

Carica Papaya Linn. Leaf Extracts Induced the Proliferation of Mice's Peritoneal Macrophages and Bone Marrow Cells, Along with Cytokine Modulation in Cell Culture

Wendwaoga Arsène Nikiema¹, Guillaume Sylvestre Sanou², André Zoulyél Da¹, Harouna Sore², Boris Honoré Amadou Ouedraogo^{1,3,4}, Kongnimissom Apoline Sondo⁴, Rawéléguinbasba Armel Flavien Kabore⁴, Rasmané Semdé^{1,3,4}, Moussa Ouedraogo^{1,3,4}

¹Laboratoire de Développement du Médicament, École Doctorale Sciences et Santé, Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso; ²Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso; ³Centre d'Excellence Africain, Centre de Formation, de Recherche et d'Expertise en sciences du Médicament (CEA-CFOREM), Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso; ⁴Unité de Formation et de Recherche, Sciences de la Santé, Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso

Correspondence: Moussa Ouedraogo; Wendwaoga Arsène Nikiema, Laboratoire de Développement du Médicament, École Doctorale Sciences et Santé, Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso, Email ouemoussa10@gmail.com; nikiemarsn@gmail.com

Purpose: *Carica papaya* is used alone or with other herbs in traditional medicine to treat infectious diseases; nonetheless, comprehensive scientific validation of this claim is limited. We conducted this preliminary study to explore its immunomodulatory potential through cytokine modulation.

Methods: An aqueous ethanolic crude extract of plant leaves was evaluated for phytochemical groups, total flavonoid, and phenolic content using aluminum chloride and Folin-Ciocalteu's methods. The crude extract and its derived fractions were compared with thin-layer chromatography, and antioxidant properties were assessed using the 2,2'-diphenyl-1-picrylhydrazyl radical scavenging assay and the Ferric Ion Reducing Antioxidant Power assay. Additionally, the effects of the extracts on cell proliferation, cytokine, and nitric oxide release by mouse peritoneal macrophages and bone marrow multipotent cells in culture were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the Enzyme-Linked Immunosorbent Assay (ELISA), and the Griess reagent. The cells were collected from Swiss strain mice (12 mice).

Results: *Carica papaya* leaf crude extract contained alkaloids, tannins, flavonoids, anthraquinones, and terpenoids. The crude extract's total phenolic and flavonoid contents were 14.07±5.76 gallic acid equivalents and 22.58±3.05 quercetin equivalents per gram of extract. The crude extract showed more potent antioxidant activity. Crude extract and fractions stimulated the proliferation of peritoneal macrophages at 1–10µg/mL and bone marrow multipotent cells at 0.01–0.3µg/mL in a concentration-dependent manner. Interleukin-6 and tumor necrosis factor-α production were significantly increased, while interleukin-1β production was inhibited in treated peritoneal macrophages. Treated bone marrow cells increased granulocyte-macrophage colony-stimulating factor, stem cell factor, and thrombopoietin secretion and decreased interleukin-3 production. Nitric oxide production was 10–20 times higher in bone marrow compared to peritoneal macrophage cells.

Conclusion: *Carica papaya* leaf crude extract and fractions exhibit immunomodulatory effects by promoting cytokine release and peritoneal macrophage and bone marrow cell proliferation, explaining the use of *C. papaya* in infectious diseases in traditional medicine. Further exploration is needed for applications in immune-related diseases.

Plain Language Summary: *Carica papaya* leaf aqueous ethanolic crude extract and fractions stimulated the proliferation of mice's peritoneal macrophages and bone marrow multipotent cells in culture, increased cytokine and NO release, and showed antioxidant properties. These findings support the traditional use of the plant in treating infectious diseases and suggest potential applications in immune-related diseases.



Keywords: immunomodulator, antioxidant, cytokines, macrophages, bone marrow cells

Introduction

The last five years have reminded us that viral infections such as COVID-19 and Dengue are serious threats to human health. Dengue fever, a re-emerging infectious disease, is the leading public health problem caused by arboviruses, with approximately 40% of the world's population at risk of DENV infection.¹ In 2023, 6.5 million cases were reported in more than 80 countries.² Severe forms of dengue and COVID-19 are associated with a cytokine storm, worsening these diseases.^{3,4} In the pathogenesis of dengue infection, the immune system plays a significant role. The activation of immune cells (dendritic cells, mast cells) leads to the release of cytokines (TNF- α , IL-6), which worsen the disease. Dengue virus induces bone marrow hypoplasia and a decrease in megakaryocytes, leading to thrombocytopenia.^{5,6}

Current treatment of these viral infections is symptom-based. Modulating the immune system with natural products remains a therapeutic option.⁷

The use of medicinal plants is gaining renewed interest in developed countries and remains the main therapeutic approach in developing countries. Medicinal plants have been shown to treat several infectious diseases by modulating the immune system.⁸ These plants belong to *Astragalus membranaceus*, *Echinacea purpurea*, and *Allium sativum*. The therapeutic effect is mainly attributed to the bioactive properties of molecules such as alkaloids, polysaccharides, flavonoids, and terpenoids. Astragaloside IV, from *A. membranaceus*, has been shown to increase T- and B-cell proliferation and antibody production, and to reduce the release of pro-inflammatory cytokines (TNF- α , and IL-1 β). The polysaccharide PG2 suppressed the expression of programmed cell death protein ligand 1 (PD-L1), which contributes to cancer immunotherapy; thereby, this could be used as a practical approach when combined with other treatments.⁹ *A. sativum* as reported to have immunomodulatory and antiviral activity by suppressing IL-6, MCP-1, TNF- α , and NF- κ B and inhibiting human immunodeficiency virus (HIV) virus adhesion to host cells.^{10,11}

The public highly appreciates *C. papaya* for the treatment of dengue fever.¹² *C. papaya* leaves contain tannins, saponins, alkaloids (such as carpain and papain), flavonoids (including quercetin, kaempferol, kaempferol-3-rutinoside, quercetin-3-(2G-rhamnosylrutinoside), quercetin-3-rutinoside, kaempferol-3-(2G-rhamnosylrutinoside), and myricetin-3-rhamnoside), phenolic acids (such as protocatechuic acid, 5,7-dimethoxy coumarin, caffeic acid, p-coumaric acid, and chlorogenic acid), anthraquinones, steroids, resins, and glycosides. Meanwhile, the shoots contain minerals like calcium, iron, magnesium, potassium, zinc, and manganese.^{13–19} The leaves of *C. papaya* are used to treat dengue. One of its effects is to increase the number of blood platelets.²⁰ However, the mechanism underlying this antithrombopenic effect remains under discussion. One area worth exploring is the involvement of hematopoietic cytokines. Basic platelet production depends on thrombopoietin (TPO). TPO is continuously produced and is mainly regulated by platelet receptor-mediated uptake. IL-6 has been shown to increase platelet production by elevating TPO levels.²¹

Because bone marrow cells are a source of immune-competent cells, such as macrophages and other blood cells, such as platelets, readily accessible mouse cells were used. Our study aimed to assess the immunomodulatory properties of aqueous ethanolic crude extract and fractions from fresh leaves of *C. papaya*, supporting the extract's potential to alleviate previously reported induced thrombocytopenia by modulating cytokine production and immune cell proliferation.

Materials and Methods

Reagents

Mouse IL-3 ELISA Kit (Cat n#RAB0295), Mouse IL-6 ELISA Kit (Cat n#RAB0308), Mouse IL-1 β ELISA Kit (Cat n#RAB0274), Mouse Tumor Necrosis Factor α ELISA Kit (Cat n#RAB0477), Mouse SCF ELISA Kit (Cat n#RAB0329), Mouse Granulocyte-Macrophage Colony Stimulating Factor/CSF2 ELISA Kit (Cat n#RAB0101), and Mouse Thrombopoietin ELISA Kit (Cat n#RAB0464) were obtained from Sigma Aldrich France.

Lipopolysaccharides *Escherichia coli* O111:B4 (Cat n#L2630), Concanavalin A from *Canavalia ensiformis* (Jack bean) (Cat n#C5275) were purchased from Sigma Aldrich, France.

Cell proliferation kit I (MTT) (Cat. n# 11465007001) was purchased from Sigma-Aldrich, France. Corning® 96 Well TC-Treated Microplates (Cat n#CLS3596). Nitrite assay kit (Griess reagent) (Cat n#MAK367-1KT), tannic acid (Cat n#SZBF1130V), 2,2'-diphenyl-1-picrylhydrazyl, Folin and Ciocalteu's reagent (Cat n#BCBS9142V), trichloroacetic acid (Cat n#102425067) were obtained from Sigma Aldrich, Co., 3050 Spruce Street, St Louis.

Cell Culture Media

RPMI-1640 medium (Cat n#R0883), DMEM medium (Cat n#D6429), DMEM complete medium (Cat n#SLM-241), Penicillin-streptomycin (Cat n#P4333), and fetal bovine serum (Cat n#F7524) were purchased from Sigma Aldrich, France.

Plants Leave Collection and Identification

The plant leaves used in this study were collected in Saaba (N: 12°21'48.5, W: 1°24'59) on the outskirts of Ouagadougou, the capital of Burkina Faso. Prof. Amade OUEDRAOGO of the Plant Biology and Ecology Laboratory at the University Joseph KI-ZERBO of Ouagadougou identified the plant. Voucher specimens were deposited with the reference number 6987/18027 for *Carica papaya* Linn. (Caricaceae).

Animals and Cell Lines

For the experimental study, 12 female albino Swiss mice, 6–8 weeks old, were used. The mice were purchased from the International Centre for Research and Development of Livestock in the Subhumid Zone (CIRDES) of Bobo-Dioulasso, Burkina Faso. All mice were housed in animal cages at a constant temperature ($24\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) on a 12 h light/dark cycle, with food and water available.

All animal welfare and experiments followed the Guide for the procedures approved by the Animal Ethics Committee, Joseph KI-ZERBO University of OUAGADOUGOU (Approval No. CE-UJKZ/2024-07). The mice were used to collect peritoneal macrophages and bone marrow hematopoietic cells, and were handled in accordance with international animal welfare standards as recommended by the European Union on Animal Care (EEC 86/609, UE 2010/63).

Crude Extract Preparations and Fractionation

Aqueous ethanolic is used as an extraction solvent of the fresh leaf because it afforded significant protection against cyclophosphamide-induced thrombocytopenia, compared to aqueous maceration as reported previously. After crushing the fresh leaves, 1000 mL of 80% (v/v) ethanol was added. The extraction was performed at $25\text{ }^{\circ}\text{C}$ for 24 hours with continuous stirring. The extract was filtered through gauze and cotton. The filtrate was then centrifuged at 3000 rpm for 10 minutes. The collected supernatant was concentrated using a vacuum rotary evaporator (BUCHI R-114 Labortechnik AG) and divided into two parts. One part was freeze-dried using a laboratory freeze-dryer (Alpha 1–4 LSCbasic, CHRIST; $T = -55\text{ }^{\circ}\text{C}$; $P = 0.06\text{ mbar}$) for 72 h to obtain a crude extract, while the other part was used for fractionation.

