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Original Research Article

In vitro Immunomodulatory and Hepatoprotective Activities of Selected Indonesian Medicinal Plants

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ABSTRACT

Bioactive compounds from plant extracts play a crucial role in drug discovery. These compounds can affect various physiological functions, including immunomodulation and hepatoprotection. This study sought to investigate the potential of selected Indonesian medicinal extracts possessing anti-hepatitis C activity as hepatoprotective and immunomodulatory agents. Eight plant extracts (*Acacia mangium*, *Curcuma xanthorrhiza*, *Ficus carica*, *Piper crocatum*, *Phyllanthus niruri*, *Piper betle*, *Ruta angustifolia*, *Sida rhombifolia*) were used in the study. Phagocytosis assay was performed on primary *Mus musculus* macrophages treated with each extract at various concentrations (Half-maximal Inhibitory Concentration (IC50), 1/2 IC_{50}, 1/4 IC_{50}, and 1/8 IC_{50}) to obtain the phagocytosis percentage. Carbon tetrachloride (CCl_4)-induced damage in human hepatocellular carcinoma (HepG2) cells was also treated with extracts at the same gradient concentrations, and the hepatoprotective potential of the extracts was analysed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. *Mitochondrial aspartate transaminase* (*mAST*) messenger ribonucleic acid (mRNA) expression was also analysed. The results revealed that the macrophage phagocytosis percentage was significantly higher than controls in the treatment groups receiving all extracts except for *P. niruri*. All extract-treated groups also showed higher IC_{50} values of CCl_4 and higher cell viability. The extracts also reduced the expression of *mAST* compared to the sick control. In conclusion, all extracts showed *in vitro* hepatoprotective and immunomodulatory properties except for *P. niruri*, which only exhibited hepatoprotective effects without immunomodulation abilities.

Keywords: Anti-hepatitis C virus, Medicinal plants, Mitochondrial AST, Phagocytosis

Introduction

Hepatitis C virus (HCV) is one of the main problems associated with liver disease. Globally, an estimated 130 million people have been infected and face the potential of developing chronic liver disease, cirrhosis, and hepatocellular carcinoma.[1] It has been reported that the largest share of HBV and HCV infections worldwide was in the Asia Pacific region.[2] In Indonesia, the estimated rate of HCV antibody presence among the general population is around 1.0%, whereas among individuals who use drugs, it can reach as high as 90%. As a result, Indonesia ranks among the countries with the highest number of HCV cases globally, affecting an estimated 2.5 million people.[3]

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The hepatitis C virus is a ribonucleic acid (RNA)-enveloped virus classified under the *Flaviviridae* family. This virus is grouped into a 9.6kb single-stranded RNA (ssRNA) virus that produces a precursor polyprotein consisting of 3,000 amino acids.[4] HCV is primarily transmitted through infected blood (bloodborne virus) that might be spread from unsafe injections, unscreened blood transfusions, and sexual practices that lead to blood exposure. Inside the infected body, HCV multiplies within the cytoplasm of hepatocytes, generating between 10^{10} to 10^{12} virions daily with a half-life of 2 to 3 hours.[5] Currently, there is no effective vaccine against hepatitis C, but it can be treated with antiviral medications to cure the disease and prevent long-term liver damage. Drug combinations that are classified as direct-acting antivirals (DAAs) can increase the patient's recovery rate, but there are some drawbacks, such as side effects, resistance, and price, and limited access.[4, 6, 7] Therefore, efforts to find leads with anti-hepatitis C need to be explored.

Medicinal plants have long been used to treat a variety of ailments. Utilization of natural products begins with traditional use to the standardization of active plant-derived compounds. A wide variety of active phytochemicals, such as flavonoids, terpenoids, lignins, sulphides, polyphenolics, coumarins, saponins, furyl compounds, alkaloids, polylines, thiophenes, proteins, and peptides, have been recognized for their ability to inhibit a range of viruses, thus showing that medicinal plants have great potential as antiviral medication.[8]

Indonesia has diverse medicinal plants. It is home to about 80% of the medicinal plant species found in Southeast Asia, with only 2,000 to 7,500 medicinal plants having been used out of 30,000-40,000 species within the country.[9] Wahyuni *et al*. uncovered the potential anti-HCV activities of leaf and stem extracts from medicinal plants found in East Java, including *Toona sureni*, *Melicope latifolia*, *Melanolepis multiglandulosa*, and *Ficus fistulosa*.[4] Several other plants were also noted to show anti-HCV properties. The leaf extract of *Ruta angustifolia* contains chalepin and pseudane IX that can inhibit HCV replication. The curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin compounds from the rhizome of *Curcuma* species can inhibit HCV from entering the cells and cell-to-cell transmission of the virus. The leaves of *F. fistulosa* and *Phyllanthus amarus* and the barks of *Acacia nilotica* and *Piper cubeba* also showed the potential to inhibit HCV.[10] Considering the anti-hepatitis activities of those medicinal plants, eight local medicinal plants from the genera that have been documented to exhibit anti-HCV properties, including *A. mangium*, *C. xanthorrhiza*, *F. carica*, *Phyllanthus niruri*, *Piper betle*, *Piper nigrum*, *R. angustifolia*, and *Sida rhombifolia* were selected. In this study, we aimed to explore the immunomodulatory and hepatoprotective activities of those plants in the search for potential hepatitis C medication.

