes, and (C) $CD14^-$ non-monocytes. The values were presented as mean ± SD of data in three times independently. (*) *p*-value < 0.05 compared to 0.5% DMSO, (#) *p*-value < 0.05 compared between groups

Cytotoxic of P. indica (L.) Less. extracts on THP-1 cells by MTT assay

The toxicity of crude extracts on THP-1 cells was assessed at 24 and 48 hours using the MTT assay across concentrations from 0 to 400 µg/mL; no cytotoxic effects were observed at either time point (Figures 5A-E). Notably, root extract concentrations between 100-400 µg/mL significantly induced THP-1 cell proliferation at the 24-hour mark. Additionally, esculentin demonstrated nontoxicity at concentrations ranging from 0 to 40 µg/mL over both time intervals (Figure 5G). However, vanillin significantly reduced cell numbers at 48 hours for concentrations between 50-1,000 µg/mL but exhibited no toxicity at 24 hours (Figure 5F). Previous studies have also reported no toxicity of *P. indica* extracts in cell lines including human oral squamous carcinoma cell lines, rats,[19,20] and human clinical trials.[21]

Anti-inflammatory and antiviral activity of crude extract-stimulated in THP-1 by RT-qPCR

Crude extract concentrations ranging from 0 to 400 µg/mL, along with vanillin at concentrations up to 50 µg/mL and esculentin at up to 40 µg/mL showed no toxicity towards cells; thus, concentrations of 25 and 50 µg/mL for crude extracts and vanillin, along with 2.5 and 5 µg/mL for esculentin were selected for analyzing anti-inflammatory and antiviral properties.

The anti-inflammatory effects of *P. indica* crude extracts on THP-1 cells induced by LPS were evaluated through mRNA expression levels of pro-inflammatory cytokines such as IL-6 and TNF-*α* as well as anti-inflammatory cytokine TGF-β expression levels. IL-6 expression was undetectable across all crude extract treatments compared to controls (data not shown). The stem extract at a concentration of 25 µg/mL along with esculentin at a concentration of 5 µg/mL stimulated TNF-*α* expression compared to the negative control group; however, TNF-*α* expression was lower in response to flower extract at a concentration of 50 µg/mL as well as vanillin at both tested concentrations compared to controls. TGF-β expression was significantly upregulated in LPS-treated cells compared to controls when treated with root extract at a concentration of 25 µg/mL as well as stem extract at both tested concentrations; flower extract at a concentration of 50 µg/mL also upregulated TGF-β expression when compared to untreated LPS controls but showed no significant difference relative to control groups. Moreover, root extract at a concentration of 50 µg/mL alongside leaf extract at a concentration of 50 µg/mL inhibited TGF-β expression in LPS-treated cells while maintaining higher expression levels than controls; bark extract at a concentration of 50 µg/mL along with vanillin at a concentration of 25 µg/mL downregulated TGF-β expression more than control groups when treated with LPS (Figure 6B). These results suggest that *P. indica* extracts possess varying degrees of anti-inflammatory properties: particularly interesting is that the leaf extract displayed notable anti-inflammatory effects by reducing TNF-*α* expression (a pro-inflammatory cytokine) and upregulating TGF-β (an anti-inflammatory cytokine).[22–24] These findings are in line with prior studies highlighting the anti-inflammatory effects of *P. indica*, including inhibition of histamine-induced inflammation and endothelial inflammation mediated by TNF-*α*.[6] Targeting TNF-*α* is particularly promising for managing inflammatory diseases.[25] While both stem extract exhibited dual anti-inflammatory and antiviral properties.