To separate the crude ethanol extracts of the leaves into fractions with different polarities, we chose liquid-liquid extraction, which separates the molecules based on their physicochemical properties between two immiscible liquid phases.

The fractionation was performed using solvents of increasing polarity, namely hexane, ethyl acetate, and butanol. The collected extracts were concentrated using a vacuum rotary evaporator and dried thoroughly in an oven. The aqueous residual was lyophilized.

Phytochemical Screening

Colorimetric Tests

The crude extract was qualitatively tested for flavonoids, tannins, saponins, alkaloids, coumarins, anthraquinones, steroids, and terpenoids using the method described by Deepti et al.²²

Characterization by Thin Layer Chromatography

We performed a phytochemical screening using TLC to identify the chemical families present in the crude ethanolic extracts and fractions.

Briefly, 10 mg of a lyophilized crude extract was dissolved in 1 mL of water. Chlorophyll was removed with activated charcoal. Quercetin and rutin were used as references. A 0.5 µg/mL methanolic solution of quercetin and rutin was prepared. Five (05) microliters of samples were, respectively, deposited on an aluminum-coated silica gel 60 F 254 TLC plate (Aluminum TLC plates, Silica gel 60 F254, Cat#1.05554, Merck).

The plate was air-dried and then inserted into a chamber presaturated with an optimized mobile phase, a mixed solvent of Ethyl acetate/Formic acid/Acetic acid/Water 100/11/11/20 (v/v/v/v). The plate was eluted at room temperature (25°C). After migration, the plate was removed, air-dried, and heated at 105 °C for 2 min before being sprayed with Neu's reagent, consisting of a mixture of a 1% methanolic solution of 2-aminoethyl diphenyl borate acid and a 5% ethanolic solution of PEG-4000 in the proportion 10/8 (v/v).

The plate was read using a 366 nm UV lamp (VL-6.LC Vilber RRID: SCR_023580).

The retardation factor (Rf) was calculated as follows:

$$Rf = \frac{\text{distance from starting line to middle of spot}}{\text{distance from starting line to solvent front}}$$

Estimation of Total Flavonoid Content

The total flavonoid content (TFC) of the *C. papaya* leaf crude extract was evaluated using the aluminum chloride colorimetric assay (Cat n#18B274122, VWR chemicals, Belgium) as described by Hayat et al.²³ Quercetin was used as a reference. An aliquot of 100 µL of plant extract (200, 100, and 50 µg/mL w/v) was added to 100 µL of aluminum chloride (2% w/v, in methanolic solution) in 96-well microplates (96w-BRAND plate-F). The samples were incubated for 10 min, and absorbance was read at 415 nm. The TFC was estimated using a quercetin standard curve and expressed as mg of quercetin equivalent (QE) per g of extract (mg QE/g).

Estimation of Total Phenolic Content

The total phenolic content of the leaf crude extract was determined using Folin-Ciocalteu's reagent of Singleton et al.²⁴ A volume of 30 µL of extract at concentrations of 200, 100, and 50 µg/mL was mixed with 125 µL of distilled water, 10 µL of Folin-Ciocalteu's, and neutralized with 30 µL of 7.5% sodium carbonate (Na₂CO₃). The reaction mixture was incubated at room temperature for 30 minutes to develop the color. The absorbance was read at 760 nm using a spectrophotometer (Agilent Biologic Technologies, USA). Gallic acid was used as the reference standard. The TPC was determined from a standard curve of gallic acid and expressed as mg of gallic acid equivalent per g of extract (mg GAE/g).

Determination of Antioxidant Activity Using in vitro Methods

2,2-Diphenyl-1-Picrylhydrazyl (DPPH•) Radical Scavenging Capacity Assay

The method described by Rice-Evans was used.²⁵ A volume of 100 µL of DPPH solution (60 µmol/L) was mixed with 100 µL of plant extract (1–300 µg/mL). The reaction mixture was incubated at room temperature in the dark for 30 min, and the absorbance was read at 517 nm. We used quercetin as a reference.²⁶ The IC₅₀, the concentration of extract required to inhibit 50% of the DPPH free radicals, was determined.

Ferric Ion Reducing Antioxidant Power Assay

We used the method described by González-Palma et al.²⁷ To measure ferric ion reducing antioxidant power with slight modifications, plant extract at concentrations of 200, 500, and 1000 µg/mL was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% potassium hexacyanoferrate. The mixture was incubated at 50°C for 30 minutes. Afterwards, 1.25 mL of trichloroacetic acid (10% w/v) and 250 µL of ferric chloride (0.1% w/v) were added, and the mixture was incubated for 30 minutes; the absorbance was then measured at 700 nm. Antioxidant activity, as measured by reducing power, is expressed as mg of ascorbic acid equivalent per 100 mg of dry extract (mg EAA/100 mg).

In vitro Immunomodulatory Activity

Cells Collection

Murine Peritoneal Macrophages

Murine peritoneal macrophages (PM) were isolated according to the procedure described by Ray and Dittel.²⁸ Three groups of three mice were treated with 1 mL of 3% (w/v) thioglycolate by intraperitoneal injection to induce peritoneal macrophage collection, as described previously.²⁹ Seventy-two (72) hours later, the mice were euthanized with thiopental (50 mg/kg, i.p.), and the macrophages were harvested by rinsing the peritoneal cavity with 5 mL of cooled RPMI 1640 medium. The cell suspension was centrifuged at 1500 rpm for 10 minutes twice at 20°C. The cells were washed twice with RPMI 1640 and resuspended in RPMI culture medium supplemented with 10% FBS and 1% Penicillin-Streptomycin. The percentage of viable cells was determined using trypan blue exclusion. Cells with viability greater than 90% were cultured.

Murine Bone Marrow Cells

Two groups of three female Swiss albino mice were euthanized by thiopental (50 mg/kg i.p.) injection.³⁰ The femur bones were excised under sterile conditions and placed in Dulbecco's Modified Eagle's Medium (DMEM). The bones were cut at both ends, and the bone marrow was flushed with 5 mL of media. Bone marrow cells of each mouse were collected separately. Cell suspension was centrifuged at 1500 rpm for 10 min at 5°C, and the cells were washed with DMEM medium. Cell viability was assessed by trypan blue solution count. Cells with a viability greater than 90% were cultured.

Cell Culture

Murine Peritoneal Cell Culture

Mouse peritoneal macrophages were seeded at 5×10^4 cells per well on Corning[®] 96 Well TC-Treated Microplates (cat no. CLS3596). After a two-hour preincubation at 37°C, 95% humidity, and 6.5% CO₂, at pH7.2–7.4, the cells were stimulated with 50 µL of plant extracts (0.01, 0.03, 0.1, 0.3, 1, 3, 10 µg/mL) or Lipopolysaccharides (LPS) (10 µg/mL).³¹ The plates were then incubated for 72 hours under the same conditions.

Murine Bone Marrow Cell Culture

Isolated bone marrow cells were cultured in 96-well Corning polystyrene cell culture plates. Cells were seeded at 5×10^4 cells/well in DMEM complete medium with 1% Penicillin-Streptomycin. The cells were preincubated for 2 hours at 37°C, 95% humidity, 5% CO₂ at pH7.2–7.4 before being challenged with 50 µL of different concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 10 µg/mL) of plant extracts or LPS at 10 µg/mL or Concanavalin A (CoA) at 5 µg/mL used as a standard.³² The cells were then incubated for 72 hours.

Cell Viability Assay: MTT Assay

Cell proliferation was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), a pale-yellow substrate that was reduced to the dark-blue formazan product in living cells. Hence, 10 µL of MTT labeling reagent (5 mg/mL) was added to each well, and the microplate was incubated for 4 hours at 37 °C in a 95% humidity, 6.5% CO₂ atmosphere. At the end of the incubation period, 100 µL of solubilization buffer was added, and the microplate was incubated overnight. The absorbance was read with a microplate reader at 550nm. Cell viability was calculated as: cell viability % = (absorbance of treated cells/absorbance of control cells) × 100%. Cell viability above 100% was interpreted as proliferation, and when below 100% as cytotoxicity.^{33,34}

Cytokine Release Assay on Peritoneal Macrophages

Basal cytokine levels in peritoneal macrophages in culture were assessed with or without challenge with plant extracts, LPS, or concanavalin A at concentrations tested in the MTT assay. Cytokine levels were measured using ELISA kits for mouse TNF-α, IL-1β, and IL-6. The culture supernatant was separated after sample processing, as in the MTT assay. The supernatant was centrifuged at 400g for 10 minutes at 5°C, and cytokine levels were determined according to each kit's

instructions. A portion of the culture supernatant was added to 96-well plates precoated with monoclonal antibodies specific for the target cytokine, and the plates were incubated at room temperature for 2.5 hours. The wells were then washed, and HRP-conjugated secondary antibodies were added; the mixture was incubated for 1 hour at room temperature. After washing, the substrate solution was added, and the reaction was allowed to proceed for 45 minutes. The stop solution was added after 30 minutes, and absorbance was measured at 450 nm. Cytokine concentrations in the samples were estimated using a standard curve generated from serial dilutions of standards.

Cytokine Release Assay on Murine Bone Marrow Cells

The concentrations of IL-3, TPO, SCF, and GM-CSF in the culture supernatant were measured using an ELISA Kit. The culture supernatant was added to 96-well plates pre-coated with monoclonal antibodies specific for each target cytokine, and the plates were incubated at room temperature for 2.5 hours. The wells were then washed, and HRP-conjugated secondary antibodies were added; the mixture was incubated for 1 hour at room temperature. After rewashing the wells, the substrate solution was added for 45 minutes. Finally, the stop solution was added after 30 minutes, and absorbance was measured at 450 nm. Cytokine concentrations in the samples were estimated from a standard curve constructed from serial dilutions of the standards.

NO Production Assay

The nitric oxide assay was performed as previously described, with minor modifications. After pre-incubating peritoneal cells (5.104 cells/well) with plant extracts or LPS (10 µg/mL) for 72 hours, the amount of nitrite in the culture medium was measured as an indicator of NO production. The nitrite levels, a stable metabolite of NO, were determined using a Griess reagent kit. Briefly, 50 µL of cell culture medium was mixed with 100 µL of Griess reagent (10 µL Griess reagent I + 10 µL Griess reagent II + 80 µL buffer). The mixture was then incubated at room temperature for 10 minutes, and the absorbance at 540 nm was measured with a microplate reader. Fresh culture medium served as the blank in each experiment. The nitrite concentration was determined from a sodium nitrite standard curve.

Statistical Analysis

The values are presented as mean ± standard deviation (S.D). The data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc tests, with $p < 0.05$ considered statistically significant, using GraphPad Prism version 5.03 (GraphPad Software, Inc., San Diego, California).