Materials And Methods

Materials

The human hepatocellular carcinoma (HepG2) cell line (Cat. #HB-8065, ATCC, Virginia, USA) was obtained from the Integrated Laboratory for Research and Testing, Universitas Gadjah Mada while the primary macrophages were isolated from the peritoneal cavity of 8-12-week-old male *Mus musculus* (obtained from the Faculty of Pharmacy, Universitas Gadjah Mada). The eight plants used in the study were collected in January 2023 from East Java, Indonesia. Plant identification and leaf specimen deposition were conducted by UPT Laboratorium Herbal Materia Medica Batu. The binomial nomenclatures and specimen numbers of the plants were as follows: *Acacia mangium* (074/444/102.20-A/2022), *Curcuma xanthorrhiza* (074/322A/102.7/2019), *Ficus carica* (074/443/102.20-A/2022), *Piper crocatum* (074/441/102.20-A/2022), *Piper betle* (000.9.3/2862/102.20/2023), *Ruta angustifolia* (074/442/102.20-A/2022), *Sida rhombifolia* (074/311A/102.7/2019), and *Phyllanthus niruri* (074/320A/102.7/2019).

Cell lines and culture

The HepG2 cells were grown in Dulbecco's modified Eagle solid medium (DMEM) (Cat. #11965-092, Gibco, New York, USA) and the macrophages were cultured in Roswell Park Memorial Institute (RPMI) medium (Cat. #11875093, Gibco) supplemented with Fetal Bovine Serum (FBS) (Cat. #16000044, Gibco), 1% Penicillin-Streptomycin (Cat. #15140122, Gibco), and Amphotericin (Cat. #15290018, Gibco). HepG2 was grown in 96-well plates with a density of 5 x 10^6 and incubated for 24 hours at 37 °C in an incubator containing 5% carbon dioxide (CO2).

Extract preparation

Eight plant extracts, namely *A. mangium* (A1), *C. xanthorrhiza* (C1), *F. carica* (F1), *P. crocatum* (P1), *P. niruri* (P2), *P. betle* (P3), *R. angustifolia* (R1), and *S. rhombifolia* (S1), were prepared by maceration using 96% ethanol. Two kilograms of leaves from each plant were washed under running water, drained, and then cut into small pieces. The pieces were air-dried and then crushed with a blender and sifted to obtain powder. As much as 500 grams of each leaf powder was macerated with 1 liter of 96% ethanol for 24 hours. The maceration process was repeated 3 times. The macerated powder was then filtered and squeezed to obtain the filtrate. The filtrate was concentrated using a rotary vacuum evaporator to obtain a thick extract. An *in vitro* cytotoxicity test was carried out to see the safety of the extract and to determine the concentration of the extract to be used for further studies.

Macrophage phagocytosis assay

Macrophage cells were isolated and grown in a 24-well plate (5 x 10^5 cells/well), cultured in RPMI medium supplemented with 10% FBS, 1% Pen Strep, and 0.5% Amphotericin B for 24 hours. Cells were then treated and incubated for 24 hours with plant extracts at the half-maximal inhibitory concentration (IC50), 1/2 IC50, 1/4 IC50, and 1/8 IC50 obtained from the MTT Assay. Details of the concentrations for each extract are shown in Table 1. Subsequently, cells were washed with RPMI, and latex bead suspension (5 x 10^6/well) (Sigma MCLS; Cat no. LB30-1ML) was added for 30 minutes. Cells were washed with PBS and then stained with Giemsa. The percentage of latex bead-phagocytosing macrophages was calculated from a total of 200 cell populations.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Table 1: Plant Extract Concentrations

No	Plant Extract	Concentration (mg/mL)		No	Plant Extract	Concentration (mg/mL)	
1	A1	IC50	0.24270	5	P2	IC_{50}	0.19730
		1/2 IC50	0.12135			1/2 IC_{50}	0.09865
		1/4 IC50	0.06068			1/4 IC_{50}	0.04933
		1/8 IC50	0.03033			1/8 IC_{50}	0.02467
2	C1	IC50	0.01317	6	P3	IC_{50}	0.06017
		1/2 IC50	0.00659			1/2 IC_{50}	0.03010
		1/4 IC50	0.00330			1/4 IC_{50}	0.01504
		1/8 IC50	0.00165			1/8 IC_{50}	0.00752
3	F1	IC50	0.21230	7	R1	IC_{50}	0.31520
		1/2 IC50	0.10615			1/2 IC_{50}	0.15760
		1/4 IC50	0.05308			1/4 IC_{50}	0.07880
		1/8 IC50	0.02654			1/8 IC_{50}	0.03940
4	P1	IC50	0.25250	8	S1	IC_{50}	0.03250
		1/2 IC50	0.12625			1/2 IC_{50}	0.01625
		1/4 IC50	0.06313			1/4 IC_{50}	0.00813
		1/8 IC50	0.03156			1/8 IC_{50}	0.00406

A1: *A. mangium*; C1: *C. xanthorrhiza*; F1: *F. carica;* P1: *P. crocatum*; P2: *P. niruri*; P3: *P. betle*; R1: *R. angustifolia*; S1: *S. rhombifolia*.