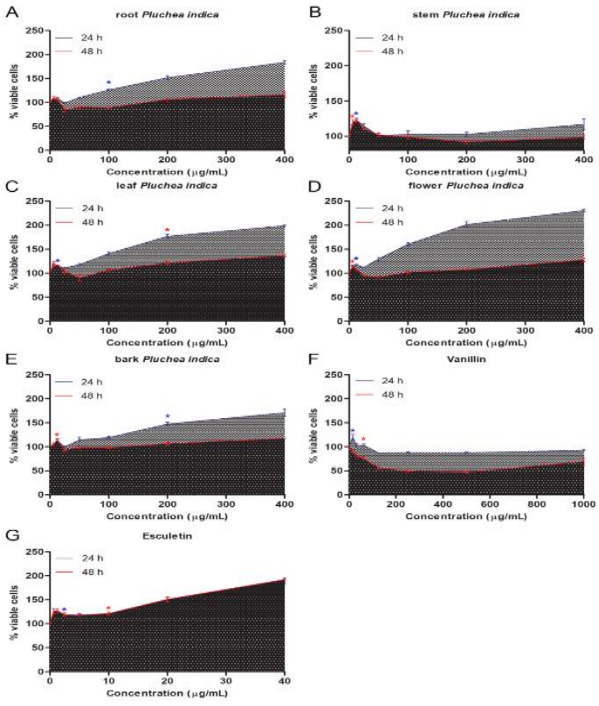


Figure 5: Percentage viability of THP-1 cells treated with crude extracts of *Pluchea indica* (L.) Less. and compounds. Percentage of viable cells of THP-1 cells were treated with (A) Root extract, (B) Stem extract, (C) Leaf extract, (D) Flower extract, (E) Bark extract, (F) Vanillin, and (G) Esculentin for 24 and 48 h. The values were presented as mean ± SD of data in three times independently. (*) *p*-value < 0.05 compared to control

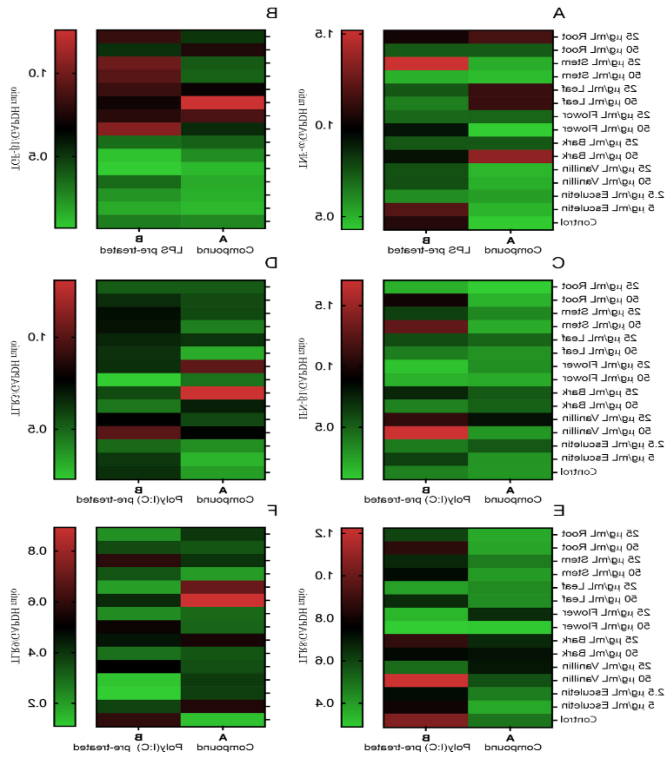


Figure 6: The expression of (**A**) pro-inflammatory cytokine (TNF-*α*), (**B**) anti-inflammatory (TGF-β), and (**C**) antiviral cytokines include IFN-β, (**D**) TLR3, (**E**) TLR8, and (**F**) TL9 on THP-1 cells treated with *Pluchea indica* (L.) Less. extracts were determined by quantitative RT-PCR