Results

Phytochemical Screening

Colorimetric Assay of Crude Extract

C. Papaya leaf crude aqueous ethanolic extract contains several phytochemical compounds (Table 1).

Table 1 Phytochemical Groups Characterized in the Crude Aqueous Ethanolic Extract of Fresh Leaves of *C. Papaya*

Chemical Groups	Results
Alkaloids	+
Flavonoids	++
Tannins	++
Saponins	–
Steroids and terpenoids	+
Anthraquinone	+
Coumarins	–

Notes: (+) weak-, (++) moderate- presence
(–) Not detected.

Thin-Layer Chromatography

The TLC fingerprints (Figure 1) obtained for different extracts after development with polyphenol-specific reagents indicate the presence of flavonoids and other polyphenolic compounds in the extract. The yellow, green, and blue spots under 365 nm UV light indicate the presence of flavonoids, and the cyan color indicates the presence of polyphenols.

Total Phenolic and Flavonoid Content

The total phenolic content was 14.07 ± 5.76 mg GAE/g of extract, determined from the standard curve of gallic acid ($y = 0.007x + 0.01$, $R^2 = 0.99$). The standard curve for quercetin to determine TFC was $y = 0.014x + 0.04$ ($R^2 = 0.99$), yielding a TFC of 22.58 ± 3.05 mg QE/g of extract.

Antioxidant Activity

2,2-Diphenyl-1-Picrylhydrazyl (DPPH•) Radical Scavenging Capacity Assay

Plant extract was tested using the DPPH free radical scavenging assay. Its antioxidant activity was compared to quercetin as a reference (Figure 2). The curves allowed determination of the IC_{50} ($\mu\text{g/mL}$) of quercetin for the standard (4.9 ± 0.8),

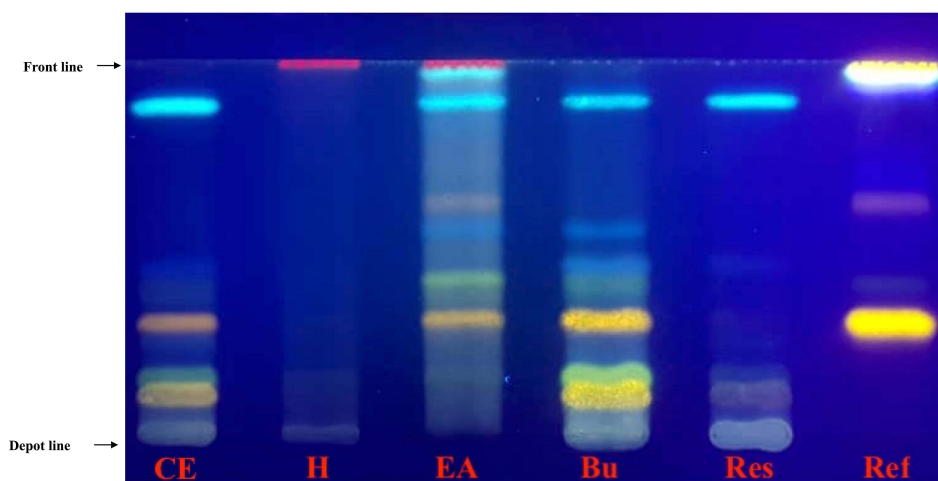


Figure 1 Thin-layer chromatography fingerprints of *C. papaya* leaf ethanolic crude extract and derived fractions on Silica 60 F_{254} Elution with Ethyl acetate -Formic acid - Acetic acid -Water (10:1:1:2). The plates were developed with NEU+PEG reagent. Ref: Reference (Quercetin + Tannic acid), CE: Crude extract, (H) Hexane fraction, EA: Ethyl acetate fraction, Bu: n-Butanol fraction, Res: Residual fraction. Arrows indicate the sample depot and the solvent front line, respectively. The TLC yielded 10 spots in the leaf crude extract and fractions, and 3 spots in the mixture of references: spot 1 (red1, 0.99, EA, H); Spot 2 (Cyan1, 0.99, CE, EA, Bu, Res); spot 3 (Yellow1, 0.98, Ref); spot 4 (Cyan2, 0.91, EA); spot 5 (Yellow2, 0.63, EA); spot 6 (Red2, 0.63, Ref); spot 7 (Blue1, 0.55, EA, Bu); spot 8 (Blue2, 0.54, CE, Bu, Res); spot 9 (Green1, 0.41, EA, Bu); spot10 (Yellow3, 0.30, CE, EA, Bu, Ref); spot 11 (Green2, 0.16, CE, EA, Bu); spot 12 (Yellow4, 0.10, CE, Bu, Res) and spot 13 (Neutral, 0.10, Res).

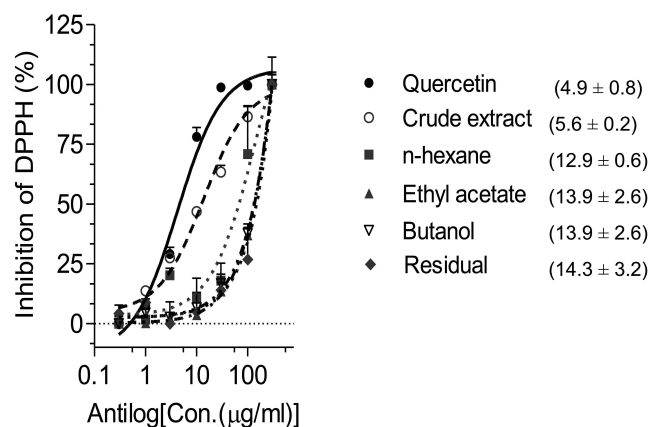


Figure 2 Effect of *C. papaya* aqueous ethanolic leaf crude extract and derived fractions on DPPH free radical. The IC_{50} ($\mu\text{g/mL}$) of crude extract and derived fractions are indicated in parentheses.

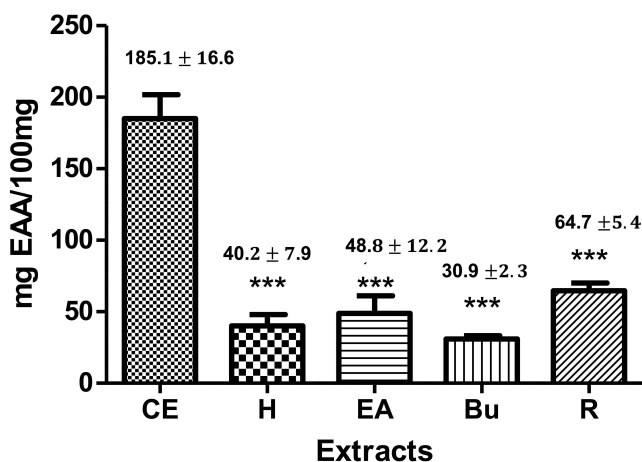


Figure 3 Ferric ion Reducing Antioxidant Power (FRAP) of *C. papaya* aqueous ethanolic leaf extract and derived fractions. *** $p < 0.001$ CE vs H, EA, Bu, R. **Abbreviations:** CE, Crude extract; H, hexane; EA, Ethyl Acetate; Bu, butanol; R, aqueous Residual, fractions.

crude extract (5.6 ± 0.2), n-hexane (12.9 ± 0.6), Ethyl acetate (13.9 ± 2.6), n-butanol (13.9 ± 2.6), and aqueous residual (14.3 ± 3.2) fractions.

Ferric Ion Reducing Antioxidant Power Assay

The crude extract showed higher activity than the fractions (Figure 3). The difference between extracts was significant ($p < 0.001$).

In vitro Immunomodulatory Activity of Plant Extract and Fractions

Effect on Mouse Peritoneal Cell Proliferation

We evaluated the effect of the crude extract and fractions on mouse peritoneal cell proliferation at 0.01–10 $\mu\text{g/mL}$ for 72h with LPS as reference mitogens (Figure 4). LPS induced cell proliferation at 10 $\mu\text{g/mL}$.

C. papaya L. crude extract induced macrophage cell proliferation dose-dependently (0.01–10 $\mu\text{g/mL}$). The maximal effect was observed at a concentration of 1 $\mu\text{g/mL}$.

The hexane fraction showed a significant ($p < 0.001$) stimulation of PM proliferation. The stimulation was 2-fold higher than that of crude extract and other fractions.

The ethyl acetate fraction showed maximal stimulation of cell proliferation at 3 and 10 $\mu\text{g/mL}$. PM treated with the butanol fraction showed significant stimulation at 0.3 $\mu\text{g/mL}$ ($p < 0.05$). Other concentrations did not stimulate significantly. The residual aqueous fraction stimulated significant ($p < 0.001$) proliferation at 0.03–10 $\mu\text{g/mL}$.

Effect on Peritoneal Macrophage Cells Cytokines Release: IL-6, IL-1 β , TNF- α

LPS at 10 $\mu\text{g/mL}$ triggered the release of cytokines (IL-6, IL-1 β , TNF- α) in the cell culture supernatant of peritoneal macrophages. We assessed the effects of plant extracts on cytokine release after 72 hours (Figure 5). LPS significantly increased IL-6, IL-1 β , and TNF- α levels compared with controls ($p < 0.001$). *C. papaya* crude extract notably ($p < 0.05$) enhanced the production of IL-6 and TNF- α at 10 $\mu\text{g/mL}$. Conversely, IL-1 β production was substantially reduced at the same concentration. The hexane fraction displayed a significant ($p < 0.01$) decrease in IL-6 and IL-1 β production after 72 hours at 1, 3, and 10 $\mu\text{g/mL}$. TNF- α levels were elevated at 1 $\mu\text{g/mL}$. The ethyl acetate fraction ($p < 0.01$) markedly promoted IL-6 production at 3 $\mu\text{g/mL}$, while IL-1 β production was suppressed. The impact on TNF- α release was not significant.

At 1, 3, and 10 $\mu\text{g/mL}$, the butanol fraction does not affect IL-6 release. Concentrations of 3 and 10 $\mu\text{g/mL}$ significantly inhibit IL-1 β release. TNF- α secretion was significantly ($p < 0.01$) stimulated at 1 and 10 $\mu\text{g/mL}$. The aqueous residual fraction significantly ($p < 0.01$) increased IL-6 and TNF- α release at 10 $\mu\text{g/mL}$. IL-1 β production was significantly inhibited ($p < 0.01$).