The hepatoprotective potential of the extracts was investigated against damage induced by carbon tetrachloride (CCl_4) in HepG2 cells. Treatment groups were divided into CCl_4 and the combination of CCl_4 with each extract. The CCl_4 gradients used were 0, 0.1, 0.2, 0.3, 0.4, and 0.5% (v/v), while the concentration of the extract used was 1/2 IC50. HepG2 cells were grown on 96-well plates at a density of 20,000 cells/well and incubated for 24 hours at 37 °C. CCl_4 or a combination of CCl_4 and each extract concentration was given to HepG2 cultures in a volume of 100 µL each and incubated for 24 hours. The supernatant was discarded, and 100 µl/well of MTT reagent (0.5 mg/ml) was added, followed by incubation for 4 hours. 100µl SDS 10% was added and incubated overnight in the dark at room temperature. Sample absorbance was quantified at a wavelength of 595 nm using a microplate reader (Cat. #1681130, BioRad, California, USA). The data obtained were then used to determine the IC50 values and cell viability.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)
qRT-PCR was used to assess the relative mRNA expression of *mitochondrial aspartate aminotransferase* (*mAST*) as a hepatocyte damage-related gene. The samples used were HepG2 cells that had been damaged by CCl_4 (16.68 ppm) for 24 hours, then were treated with each extract at concentrations of IC50, 1/2 IC50, 1/4 IC50, and 1/8 IC50 (Table 1). Isolation of RNA was performed using FavorPrep™ Tissue Total RNA Mini Kit (Cat. #FATRK 001-1, Favorgen, Ping Tung, Taiwan), and cDNA synthesis with ReverTra Ace® qPCR RT Master Mix with gDNA remover (Cat. #FSQ-301, Toyobo, Osaka, Japan). The primers used were *mAST* (F: 5'- AGCCTTACGTTCTGCCTAGC -3', R: 5'- GACTTCGCTGTTCTCACCCA -3') and *GAPDH* (F: 5'- ATGTTCGTCATGGGTGTGAA -3', R: 5'- GTCTTCTGGGTGGCAGTGAT -3'). qPCR was performed using ExcelTaq™ 2X Fast Q-PCR Master Mix (Cat. #TQ1200, Smobio, Hsinchu City, Taiwan). The thermal cycling conditions for the amplification of *mAST* and *GAPDH* were at 95 °C for 20 sec for template strand denaturation and activation of the enzyme, and at 95°C for 3 sec for denaturation. The annealing/extension condition was at 54°C for 30 sec. qPCR reactions were done using the BIO-RAD CFX96 Touch Real-Time PCR Detection System machine. The Ct values were analysed using the Livak method.[11]

Statistical analysis
The data obtained were analysed and statistically tested using GraphPad Prism version 9.0.0. The Shapiro-Wilk test was used to analyse data normality, followed by one-way ANOVA with a 95% confidence level, as all data followed a normal distribution.

Results and Discussion

This study investigated the ability of eight Indonesian plant extracts to modulate the immune system through macrophage phagocytosis activity and their protective ability against CCl_4-induced damage in liver cells. The findings revealed that the macrophage phagocytosis percentage was markedly higher than controls in the treatment groups receiving all extracts except for *P. niruri*. All extract-treated groups also showed higher IC_{50} values of CCl_4 and higher cell viability. The extracts also reduced the expression of *mAST* compared to the sick control.