The antiviral properties mediated through poly(I:C)-toll-like receptor (TLR)3 activation were determined via qRT-PCR analysis assessing mRNA expression levels for IFN-β, TLR3, TLR7, TLR8, and TLR9 genes within THP-1 cells treated with various extracts or compounds. IFN-β expression levels were significantly elevated in THP-1 cells treated with root extract at a concentration of 50 µg/mL; similarly elevated IFN-β levels were observed following treatment with stem extract at both tested concentrations alongside bark extract treated with a concentration of 25 µg/mL while vanillin treatment resulted in increased IFN-β mRNA levels across both tested concentrations compared to controls. TLR3 expression was upregulated in poly(I:C)-pretreated THP-1 cells following treatment with stem extracts at both tested concentrations along with vanillin treatment at a concentration of 50 µg/mL compared to controls while TLR8 mRNA levels were similarly elevated following treatment with vanillin at a concentration of 50 µg/mL whereas TLR9 mRNA expression increased following treatment with stem extract at a concentration of 25 µg/mL compared to controls. These findings indicate that *P. indica* extracts may exhibit antiviral properties through mechanisms involving the upregulation of IFN-β and TLR3 activation. The root extract exhibited unique antiviral properties by inducing IFN-β expression, critical for the innate antiviral response.[25] It has been reported that leaf of *P. indica* (Less.) aqueous extract showed antiviral activity against human immunodeficiency virus type 1 (HIV-1).[26] Monocytes have high levels of mRNA of TLR2, TLR4, and TLR8 and low levels of TLR3, TLR7, and TLR9.[27] Vanillin, identified in the extracts, promoted IFN-β, TLR3, and TLR8 expression. TLR3 detects double-stranded RNA, while TLR8 senses single-stranded RNA, both essential for antiviral defense.[28] The TLR3-TRIF pathway is particularly relevant for controlling viral infections, including SARS-CoV-2.[29] Indicates a further enhances these antiviral effects through its influence on IFN signaling pathways; thus, highlighting potential therapeutic applications for these compounds against inflammatory responses or viral infections.

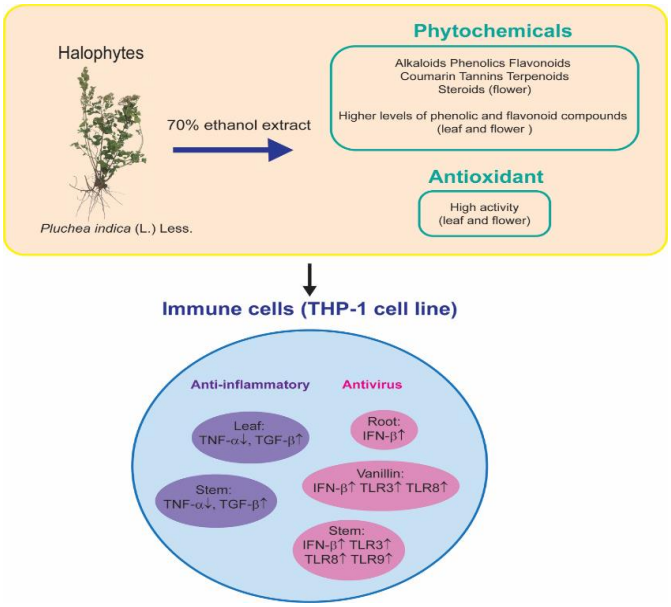


Figure 7: Summary of the effects of *Pluchea indica* (L.) Less. extract on anti-inflammatory and anti-viral properties

Conclusions

P. indica demonstrates significant pharmacological potential as a source of antioxidant, anti-inflammatory, and antiviral agents. Extracts from saline-rich environments revealed high levels of phenolic and flavonoid compounds, particularly in the leaf and flower parts. The leaf extract exhibited anti-inflammatory properties by inhibiting TNF-*α* and inducing TGF-β, while the root extract promoted IFN-β expression, suggesting possible antiviral activity. The stem extract displayed both anti-inflammatory and antiviral effects through modulation of TNF-*α*, TGF-β, IFN-β, TLR3, TLR8, and TLR9 pathways (Figure 7). These findings provide a foundation for the development of *P. indica*-derived therapeutic agents for inflammatory and viral diseases. Further *in vivo* studies are warranted to elucidate the molecular mechanisms underlying these bioactivities, assess their clinical potential and validate these *in vitro* properties.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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