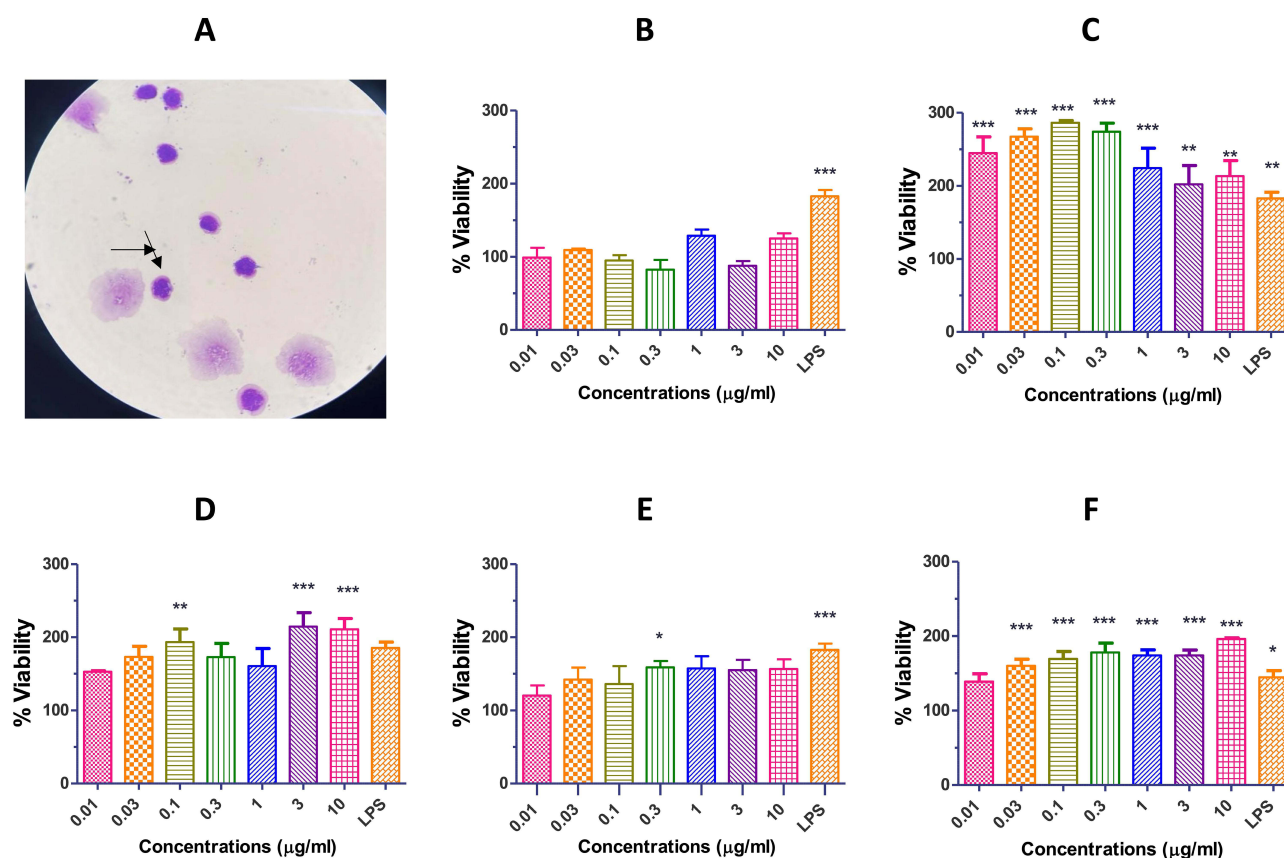


Figure 4 Viability of peritoneal macrophage cells after treatment with *C. papaya* crude extract or fractions. Non-treated peritoneal macrophage cells stained with RAL reagent, $\times 100$ (A) crude extract (B) and fractions: Hexane (C) Ethyl acetate (D) Butanol (E) and aqueous residual (F). LPS: Lipopolysaccharide. Results are expressed as Mean \pm SD (n=2 independent experiments in triplicate). Test vs LPS, ***p<0.001, **p<0.01, *p<0.05. Arrows show PM cells.

Effect of *C. Papaya* Crude Extract and Fractions on Murine Bone Marrow Cell Proliferation

As shown in Figure 6, after 72 hours of culture, LPS and concanavalin A significantly ($p<0.001$) stimulated the proliferation of murine BM cells. The highest and most significant ($p<0.001$) stimulation was observed with crude extract at 0.1 $\mu\text{g}/\text{mL}$. The n-hexane, ethyl acetate, and residual aqueous extracts showed significant ($p<0.01$) stimulation at concentrations from 0.01 to 1 $\mu\text{g}/\text{mL}$. Concentrations above 1 $\mu\text{g}/\text{mL}$ had no effect or caused inhibition. The butanol fraction stimulated cell proliferation at all concentrations, with significant activity at 0.3 and 3 $\mu\text{g}/\text{mL}$.

Effect of Plant Extract on Bone Marrow Cell Cytokine Production: IL3, SCF, GM-CSF, and TPO

After stimulation with plant extracts and fractions, the production of cytokines (IL-3, GM-CSF, TPO, SCF) by non-differentiated hematopoietic BM cells was assessed after 72 hours (Figure 7). Lipopolysaccharides (LPS) and Concanavalin A were used as reference mitogens at 10 $\mu\text{g}/\text{mL}$ and 5 $\mu\text{g}/\text{mL}$. Concanavalin A significantly ($p<0.01$) stimulated cytokine production at 5 $\mu\text{g}/\text{mL}$. LPS significantly ($p<0.05$) stimulated TPO and SCF, but did not significantly stimulate IL-3 or GM-CSF. *C. papaya* extract and fractions stimulate cytokine production differently. The crude extract of *C. papaya* significantly stimulated IL-3 and GM-CSF at 0.01 $\mu\text{g}/\text{mL}$, and SCF and TPO at 0.1 and 0.3 $\mu\text{g}/\text{mL}$, respectively. The hexane fraction significantly increased TPO and SCF production at 0.03 $\mu\text{g}/\text{mL}$. The stimulation of IL-3 and GM-CSF production was not significant. The ethyl acetate fraction significantly stimulated IL-3 and GM-CSF production at 0.01 $\mu\text{g}/\text{mL}$; however, it did not significantly stimulate SCF or TPO. The butanol fraction significantly stimulated the release of GM-CSF, TPO, and IL-3 at 0.01 $\mu\text{g}/\text{mL}$. SCF production was significantly ($p<0.05$) stimulated

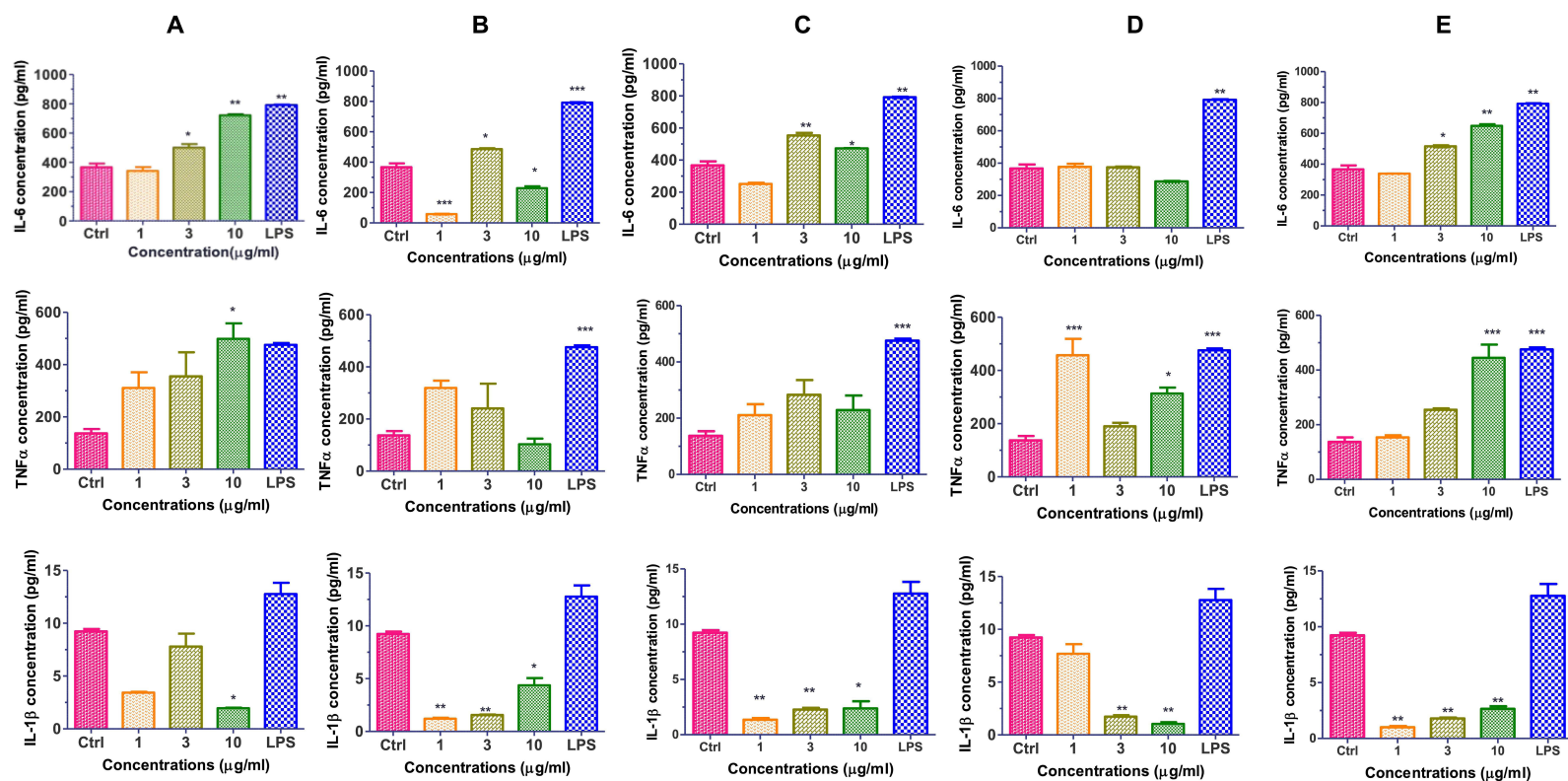


Figure 5 Effect of *C. papaya* aqueous ethanolic leaf crude extract and derived fractions on cytokine secretion in mouse peritoneal macrophage primary cells culture. Crude extract (A) hexane (B) ethyl acetate (C) n-butanol (D) and residual aqueous (E) fractions effect on IL-6, TNF α and IL-1 β production respectively. Control (Ctrl) versus test or LPS, * $p < 0.05$, ** $p < 0.01$, $p < 0.001$, **** $p < 0.0001$. LPS: Lipopolysaccharides 10 $\mu\text{g/mL}$. Results are expressed as Mean \pm SD (n=2 independent experiments in triplicate).