Macrophage phagocytic activity
The immunomodulatory capacity of the extracts was evaluated by observing the phagocytic ability of macrophages against latex beads. Figure 1 shows the morphology of macrophages that did not show phagocytic activity and macrophages that phagocytized latex beads. Based on the number of cells showing phagocytosis activity from a total of 200 cell populations, the macrophage phagocytosis percentage of every extract was generated as shown in Figure 2. The results showed that macrophage phagocytosis percentages of A1, C1, F1, P1, RI, and S1 extracts were significantly greater in comparison to the control group, mainly in the groups given the higher concentrations of the extracts. In contrast, we found that in P3, the significant increase in the phagocytosis percentage was only shown in the group given the lowest concentration of the extract. A1, C1, F1, P1, RI, and S1 extracts showed the best immunomodulation activity (as immunostimulant) at a concentration of IC50, while P3 at a concentration of 1/8 IC50. Unlike the other extracts, P2 did not increase the phagocytic activity of macrophages.
Macrophages are the most important phagocytic cells in the immune system, originating from mature monocytes that reside in tissues. As the early innate immune responses, macrophages serve as phagocytes and antigen-presenting cells (APCs) to clear invading pathogens, triggering inflammatory signals, engulfing dead cells, and presenting antigens to T lymphocytes.[12] The phagocytosis ability of macrophages has been widely used as a parameter to determine active compounds that stimulate immune responses. In this study, all extracts except for P2 increased the percentage of phagocytosing macrophages.
It has been reported that certain plants contain active substances capable of modulating body functions. Several phytochemicals, such as flavonoids, polysaccharides, lactones, alkaloids, diterpenoids, and glycosides, were shown to be responsible for the plants' immunomodulating activities.[13] From all the extracts we used in this study, previous findings have identified that they contain flavonoids that are known to act as immunomodulators, such as quercetin, which modulates leukocyte biology in a stimulus-dependent manner, and epicatechin found in *A. mangium* which increases interleukin 2 (IL-2) secretion. Crude polysaccharide enhances macrophage immune activity through targeted activation of nuclear factor-kappa B (NF-κB), and benzofuran from *C. xanthorrhiza* regulates the innate immune activity of phagocytic cells. Various flavonoid contents have also been reported in other types of extracts that play a role in activating natural killer cells to promote Interferon-γ (IFN-γ) production. IFN-γ activates macrophages, thereby enhancing their phagocytic activity.[13-20] Further isolation of the active compounds from these extracts needs to be done to pinpoint the substances involved in immunomodulation.

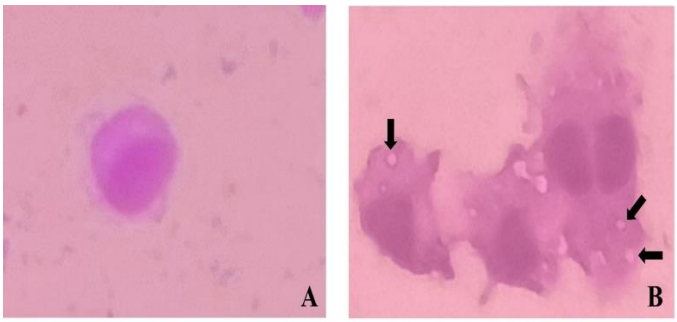

Figure 1: Phagocytosis activity of macrophages after incubation with latex bead (magnification 400x). Non-phagocytic macrophages (A) and macrophages that phagocytose latex beads (shown in black arrow) (B).

Hepatoprotective potential of extracts against CCl_4-induced damage
The hepatoprotective potential of the extract was evaluated against CCl_4-induced hepatocyte damage in HepG2 cells. Through the MTT assay, the sigmoid curves (Figure 3) and the IC_{50} values of all extracts were obtained (Table 2). From the data, the CCl_4 group showed the lowest IC50 value compared to all CCl_4-extract groups. A higher IC_{50} value indicates that a larger concentration of CCl_4 is required to inhibit the biological or biochemical function of HepG2 cells, implying that the extract had the ability to protect cells from damage caused by CCl_4. The cell viability assay (Figure 4) showed a linear trend, indicating that all extracts could reduce cell apoptosis induced by CCl_4. The S1, P3, and R1 extracts did not exhibit any significant change in cell viability compared to the control group. However, the C1, F1, P1, and P2 groups displayed reduced viability than the control group but still showed protection against CCl_4-induced apoptosis. It is noteworthy that the A1 group exhibited even greater viability than the control group.

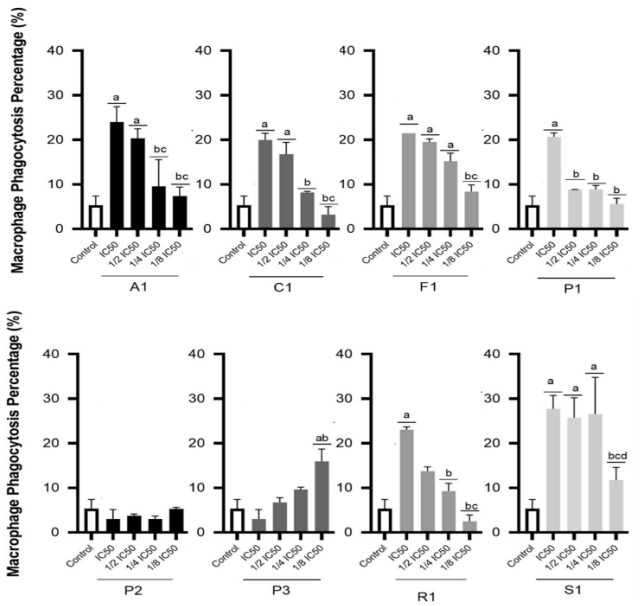

Figure 2: Macrophage phagocytosis percentage of all groups. A1: *A. mangium*; C1: *C. xanthorrhiza*; F1: *F. carica;* P1: *P. crocatum*; P2: *P. niruri*; P3: *P. betle*; R1: *R. angustifolia*; S1: *S. rhombifolia*; $P \leq 0.05$; [a]significantly different from control; [b]significantly different from IC_{50} of respective extract group; [c]significantly different from 1/2 IC50 of respective extract group; [d]significantly different from 1/4 IC_{50} of respective extract group.