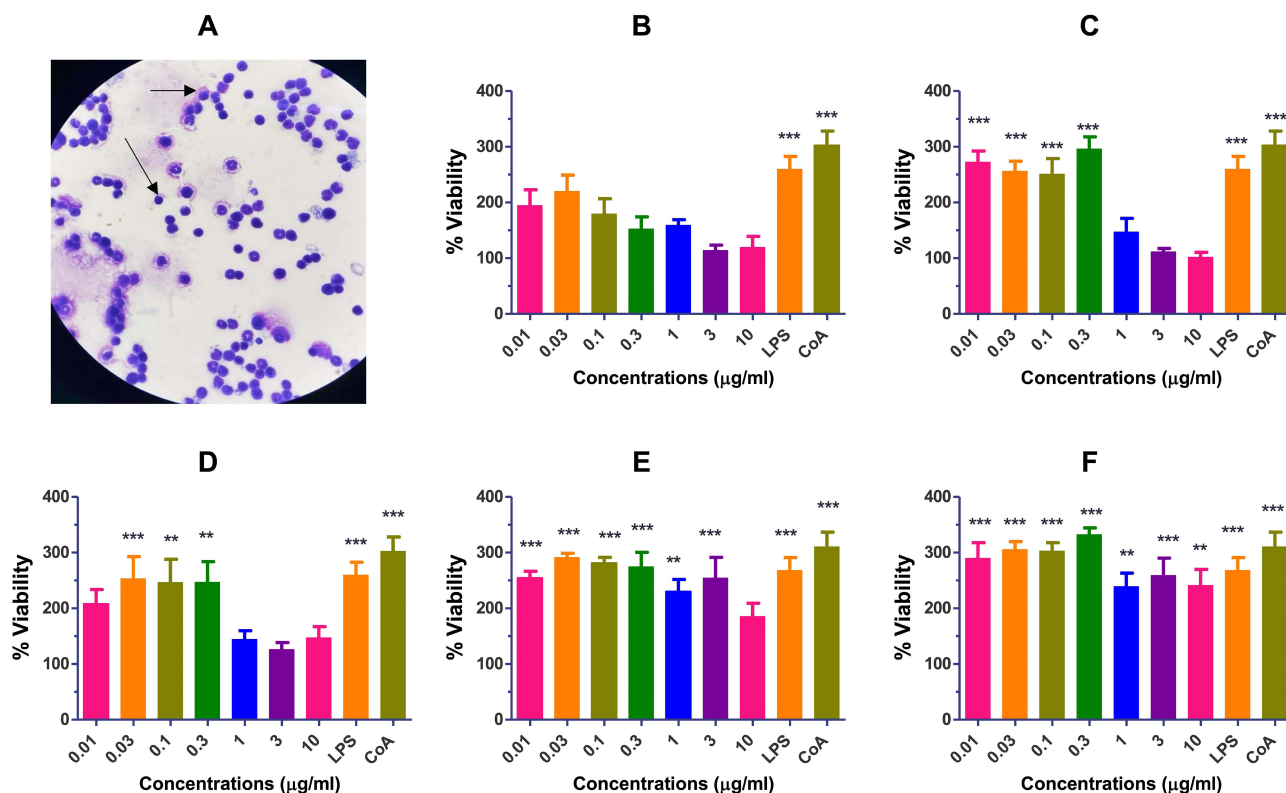


Figure 6 Viability of bone marrow cells after treatment with *C. papaya* crude extract or fractions. Non-stimulated cells, stained with RAL reagent, magnification $\times 40$ (A) crude extract (B) and fractions: hexane (C) ethyl acetate (D) butanol (E) and aqueous residual (F). Results are expressed as Mean \pm SD ($n = 2$ independent experiments in triplicate). Test versus CoA: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Arrows show the BM cells.

Abbreviations: Co A, Concanavalin A; LPS, Lipopolysaccharide.

with increasing concentrations (0.01–0.1 $\mu\text{g/mL}$). The aqueous residual fraction had no significant effect on GM-CSF and IL-3 production but significantly stimulated TPO and SCF at 0.01–0.1 $\mu\text{g/mL}$.

Effect on NO Production

Effect on Mouse Peritoneal Macrophages

Lipopolysaccharide at 10 $\mu\text{g/mL}$ significantly stimulated NO production. When mouse peritoneal cells were treated with plant extract alone, the amount of NO increased in a concentration-dependent manner (Figure 8). The crude extract of *C. papaya* significantly stimulated NO production ($p < 0.01$) at 3 and 10 $\mu\text{g/mL}$. The hexane fraction of *C. papaya* did not considerably increase NO production. The ethyl acetate, butanol, and residual aqueous fractions from *C. papaya*.

Effect on Murine Bone Marrow

Concanavalin A at 5 $\mu\text{g/mL}$ had no significant effect. Crude extracts of *C. papaya* stimulated nitric oxide release in a concentration-dependent manner (Figure 8). A ceiling effect was observed with *C. papaya* at 0.03 $\mu\text{g/mL}$. A concentration of 0.1 $\mu\text{g/mL}$ of the hexane fraction resulted in maximal stimulation. Concentrations of 0.1 $\mu\text{g/mL}$ and 0.3 $\mu\text{g/mL}$ of the ethyl acetate fraction significantly stimulated NO production. The n-butanol fraction also stimulates NO production, depending on the concentration. A concentration of 0.03 $\mu\text{g/mL}$ of the residual aqueous fraction significantly affects NO release.

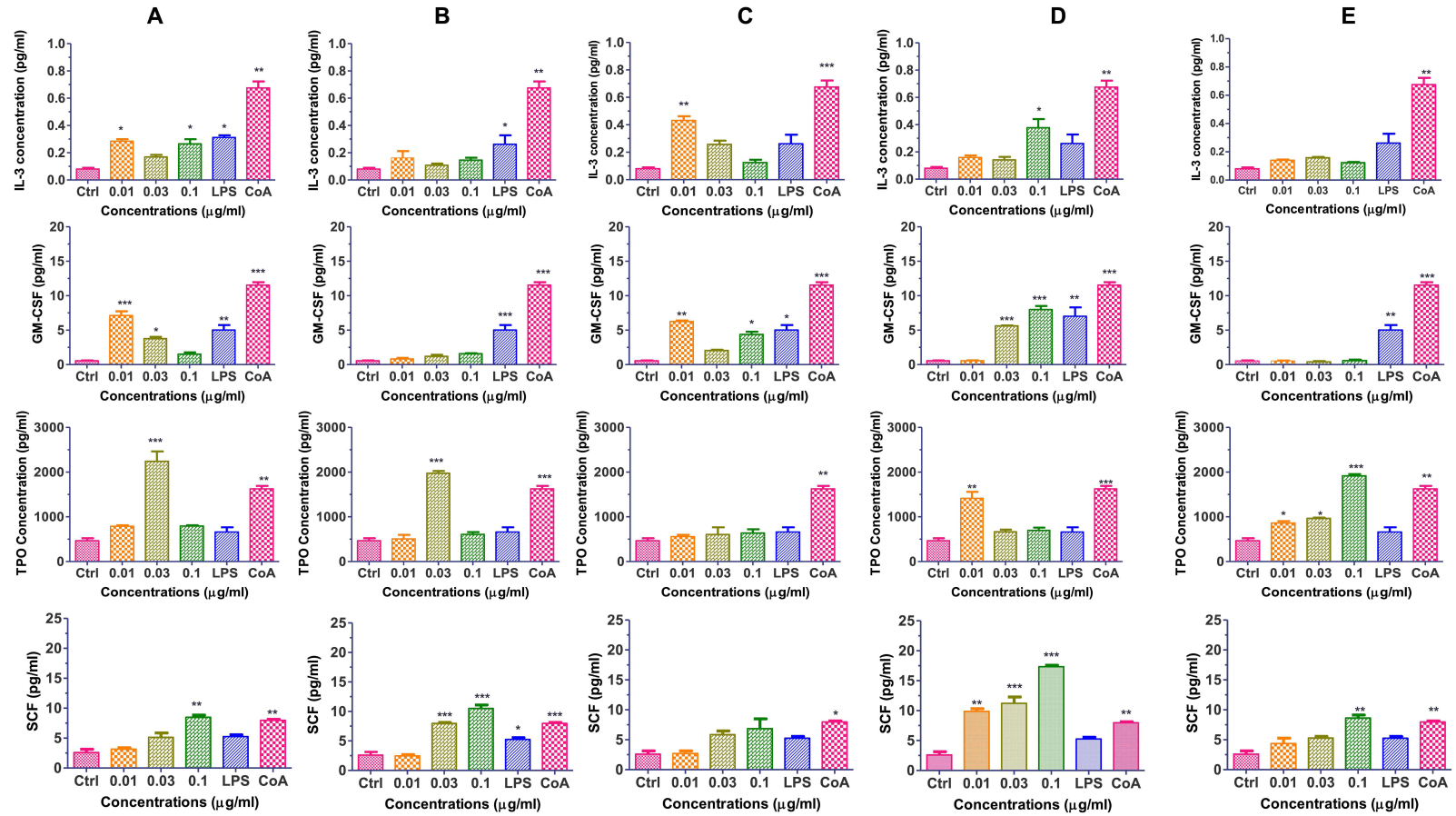


Figure 7 Effect of *C. papaya* aqueous ethanolic leaf crude extract and derived fractions on cytokine secretion in mouse bone marrow primary cells culture. Crude extract (A) hexane (B) ethyl acetate (C) butanol (D) and aqueous residual (E) fraction on IL-3, GM-CSF, TPO, and SCF production, respectively. Results are expressed as Mean \pm SD (n=2 independent experiments in triplicate). Control (Ctrl) vs test (*p<0.05, **p<0.01, ***p<0.001).

Abbreviations: LPS, lipopolysaccharides 10 μ g/mL; CoA, concanavalin A.