CCl_4 is a well-known hepatotoxic agent, it is frequently used to cause liver injury *in vitro* and *in vivo*. The mechanism of action of CCl_4 is closely related to its metabolic processes. The liver is responsible for metabolizing nutrients, drugs, and xenobiotics. This process is mainly carried out by a set of enzymes known as cytochrome P-450. However, during the metabolism of CCl_4 by cytochrome P-450, free radicals are generated, which can lead to lipid peroxidation. When lipids, especially unsaturated phospholipids, break down, it harms both intracellular and plasma membranes. Reactive aldehydes, which are the products of this breakdown, spread throughout the cell and cause more damage, including an increase in membrane permeability, causing apoptosis.[21]

Plant materials contain a wide range of natural antioxidants, including polyphenols (such as phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (including xanthophylls and carotenes), and vitamins (vitamins E and C).[22] It is conceivable that the antioxidant properties of the extracts used in this study could have contributed to the inhibition of oxidative damage caused by the free radicals generated during CCl_4 metabolism, for instance, the high concentration of phenolic compound present in *A. mangium*, *F. carica*, and *P. crocatum* (flavonoids, tannins, and alkaloids), and the rest of the extracts that showed strong antioxidant activity.[23-30]

mRNA expression of mAST

The relative mRNA expression of *mAST* as a hepatotoxicity marker is shown in Figure 5. Overall, the treatment of various extracts on HepG2 cells damaged by CCl_4 resulted in lower relative expression of the gene compared to the sick control. Most extract concentrations did not show significant differences in expression compared to controls. The only exception was found in group A1, where only the concentration of 1/4 IC50 showed no significant differences.

Aspartate aminotransferase is a catalytic enzyme involved in transferring α-amino groups from aspartate and alanine to the α-keto group of ketoglutaric acid to produce oxalic acid and pyruvic acid, which are key intermediates in the citric acid cycle. In general, AST is reported to be concentrated in the liver but is also expressed in other parts, such as the heart, skeletal muscle, kidney, brain, and red blood cells.[31] Elevation of this enzyme (along with the ALT enzyme) has been widely used as a biomarker of hepatocellular injury.[32] In humans, AST is found in two separate forms that differ genetically and immunologically, one located in the cytoplasm (cytoplasmic AST/cAST) and the other in the mitochondria (mitochondrial AST/mAST). These two isoenzymes catalyze the same reactions, even though they have different kinetics.[33] In this study, the treatment of various extracts on CCl_4-induced HepG2 damage resulted in lower relative expression of *mAST* compared to the sick control. It appeared that all extracts could suppress the expression of the gene encoding the mAST enzyme. It indicated that the extracts have the potential to act as a hepatoprotector through the downregulation of the *mAST* gene. This hepatoprotective activity may be associated with the antioxidant capabilities of the extracts, which can eliminate the production of free radicals, thereby preventing any potential damage to the liver.[34] Polyphenols, carotenoids, and vitamins that exist in these extracts were previously reported as strong antioxidants.[35,36]