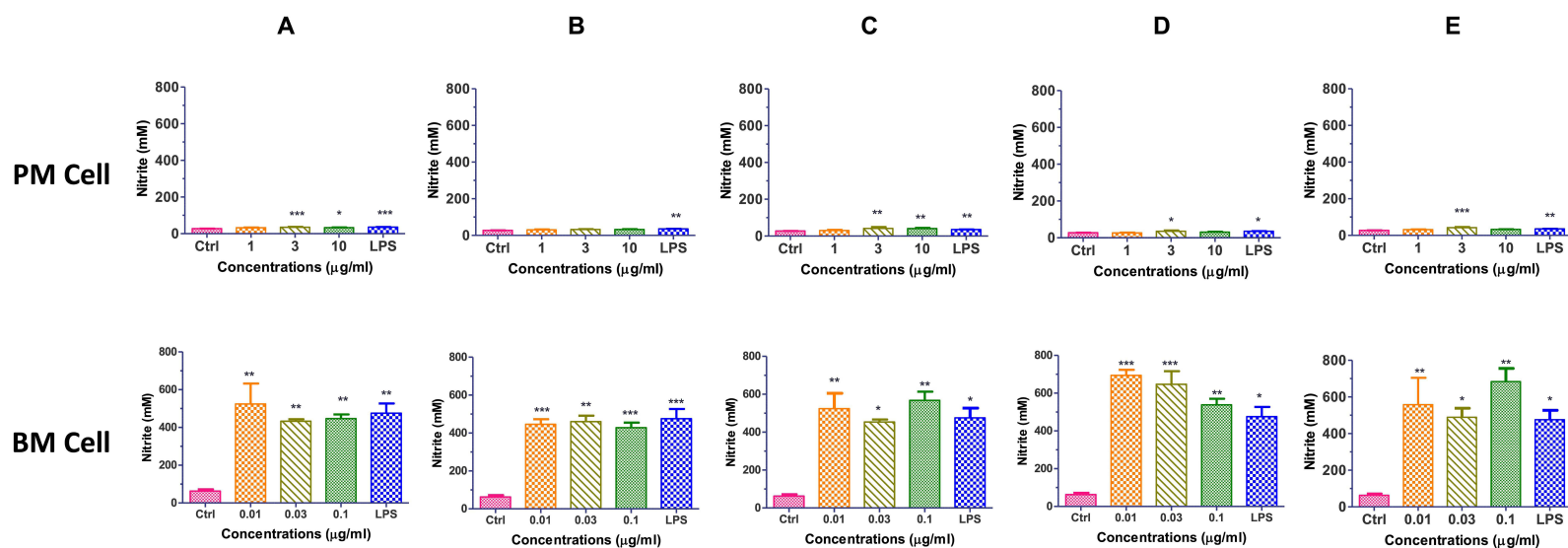


Figure 8 Effect of *C. papaya* aqueous ethanolic leaf crude extract and derived fractions on nitric oxide (NO) production in mouse bone marrow primary cells culture. Crude extract (A) hexane (B) ethyl acetate (C) butanol (D) and aqueous residual (E) fractions on NO production, respectively, by BM and PM cells. Data are presented as the mean \pm SD from at least three independent experiments (n=3 duplicates). Test versus control, * p < 0.05, **p < 0.01, and ***p < 0.001. **Abbreviations:** LPS, lipopolysaccharide; ctrl, control; BM, bone marrow cell; PM, peritoneal macrophage cell.

Discussion

Phytochemical Screening

Carica papaya leaves contain secondary metabolites, such as tannins, alkaloids, flavonoids, anthraquinones, steroids, and terpenoids.

The leaves had higher TFC than TPC. Conversely, Asghar et al³⁵ showed similar flavonoid content but a threefold higher phenolic content in papaya leaves. The levels of phenolic and flavonoid compounds depend on the plant's location, the part of the plant, and the extraction solvent used.^{35,36} Flavonoids are important secondary metabolites of plant extracts and are a subclass of the polyphenol family. The TLC analysis revealed green, yellow, and blue spots, indicating the presence of flavonols and flavones,³⁷ and cyan spots due to caffeic and chlorogenic acids.¹³ These compounds have been reported to have biological activities, including anti-inflammatory, antibacterial, anticancer, immunomodulatory, and antioxidant effects.³⁸

Antioxidant Activity

Polyphenols are considered indicators of the antioxidant potential of natural substances. Several tests can be used to assess the antioxidant capacity of plant extracts. The DPPH free radical scavenging test is most commonly used. It involves a stable, purple-colored radical that turns yellow during electron transfer. This reduction reaction is used to measure antioxidant strength.

Our study showed that the crude extract was more active than the n-hexane fraction. The ethyl acetate, butanol, and aqueous residual fractions had similar reducing power. Antioxidant activity may be linked to phenolic compound content. The crude extract had the highest reducing power in the FRAP antioxidant test. The differences between it and the fractions are significant.

As part of the innate immune system, Macrophages produce reactive oxygen species (ROS) during their immune response, including against invading pathogens. Antioxidants are essential for controlling ROS production, and a deficiency can impair macrophage function.³⁹ Macrophages also use a complex network of self-defense mechanisms, including antioxidant pathways, to survive in inflammatory environments. Antioxidant activity helps protect the immune system.

Plant Extract Effect on Cell Viability and Cytokine Production

Modulating various immune system functions is one of the ways medicinal plants provide their health benefits and therapeutic effects. Concerning immune cells, this may include stimulating their proliferation and growth, as well as altering the microenvironment in which they develop. Plants can therefore influence cell proliferation or survival by modulating growth factors, such as cytokines. Cytokines are key regulators of cell-to-cell interactions, growth, and differentiation.^{40,41} Based on their function, they can be classified as interleukins, tumor necrosis factors, and colony-stimulating factors. They play a crucial role in regulating inflammatory and immune responses. Along with immune cells, they control both innate and adaptive immunity.

We conducted an in vitro study to evaluate the effect of *C. papaya* extracts on immune cells and cytokine production. Two cell lines were used: mouse PM and undifferentiated mouse BM cells. This helps explain the immunomodulatory potential of the selected plant.

Macrophages are immune cells that are highly specialized in phagocytosis. They therefore serve a defensive role against pathogens and tumor cells.⁴² They are produced in the BM and released into the bloodstream.⁴³ They are effector cells for pro-inflammatory cytokines (IL-1 β , IL-6) and TNF- α . These cytokines are involved in most chronic inflammatory diseases, including infectious diseases. They also include cancer, type 2 diabetes, rheumatoid arthritis, neurodegenerative disorders, cardiovascular diseases, and pulmonary conditions.

In our study, mouse peritoneal cells treated with the plant extract showed concentration-dependent stimulation. The response was similar to that observed with LPS at 10 μ g/mL. The production of cytokines in cells treated with the plant extract was modulated.

The concentration of TNF- α released in the supernatants of macrophages stimulated with the crude extract at a concentration of 10 $\mu\text{g/mL}$ of *C. papaya* was significantly ($p < 0.01$) higher than in the supernatants of unstimulated control cultures. The n-hexane and butanol fractions showed a greater response at a lower concentration (1 $\mu\text{g/mL}$). The ethyl acetate fraction and the residual aqueous layer at 3 $\mu\text{g/mL}$ also showed increased TNF- α levels. TNF- α is a pro-inflammatory mediator released by activated macrophages and plays an essential role in non-specific immune responses to infections and tumors.⁴⁴ Therefore, the increase in Phagocytosis, lysosomal enzyme activity, and TNF- α release by bone marrow-derived macrophages stimulated by the extract are significant observations and could help explain the claimed benefits of extracts in treating immune-based diseases.

The crude extract of *C. papaya* significantly inhibited IL-1 β production at 10 $\mu\text{g/mL}$. Then n-hexane, ethyl acetate, butanol, and residual fractions inhibited production at 3 and 10 $\mu\text{g/mL}$. IL-1 β is a pro-inflammatory cytokine that initiates and promotes several autoimmune diseases, mainly through its stimulatory effect on CD4⁺ cells.⁴⁵ The sources of IL-1 β are monocytes, DCs, and macrophages. Caspase-1 is an enzyme that enables the maturation of IL-1 β . Its activation triggers overexpression of IL-1 β , initiating inflammatory pathways associated with neuronal damage and cell death.⁴⁶ Elevated levels of IL-1 β are related to central nervous system infections, brain damage, and neurodegenerative diseases. Reducing IL-1 β levels in these cases could be beneficial.

We also observed an increase in IL-6 release from peritoneal macrophages in vitro upon treatment with increasing doses of the *C. papaya* crude extract and its residual aqueous fraction. Under normal physiological conditions, IL-6 is produced in various tissues and cell types, including macrophages, lymphocytes, fibroblasts, keratinocytes, and osteoblasts.⁴⁷ As a pleiotropic cytokine, IL-6 acts on hepatocytes in the liver to stimulate increased expression of constitutively produced thrombopoietin (TPO), one of the main cytokines that induce megakaryopoiesis or thrombopoiesis, leading to higher platelet counts.

C. papaya has been shown to increase platelet counts.⁴⁸ *C. papaya* is also effective in alleviating the symptoms related to dengue.⁴⁹ However, no studies have elucidated the mechanism of action that induces megakaryocytopoiesis. The most likely mechanism involves cytokines such as IL3, IL6, SCF, GM-CSF, and TPO. We also undertook this study to elucidate the effect of extracts on the release of these cytokines in undifferentiated hematopoietic stem cells in mice. We isolated stem cells using a method that preserves their original niche with minimal disturbance.⁵⁰ These cells can self-renew and differentiate into multiple lineages.

We observed that the extracts increased BM cell viability in vitro, with a concentration-dependent increase in cell proliferation. In the same conditions, *C. papaya* extracts significantly stimulate SCF production. Previous studies have shown that SCF acts synergistically with other cytokines, such as TPO, to enhance the proliferation of immature progenitor cells, thereby contributing to platelet production.⁵¹ TPO is the primary cytokine involved in megakaryopoiesis and thrombopoiesis. Other cytokines, GM-CSF, G-CSF, IL-3, and EPO, also act synergistically with TPO to regulate hematopoiesis in the bone marrow. The crude extract of *C. papaya* and the hexane fraction significantly increased TPO production. The butanol and residual fractions were stimulated at 0.01 and 0.1 $\mu\text{g/mL}$.

The extracts may enhance platelet production by first elevating IL-6 expression in stem cells and leukocytes, thereby increasing TPO production in the liver, and thereby increasing the rate of platelet formation. In an in vitro study, Aziz et al demonstrated that 5% and 10% *C. papaya* juice significantly increased IL-6 and SCF secretion but did not markedly affect IL-3 and TPO levels.⁵² In another model, an aqueous extract of *C. papaya* stimulated the production of TNF- α , IL1 β , NO, and PGE2 and increased phosphorylation of ERK and JNK.⁵³

Therefore, the immunomodulatory mechanism involves the MAPK pathway. These results contradict other studies that, in an in vivo model, have shown that high-dose administration significantly reduces TNF- α and IL-6 production.^{32,53} This contradictory result could be explained by the experimental models used and the pleiotropic effects of the cytokines.⁴⁰ In the same study, the extract stimulated bone marrow cell proliferation at 31.25 $\mu\text{g/mL}$ without a mitogen. We used LPS and Concanavalin A as reference mitogens. LPS is a bacterial endotoxin with a paradoxical immunomodulatory effect. While low concentrations stimulate cell proliferation, high concentrations are cytotoxic.⁵⁴ Plant mitogens, such as lectins, are glycoproteins that trigger mitosis by binding to cell receptors. Concanavalin A, like phytohemagglutinin A, is a lectin. It prefers T lymphocytes, whereas bacterial lipoproteins, including LPS, tend to target B lymphocytes and macrophages.⁵⁵ The proliferative effect of the crude extract and fractions on PM and BM cells at low

concentrations might be due to mitogens or growth factors. The effect could also result from endotoxins, which could cause false positive results. All extracts and fractions were filtered through a 0.22-micron filter to prevent this and ensure accurate results. Therefore, the immunomodulatory mechanism involves the MAPK pathway. These results contradict other studies that, in an in vivo model, have shown that high-dose administration significantly reduces TNF- α and IL-6 production.^{32,53} This contradictory result could be explained by the experimental models used and the pleiotropic effects of the cytokines.⁴⁰ In the same study, the extract stimulated bone marrow cell proliferation at 31.25 $\mu\text{g}/\text{mL}$ in the absence of a mitogen. We used LPS and Concanavalin A as reference mitogens. LPS is a bacterial endotoxin with a paradoxical immunomodulatory effect. While low concentrations stimulate cell proliferation, high concentrations are cytotoxic.⁵⁴ Plant mitogens, such as lectins, are glycoproteins that trigger mitosis by binding to cell receptors. Concanavalin A, like phytohemagglutinin A, is a lectin. It prefers T lymphocytes, whereas bacterial lipoproteins, including LPS, tend to target B lymphocytes and macrophages.⁵⁵ The proliferative effect of the crude extract and fractions on PM and BM cells at low concentrations might be due to mitogens or growth factors. The effect could also result from endotoxins, which can cause false-positive results. All extracts and fractions were filtered through a 0.22-micron filter to prevent this and ensure accurate results.