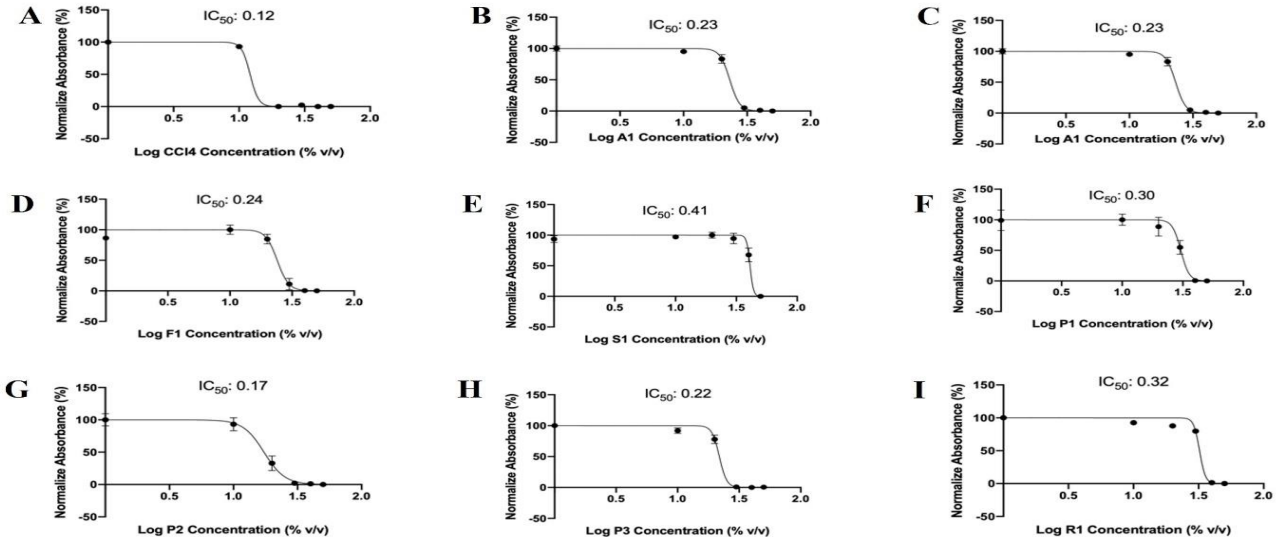

Figure 3: MTT Assay sigmoid curve of CCl_4 (A) and the combination of CCl_4 with A1 (B), C1 (C), F1 (D), S1 (E), P1 (F), P2 (G), P3 (H), and R1 (I) plant extract. A1: *A. mangium*; C1: *C. xanthorrhiza*; F1: *F. carica;* P1: *P. crocatum*; P2: *P. niruri*; P3: *P. betle*; R1: *R. angustifolia*; S1: *S. rhombifolia.*

Table 2: IC50 value of CCl_4 and the combination CCl_4-plant extract

No	Treatment group	IC50 (% v/v)
1	CCl_4	0.12
2	CCl_4 + A1	0.23
3	CCl_4 + C1	0.22
4	CCl_4 + P1	0.30
5	CCl_4 + P2	0.17
6	CCl_4 + P3	0.22
7	CCl_4 + F1	0.24
8	CCl_4 + R1	0.32
9	CCl_4 + S1	0.41

A1: *A. mangium*; C1: *C. xanthorrhiza*; F1: *F. carica;* P1: *P. crocatum*; P2: *P. niruri*; P3: *P. betle*; R1: *R. angustifolia*; S1: *S. rhombifolia*.

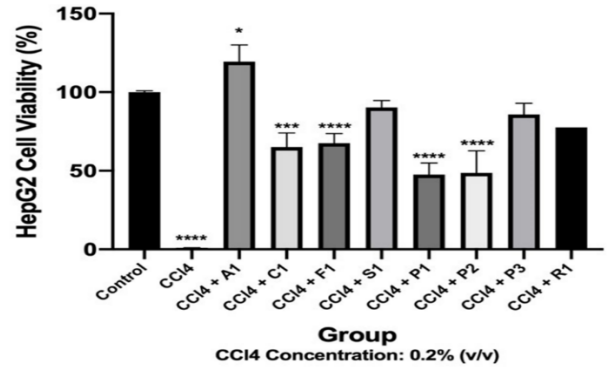

Figure 4: Cell viability percentage of the control group, CCl_4, and the combination CCl_4-plant extract. CCl4 concentration 0.2% (v/v). Data are presented in mean ± SD; *significantly different from the control group with $P \leq 0.05$; *** $P \leq 0.001$; **** $P \leq 0.0001$.

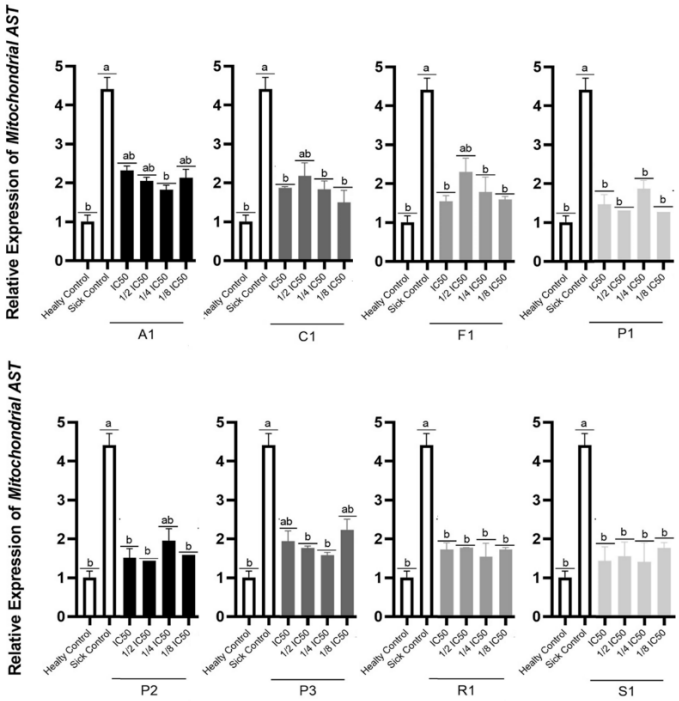

Figure 5: Relative mRNA expression of *mAST* in HepG2 cells after extract treatment, along with healthy and sick control group. A1: *A. mangium*; C1: *C. xanthorrhiza*; F1: *F. carica;* P1: *P. crocatum*; P2: *P. niruri*; P3: *P. betle*; R1: *R. angustifolia*; S1: *S. rhombifolia*; $P \leq 0.05$; [a]significantly different from control; [b]significantly different from IC_{50} of respective extract group.