Additionally, all procedures were conducted under aseptic conditions. This confirms that the observed proliferative effects are attributable to the biologically active phytochemicals in the extract and its fractions. Phytochemical analysis using thin-layer chromatography showed the presence of polyphenols, alkaloids, and terpenoids in the leaves. These compounds could account for the immunomodulatory effects. In fact, alkaloids have been demonstrated to enhance the production of TNF- α , IL-1- β , and IL6.^{56,57} Additionally, polysaccharides, terpenoids, and proteins activate splenocytes, thymocytes, PBMCs, and DCs through different pathways.^{58–60}

Plant Extract Effect on NO Production

The activation of macrophages is typically linked to increased production of immune mediators. To determine whether the plant extract influences this production, the conditioned media from the treated cells were collected for analysis. Our study has demonstrated that the extract can activate macrophages and bone marrow cells, thereby enhancing nitric oxide production.

NO is an important, biologically active signaling molecule that acts as a signal transducer in the central nervous system. It is produced via the action of iNOS in response to pro-inflammatory cytokines and lipopolysaccharides.⁶¹ The pathophysiology involves multiple systems, including immune and inflammatory responses. In the immune system, the stimulation and activation of macrophages result in a significant release of NO. The NO released can kill microorganisms, bacteria, parasites, and tumor cells.⁶² It can trigger a protective inflammatory response. NO is an important, biologically active signaling molecule that acts as a signal transducer in the central nervous system. It is produced by iNOS in response to pro-inflammatory cytokines and lipopolysaccharides.⁶¹ The pathophysiology involves multiple systems, including immune and inflammatory responses. In the immune system, macrophage stimulation and activation lead to a significant release of NO. The NO released can kill microorganisms, bacteria, parasites, and tumor cells.⁶² It can trigger a protective inflammatory response.

The increase in NO production induced by plant extracts was dose-dependent, suggesting that they act as immune mediators or modulators. Previous studies have shown that the potential immunomodulatory effects of leaf crude extract and fractions are associated with increased NO production.^{63,64} These results contradict those of Inam et al, who demonstrated that *C. papaya* leaf extracts reduced iNOS expression in an OVA-induced asthma model in mice.⁵³ The study model and the extraction solvent used to prepare the extracts may explain these conflicting results.

Previous in vivo toxicity studies showed no hepatic or renal toxicity following oral administration of a 0.72 mL/100 g bw dose in rats.³² The extract also showed no significant toxic effects in mice. Oral administration of the extract to rats significantly increased platelet, red blood cell, and white blood cell counts.⁶⁵

Conclusion

The present preliminary study showed that the crude extract and the n-hexane, ethyl acetate, n-butanol, and residual aqueous fractions effectively modulated non-functional immune responses. They increased the proliferation of

macrophages and hematopoietic cells in mouse bone marrow. They enhanced the production of TNF, IL-6, and NO in peritoneal macrophages, whereas IL-1 was inhibited. The production of IL-3, SCF, GM-CSF, and TPO was stimulated after 72 h of culture, with the extent of stimulation depending on the concentration.

The immunomodulatory properties of plant crude extract and fractions are due to several active secondary metabolites, mainly alkaloids, flavonoids, tannins, anthraquinones, and terpenoids.

C. papaya leaf extracts may be potential candidates for further research for the development of a plant-based therapeutic agent to modulate the immune system in many diseases. The stimulatory effect of crude extract, hexane, and ethyl acetate fractions on IL-6, TPO, and SCF production could be promising for ameliorating thrombocytopenia observed in severe dengue. Further preclinical *in vivo* studies, including acute and chronic toxicity assessments, must be conducted before clinical evaluation of *C. papaya* leaf extracts for immune-based diseases.

Abbreviations

BMC, bone marrow cells, DC, dendritic cells, DPPH, 2,2-Diphenyl-1-picrylhydrazyl, FRAP, Ferric ion Reducing Antioxidant Power, ELISA, Enzyme-Linked Immunosorbent Assay, GM-CSF, Granulocyte-Macrophage Colony Stimulating Factor, iNOS, inducible nitric oxide synthase, LPS, Lipopolysaccharides, MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, NO, Nitric oxide, PBMC, Peripheral Blood Mononuclear Cells, PM, peritoneal macrophages, SCF, stem cell factor, TPO, Thrombopoietin.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from a request addressed to the corresponding author at email: ouemoussa10@gmail.com

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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References

1. Pourzangiabadi M, Najafi H, Fallah A, Goudarzi A, Pouladi I. Dengue virus: etiology, epidemiology, pathobiology, and developments in diagnosis and control - A comprehensive review. *Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis.* 2025;127:105710. doi:10.1016/j.meegid.2024.105710
2. Haider N, Hasan MN, Onyango J, Asaduzzaman M. Global landmark: 2023 marks the worst year for dengue cases with millions infected and thousands of deaths reported. *IJID Reg.* 2024;13:100459. doi:10.1016/j.ijregi.2024.100459
3. Kuczera D, Assolini JP, Tomiotto-Pellissier F, Pavanelli WR, Silveira GF. Highlights for dengue immunopathogenesis: antibody-dependent enhancement, cytokine storm, and beyond. *J Interferon Cytokine Res.* 2018;38(2):69–80. doi:10.1089/jir.2017.0037
4. Khan MA, Khan ZA, Charles M, et al. Cytokine storm and mucus hypersecretion in COVID-19: review of mechanisms. *J Inflamm Res.* 2021;14:175–189. doi:10.2147/JIR.S271292

5. Noisakran S, Onlamoon N, Hsiao HM, et al. Infection of bone marrow cells by dengue virus in vivo. *Exp Hematol.* 2012;40(3):250–259.e4. doi:10.1016/j.exphem.2011.11.011
6. Srikiatkachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. *Semin Immunopathol.* 2017;39(5):563–574. doi:10.1007/s00281-017-0625-1
7. Mohammed MA. Fighting cytokine storm and immunomodulatory deficiency: by using natural products therapy up to now. *Front Pharmacol.* 2023;14:1111329. doi:10.3389/fphar.2023.1111329
8. Nikiema WA, Ouedraogo M, Ouedraogo WP, et al. Systematic review of chemical compounds with immunomodulatory action isolated from African medicinal plants. *Molecules.* 2024;29(9):2010. doi:10.3390/molecules29092010
9. Xiuping Y, Xiaotong Z, Rongjia Z, Ping S. Effect of astragaloside IV on the immunoregulatory function of adipose-derived mesenchymal stem cells from patients with psoriasis vulgaris. *J Tradit Chin Med Chung Tsa Chih Ying Wen Pan.* 2022;42(4):513–519. doi:10.19852/j.cnki.jtcm.20220516.002
10. Rouf R, Uddin SJ, Sarker DK, et al. Antiviral potential of garlic (*Allium sativum*) and its organosulfur compounds: a systematic update of pre-clinical and clinical data. *Trends Food Sci Technol.* 2020;104:219–234. doi:10.1016/j.tifs.2020.08.006
11. Arreola R, Quintero-Fabián S, López-Roa RI, et al. Immunomodulation and anti-inflammatory effects of garlic compounds. *J Immunol Res.* 2015;2015:401630. doi:10.1155/2015/401630
12. Ahmad N, Fazal H, Ayaz M, Abbasi BH, Mohammad I, Fazal L. Dengue fever treatment with *Carica papaya* leaves extracts. *Asian Pac J Trop Biomed.* 2011;1(4):330–333. doi:10.1016/S2221-1691(11)60055-5
13. Canini A, Alesiani D, D’Arcangelo G, Tagliatesta P. Gas chromatography–mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. *J Food Compos Anal.* 2007;20(7):584–590. doi:10.1016/j.jfca.2007.03.009
14. Musa YM. Isolation and purification of flavonoids from the leaves of locally produced *Carica papaya*. *Int J Sci Technol Res.* 2015;4:282–284.
15. Wang X, Hu C, Ai Q, Chen Y, Wang Z, Ou S. Isolation and identification caripaine in *Carica papaya* L. Leaf by HPLC-UV method. *Int J Food Prop.* 2015;18(7):1505–1512. doi:10.1080/10942912.2014.900785
16. El-Mesallamy AM, Hussein SA, Abd El Azim MH, El-Gerby M. Phenolic composition and biological activities of methanolic extract of caricapapaya. *Nat Prod Indian J.* 2015;10:91–98.
17. Anjum V, Arora P, Ansari SH, Najmi AK, Ahmad S. Antithrombocytopenic and immunomodulatory potential of metabolically characterized aqueous extract of *Carica papaya* leaves. *Pharm Biol.* 2017;55(1):2043–2056. doi:10.1080/13880209.2017.1346690
18. Nugroho A, Heryani H, Choi JS, Park HJ. Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity. *Asian Pac J Trop Biomed.* 2017;7(3):208–213. doi:10.1016/j.apjtb.2016.12.009
19. Soib HH, Ismail HF, Husin F, Abu Bakar MH, Yaakob H, Sarmidi MR. Bioassay-Guided different extraction techniques of *Carica papaya* (Linn.) leaves on in vitro wound-healing activities. *Mol.* 2020;25(3):517. doi:10.3390/molecules25030517
20. Sharma N, Mishra KP, Chanda S, et al. Evaluation of anti-dengue activity of *Carica papaya* aqueous leaf extract and its role in platelet augmentation. *Arch Virol.* 2019;164(4):1095–1110. doi:10.1007/s00705-019-04179-z
21. Kaser A, Brandacher G, Steurer W, et al. Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. *Blood.* 2001;98(9):2720–2725. doi:10.1182/blood.v98.9.2720
22. Deepti K, Umadevi P, Vijayalakshmi G, Polarao BV. Antimicrobial activity and phytochemical analysis of morinda tinctoria roxb. leaf extracts. *Asian Pac J Trop Biomed.* 2012;2(3):Supplement:S1440–S1442. doi:10.1016/S2221-1691(12)60433-X
23. Hayat S, Miana GA, Kanwal M, Ahsan Z, Tariq MJ. Determination of total flavonoid content and phenolic content, antioxidant assay, and antiepileptic activity of achillea millefolium extract. *Nat Prod Commun.* 2025;20(2):1934578X251319221. doi:10.1177/1934578X251319221
24. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: *Methods in Enzymology.* Vol 299. Elsevier; 1999:152–178. doi:10.1016/S0076-6879(99)99017-1
25. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.* 1958;181(4617):1199–1200. doi:10.1038/1811199a0
26. Kirkova D, Stremski Y, Statkova-Abeghe S, Docheva M. Quercetin hybrids—synthesis, spectral characterization and radical scavenging potential. *Molbank.* 2022;2022(1):M1329. doi:10.3390/M1329
27. González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, et al. Evaluation of the antioxidant activity of aqueous and methanol extracts of pleurotus ostreatus in different growth stages. *Front Microbiol.* 2016;7. doi:10.3389/fmicb.2016.01099
28. Ray A, Dittel BN. Isolation of mouse peritoneal cavity cells. *J Vis Exp.* 2010;(35):1488. doi:10.3791/1488
29. Hermida MDR, Malta R, de S. Santos MDPC, Dos-Santos WLC. Selecting the right gate to identify relevant cells for your assay: a study of thioglycollate-elicited peritoneal exudate cells in mice. *BMC Res Notes.* 2017;10:695. doi:10.1186/s13104-017-3019-5
30. Liu X, Quan N. Immune cell isolation from mouse femur bone marrow. *Bio-Protoc.* 2015;5(20):e1631. doi:10.21769/bioprotoc.1631
31. Choi CY, Kim JY, Kim YS, Chung YC, Hahm KS, Jeong HG. Augmentation of macrophage functions by an aqueous extract isolated from *Platycodon grandiflorum*. *Cancer Lett.* 2001;166(1):17–25. doi:10.1016/S0304-3835(01)00440-2
32. Jayasinghe CD, Gunasekera DS, De Silva N, Jayawardena KKM, Udagama PV. Mature leaf concentrate of Sri Lankan wild type *Carica papaya* Linn. modulates nonfunctional and functional immune responses of rats. *BMC Complement Altern Med.* 2017;17(1):230. doi:10.1186/s12906-017-1742-z
33. López-García J, Lehocký M, Humpolíček P, Sába P. HaCaT keratinocytes response on antimicrobial atelocollagen substrates: extent of cytotoxicity, cell viability and proliferation. *J Funct Biomater.* 2014;5(2):43. doi:10.3390/jfb5020043
34. Lewis MA, Patil K, Ettayebi K, Estes MK, Atmar RL, Ramani S. Divergent responses of human intestinal organoid monolayers using commercial in vitro cytotoxicity assays. *PLoS One.* 2024;19(6):e0304526. doi:10.1371/journal.pone.0304526
35. Asghar N, Naqvi SAR, Hussain Z, et al. Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. *Chem Cent J.* 2016;10(1):5. doi:10.1186/s13065-016-0149-0
36. Nisa FZ, Astuti M, Haryana SM, Murdiati A. Antioxidant activity and total flavonoid of *Carica papaya* L. Leaves with different varieties, maturity and solvent. *Agritech.* 2019;39(1):54. doi:10.22146/agritech.12813
37. Koala M, Ramde-Tiendrebeogo A, Ouedraogo N, et al. HPTLC phytochemical screening and hydrophilic antioxidant activities of apium graveolens L. Cleome gynandra L. and Hibiscus sabdariffa L. *Am J Anal Chem.* 2021;12(1):15–28. doi:10.4236/ajac.2021.121002