Conclusion

In conclusion, we found that *A. mangium*, *C. xanthorrhiza*, *F. carica*, *P. crocatum*, *R. angustifolia*, *S. rhombifolia*, and *P. betle* possessed *in vitro* hepatoprotective and immunomodulatory properties. However, *P. niruri* only exhibited hepatoprotective effects and lacked immunomodulation abilities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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References

1. Alter MJ, Fabiani FL. Epidemiology of hepatitis C virus infection: Prevalence and incidence. World J. Gastroenterol. 2007; 13(17):2436-2441. http://www.wjgnet.com/1007-9327/13/2436.asp
2. Udompap P, Tanwandee T, Gani R. Affordability of antiviral therapy in Asia-Pacific countries and its impact on public health outcomes. Clin Liver Dis. 2021; 16(6):249-253. doi:10.1002/cld.977
3. Trickey A, Hiebert L, Perfect C, Thomas C, El Kaim JL, Vickerman P, Schütte C, Hecht R. Hepatitis C virus elimination in Indonesia: Epidemiological, cost and cost-effectiveness modelling to advance advocacy and strategic planning. Liver Int. 2020; 40(2):286-297. doi:10.1111/liv.14232
4. Wahyuni TS, Tumewu L, Permanasari AA, Apriani E, Adianti M, Rahman A, Widyawaruyanti A, Lusida MI, Fuad A, Soetjipto, Nasronudin, Fuchino H, Kawahara N, Shoji I, Deng L, Aoki C, Hotta H. Antiviral activities of Indonesian medicinal plants in the East Java region against hepatitis C virus. Virol J. 2013; 10:259. doi:10.1186/1743-422X-10-259
5. Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. Int J. Med Sci. 2006; 3(2):47-52. doi:10.7150/ijms.3.47
6. Di Marco L, La Mantia C, Di Marco V. Hepatitis C: Standard of treatment and what to do for global elimination. Viruses. 2022; 14(3):505. doi:10.3390/v14030505

7. Hafid AF, Aoki-Utsubo C, Permanasari AA, Adianti M, Tumewu L, Widyawaruyanti A, Wahyuningsih SPA, Wahyuni TS, Lusida MI, Soetjipto, Hotta H. Antiviral activity of the dichloromethane extracts from *Artocarpus heterophyllus* leaves against hepatitis C virus. Asian Pac J. Trop Biomed. 2017; 7(7):633-639. doi:10.1016/j.apjtb.2017.06.003
8. Jassim SA, Naji MA. Novel antiviral agents: A medicinal plant perspective. J. Appl Microbiol. 2003; 95(3):412-427. doi:10.1046/j.1365-2672.2003.02026.x
9. Cahyaningsih R, Magos Brehm J, Maxted N. Setting the priority medicinal plants for conservation in Indonesia. Genet Resour Crop Evol. 2021; 68(5):2019-2050. doi:10.1007/s10722-021-01115-6
10. Wahyuni TS, Utsubo CA, Hotta H. Promising anti-hepatitis C virus compounds from natural resources. Nat Prod Commun. 2016; 11(8):1193-1200.
11. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 2001; 25(4):402-408. doi:10.1006/meth.2001.1262
12. Christofides A, Strauss L, Yeo A, Cao C, Charest A, Boussiotis VA. The complex role of tumor-infiltrating macrophages. Nat Immunol. 2022; 23(8):1148-1156. doi:10.1038/s41590-022-01267-2
13. Miteva D, Kitanova M, Velikova T. Biomacromolecules as immunomodulators: Utilizing nature's tools for immune regulation. Macromol. 2024; 4(3):610-633. doi:10.3390/macromol4030037
14. Azizah IN, Winanta A. In vitro immunomodulatory activity of fig fruit ethanol extract (*Ficus carica* Liin) against phagocytosis macrophages and lymphocyte proliferation. Majalah Obat Tradisional. 2022; 27(2):85-92. doi:10.22146/mot.70128
15. Halim MRA, Tan MSMZ, Ismail S, Mahmud R. Standardization and phytochemical studies of *Curcuma xanthorrhiza* Roxb. Int J. Pharm Pharm Sci. 2012; 4(3): 606-610.
16. Khaleghi F, Jantan I, Din L Bin, Yaacob WA, Khalilzadeh MA, Bukhari SNA. Immunomodulatory effects of 1-(6-hydroxy-2-isopropenyl-1-benzofuran-5-yl)- 1-ethanone from *Petasites hybridus* and its synthesized benzoxazepine derivatives. J. Nat Med. 2014; 68(2):351-357. doi:10.1007/s11418-013-0805-9
17. Kim AJ, Kim YO, Shim JS, Hwang JK. Immunostimulating activity of crude polysaccharide extract isolated from *Curcuma xanthorrhiza* Roxb. Biosci Biotechnol Biochem. 2007; 71(6):1428-1438. doi:10.1271/bbb.60241
18. Trivadila T, Iswantini D, Rahminiwati M, Rafi M, Salsabila AP, Sianipar RNR, Indariani S, Murni A. Herbal immunostimulants and their phytochemicals: Exploring *Morinda citrifolia*, *Echinacea purpurea*, and *Phyllanthus niruri*. Plants. 2025; 14(6):897. doi:10.3390/plants14060897
19. Wahyuni TS, Sukma NS, Permanasari AA, Aoki-Utsubo C, Widyawaruyanti A, Hafid AF. *Acacia mangium*: A promising plant for isolating anti-hepatitis C virus agents. F1000Res. 2022; 11:1452. doi:10.12688/f1000research.124947.1
20. Hosseinzade A, Sadeghi O, Biregani AN, Soukhtehzari S, Brandt GS, Esmaillzadeh A. Immunomodulatory effects of flavonoids: Possible induction of T CD4+ regulatory cells through suppression of mTOR pathway signaling activity. Front Immunol. 2019; 10:51. doi:10.3389/fimmu.2019.00051
21. Boll M, Weber LW, Becker E, Stampfl A. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Z Naturforsch C J. Biosci. 2001; 56(7-8):649-659. doi:10.1515/znc-2001-7-826
22. Loi M, Paciolla C. Plant antioxidants for food safety and quality: Exploring new trends of research. Antioxidants. 2021; 10(6):972. doi: 10.3390/antiox10060972
23. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules. 2023; 28(4):1845. doi: 10.3390/molecules28041845
24. Xavier J, Thomas S. Antioxidant activities of leaves and fruits of *Piper nigrum* and *Piper longum*. Asian J. Plant Sci. 2020; 19(2):127-132. doi:10.3923/ajps.2020.127.132
25. Xu Z, Gao P, Liu D, Song W, Zhu L, Liu X. Chemical composition and in vitro antioxidant activity of *Sida rhombifolia* L. volatile organic compounds. Molecules. 2022; 27(20):7067. doi:10.3390/molecules27207067
26. Shuib NA, Iqbal A, Sulaiman FA, Razak I, Susanti D. Antioxidant and antibacterial activities of *Ruta angustifolia* extract. J. Teknol. 2015; 77(25):101-105. doi:10.11113/jt.v77.6747
27. Lister INE, Ginting CN, Girsang E, Armansyah A, Marpaung HH, Sinaga APF, Handayani RAS, Rizal R. Antioxidant properties of red betel (*Piper crocatum*) leaf extract and its compounds. J. Nat Rem. 2019; 19(4):198-205. doi:10.18311/jnr/2019/23633
28. Mahmoudi S, Khali M, Benkhaled A, Benamirouche K, Baiti I. Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica* L. varieties. Asian Pac J. Trop Biomed. 2016; 6(3):239-245. doi:10.1016/j.apjtb.2015.12.010
29. Zhang L, Chen J, Wang Y, Wu D, Xu M. Phenolic extracts from *Acacia mangium* bark and their antioxidant activities. Molecules. 2010; 15(5):3567-3577. doi:10.3390/molecules15053567
30. Rahmat E, Lee J, Kang Y. Javanese turmeric (*Curcuma xanthorrhiza* Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Evid Based Complement Alternat Med. 2021; 2021:9960813. doi:10.1155/2021/9960813
31. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Can. Med. Assoc. J. 2005; 172(3):367-379. doi:10.1503/cmaj.1040752
32. Chen VL, Du X, Chen Y, Kuppa A, Handelman SK, Vohnoutka RB, Peyser PA, Palmer ND, Bielak LF, Halligan B, Speliotes EK. Genome-wide association study of serum liver enzymes implicates diverse metabolic and liver pathology. Nat Commun. 2021; 12(1):816. doi:10.1038/s41467-020-20870-1
33. Ndrepepa G. Aspartate aminotransferase and cardiovascular disease - A narrative review. J. Lab Precis Med. 2021; 6(January):6. doi:10.21037/jlpm-20-93
34. Aitynova A, Sevastre B, Ielciu I, Hanganu D, Olah N-K, Ibragimova N, Shalakhmetova T, Benedec D, Lyu M, Krasnoshtanov A, Gapurkhaeva T. Hepatoprotective activity and oxidative stress reduction of an *Arctium tomentosum* Mill. root extract in mice with experimentally induced hepatotoxicity. Livers. 2024; 4(4):696-710. doi:10.3390/livers4040048
35. Meziane Y, Yakoubi R, Megateli S, Chaouia C. In vitro assessment of total bioactive contents and antioxidant capacity of grape juices extracts of table and wine varieties from Algeria and their correlations. Nat Life Sci Commun. 2023; 22(3):e2023050.
36. Fitriansyah SN, Yollanda T, Riasari H. Total carotenoid content and antioxidant activity of Sawo Walanda (*Pouteria campechiana* Kunth. Baehni) extract from various solvents. Trop J. Nat Prod Res. 2023; 7(7):3478-3481. doi:10.26538/tjnpr/v7i7.28