38. Sharma A, Bachheti A, Sharma P, Bachheti RK, Husen A. Phytochemistry, pharmacological activities, nanoparticle fabrication, commercial products and waste utilization of *Carica papaya* L.: a comprehensive review. *Curr Res Biotechnol*. 2020;2:145–160. doi:10.1016/j.crbiot.2020.11.001
39. Herb M, Schramm M. Functions of ROS in macrophages and antimicrobial immunity. *Antioxidants*. 2021;10(2):313. doi:10.3390/antiox10020313
40. Oppenheim JJ, Matsushima K, Yoshimura T, Leonard EJ. The activities of cytokines are pleiotropic and interdependent. *Immunol Lett*. 1987;16(3–4):179–183. doi:10.1016/0165-2478(87)90145-3
41. Cohen MC, Cohen S. Cytokine function: a study in biologic diversity. *Am J Clin Pathol*. 1996;105(5):589–598. doi:10.1093/ajcp/105.5.589
42. Hirayama D, Iida T, Nakase H. The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis. *Int J Mol Sci*. 2017;19(1):92. doi:10.3390/ijms19010092
43. Gottlieb AA, Waldman SR, Adler F. The multiple functions of macrophages in immunity. *CRC Crit Rev Microbiol*. 1972;1(3):321–352. doi:10.3109/10408417209103870
44. Adrain C, Zettl M, Christova Y, Taylor N, Freeman M. Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE. *Science*. 2012;335(6065):225–228. doi:10.1126/science.1214400
45. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1 β secretion. *Cytokine Growth Factor Rev*. 2011;22(4):189. doi:10.1016/j.cytogfr.2011.10.001
46. Conos SA, Lawlor KE, Vaux DL, Vince JE, Lindqvist LM. Cell death is not essential for caspase-1-mediated interleukin-1 β activation and secretion. *Cell Death Differ*. 2016;23(11):1827–1838. doi:10.1038/cdd.2016.69
47. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harb Perspect Biol*. 2014;6(10):a016295. doi:10.1101/cshperspect.a016295
48. Ouedraogo M, Nikiema WA, Pagbelguem B, et al. Effects of leaf extracts of *Carica papaya* L. (Caricaceae) and *Vernonia colorata* (Willd.) Drake (Asteraceae) on induced thrombocytopenia and increased vascular permeability: an approach to symptomatic treatment of dengue. *J Phytopharm*. 2023;12(6):358–365. doi:10.31254/phyto.2023.12602
49. Rajapakse S, de Silva NL, Weeratunga P, Rodrigo C, Sigera C, Fernando SD. *Carica papaya* extract in dengue: a systematic review and meta-analysis. *BMC Complement Altern Med*. 2019;19(1):265. doi:10.1186/s12906-019-2678-2
50. Huang S, Xu L, Sun Y, Wu T, Wang K, Li G. An improved protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. *J Orthop Transl*. 2015;3(1):26–33. doi:10.1016/j.jot.2014.07.005
51. Drayer AL, Boer AK, Los EL, Esselink MT, Vellenga E. Stem cell factor synergistically enhances thrombopoietin-induced STAT5 signaling in megakaryocyte progenitors through JAK2 and Src kinase. *Stem Cells Dayt Ohio*. 2005;23(2):240–251. doi:10.1634/stemcells.2004-0153
52. Aziz J, Abu Kassim NL, Abu Kasim NH, Haque N, Rahman MT. *Carica papaya* induces in vitro thrombopoietic cytokines secretion by mesenchymal stem cells and haematopoietic cells. *BMC Complement Altern Med*. 2015;15(1):215. doi:10.1186/s12906-015-0749-6
53. Inam A, Shahzad M, Shabbir A, Shahid H, Shahid K, Javeed A. *Carica papaya* ameliorates allergic asthma via down regulation of IL-4, IL-5, eotaxin, TNF- α , NF- κ B, and iNOS levels. *Phytomedicine Int J Phytother Phytopharm*. 2017;32:1–7. doi:10.1016/j.phymed.2017.04.009
54. Xu H, Liew LN, Kuo IC, Huang CH, Goh DLM, Chua KY. The modulatory effects of lipopolysaccharide-stimulated B cells on differential T-cell polarization. *Immunology*. 2008;125(2):218–228. doi:10.1111/j.1365-2567.2008.02832.x
55. Krauss S, Buttgerit F, Brand MD. Effects of the mitogen concanavalin A on pathways of thymocyte energy metabolism. *Biochim Biophys Acta BBA - Bioenerg*. 1999;1412(2):129–138. doi:10.1016/S0005-2728(99)00058-4
56. Ahmad W, Jantan I, Kumolosasi E, Haque MA, Bukhari SNA. Immunomodulatory effects of *Tinospora crispa* extract and its major compounds on the immune functions of RAW 264.7 macrophages. *Int Immunopharmacol*. 2018;60:141–151. doi:10.1016/j.intimp.2018.04.046
57. Sharma U, Bala M, Kumar N, Singh B, Munshi RK, Bhalerao S. Immunomodulatory active compounds from *tinospora cordifolia*. *J Ethnopharmacol*. 2012;141(3):918–926. doi:10.1016/j.jep.2012.03.027
58. Sindhu RK, Goyal A, Das J, Neha Choden S, Kumar P. Immunomodulatory potential of polysaccharides derived from plants and microbes: a narrative review. *Carbohydr Polym Technol Appl*. 2021;2:100044. doi:10.1016/j.carpta.2021.100044
59. Zhang S, Li Z, Wang X, et al. Isolation, structural elucidation, and immunoregulation properties of an arabinofuranan from the rinds of *Garcinia mangostana*. *Carbohydr Polym*. 2020;246:116567. doi:10.1016/j.carbpol.2020.116567
60. Huang H, Luo SH, Huang DC, Cheng SJ, Cao CJ, Chen GT. Immunomodulatory activities of proteins from *Astragalus membranaceus* waste. *J Sci Food Agric*. 2019;99(8):4174–4181. doi:10.1002/jsfa.9650
61. Lyons CR, Orloff GJ, Cunningham JM. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. *J Biol Chem*. 1992;267(9):6370–6374. doi:10.1016/S0021-9258(18)42704-4
62. Schairer DO, Chouake JS, Nosanchuk JD, Friedman AJ. The potential of nitric oxide releasing therapies as antimicrobial agents. *Virulence*. 2012;3(3):271–279. doi:10.4161/viru.20328
63. Singh S, Kumar S, Tomar M, et al. Aqueous extract of *Carica papaya* leaf elicits the production of TNF- α and modulates the expression of cell surface receptors in tumor-associated macrophages. *Biosci Biotechnol Res Commun*. 2019;12. doi:10.21786/bbrc/12.4/35
64. Hyun S, Ko M, Hyun CG. *Carica papaya* leaf water extract promotes innate immune response via MAPK signaling pathways. *J Appl Biol Chem*. 2021;64:277–284. doi:10.3839/jabc.2021.038
65. Dharmarathna SLCA, Wickramasinghe S, Waduge RN, Rajapakse RPVJ, Kularatne SAM. Does *Carica papaya* leaf-extract increase the platelet count? An experimental study in a murine model. *Asian Pac J Trop Biomed*. 2013;3(9):720–724. doi:10.1016/S2221-1691(13)60145-8

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