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Research Paper

Avicennia officinalis L. - A Promising Source for The Creation of Medicines for Hepatoprotective and Antioxidant Activities in HepG2 Cell Line and Mice

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Abstract

Avicennia officinalis L. (AOL), a mangrove plant, is extensively used as an anti-inflammatory, anti-cancer, and antioxidant which has traditionally utilized this plant as herbal in Vietnam. In preliminary phytochemical screening, the methanol and aqueous leaf extracts showed high levels of bioactive compounds including glycosides, saponins, flavonoids, tannins, and triterpenes. To identify the major compounds in five extracts of AOL (leaves, flowers, fruits, roots, and bark), the quantitative HPLC/PDA technique was utilized. It was discovered that the total flavonoid content and total phenolic content were 52.55±0.070 and 16.59±0.030 mg/g extract, respectively. The result of antioxidant activity in vitro was shown leave and bark extract with the strongest activity (citric acid aqueous leave with EC_{50} =43.52 μg/mL in DPPH assay and tartaric acid aqueous bark extract with 30.69% of inhibition in TBRAS assay). Citric acid aqueous leaf extract (CALE) demonstrated 17.08% of the protective activity against CCl_4-induced hepatoxicity in HepG2 cells ($p < 0.05$). CALE was standardized in order to assess its potential hepatoprotective effects against a mouse model of paracetamol-induced liver injury. The extract was administered orally to the animals at two different dosage levels (200 and 400 mg/kg b.wt.). For a duration of one week, the dosage was administered once daily. By improving the blood levels of biochemical, AOL demonstrated a notable dose-dependent hepatoprotective effect. The action of the standardized AOL was found to be similar to that of silymarin. AOL collected in Vietnam has a lot of potential to become a traditional medicine source.

Keywords

Avicennia officinalis L., Antioxidant, Hepatoprotective Activity, Polyphenols, Against Paracetamol-Induced Liver Toxicity Model in Mice

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1. INTRODUCTION

Liver disease is considered a global issue, with emerging nations like Vietnam being particularly impacted. Despite the high incidence of liver disease in Vietnam, there is no national strategy or systematic screening program for those at risk. Men's high alcohol intake, chronic hepatitis B (prevalence estimated at 12%), and chronic hepatitis C (prevalence at least 2%), are risk factors. Liver cancer is now the main cause of cancer-related fatalities in Vietnam due to a number of causes (Gish et al., 2012). Synthetic pharmaceutical treatments for liver illnesses often result in severe side effects after prolonged use. Consequently, there has been growing interest in herbal medicines, and recent research has focused on describing the health-promoting properties of various plants, especially those rich in phenolics, known for their outstanding antioxidant and hepatoprotective potential (Stickel and Schuppan, 2007).

Avicennia officinalis L. (AOL) is a medium-sized evergreen mangrove shrub belonging to the Acanthaceae family. These mangroves are a unique type of salt-tolerant plant ecosystem that grows at the land-water interface (Prakashamani et al., 2019). In Vietnam, *Avicennia officinalis* L. is found in coastal provinces with mangrove areas. It is a pioneer tree species that helps in soil and forest conservation in Ca Mau province, Vietnam. Traditionally, this plant has been used in folk medicine as a therapeutic herb. Previous studies on the pharmacology of *Avicennia officinalis* L. have shown that its leaves were used to treat smallpox, alleviate joint pain, and treat snakebites (Thirunavukkarasu et al., 2011). The bark, leaves, and fruit have been used to treat skin conditions such as scabies, boils,

and skin ulcers (Sumithra et al., 2011; Noviyanto et al., 2025). Aqueous preparations of the seeds have also been used as a remedy for sore throats. According to Sumithra, the fruit of AOL has been used to treat constipation and other digestive issues. Various phytoconstituents have been identified and isolated, including triterpenoids (Assaw et al., 2020), flavonoids (Nguyen et al., 2019), phenolic acids (Van Nguyen et al., 2024), and naphthofuranquinones (Ramanjaneyulu et al., 2015).

According to previous research, a similar species, *Avicennia marina*, has a long history of use in treating hepatitis, with its antihepatotoxic properties well-documented (Namazi et al., 2013; Vellimalai et al., 2019; Azhagu Madhavan et al., 2021). To our knowledge, no comprehensive study has been conducted to evaluate the chemical composition, antioxidant potential, and hepatoprotective qualities of the entire plant of *Avicennia officinalis* L. There is a lack of information on the hepatoprotective properties and antioxidant capacity of *Avicennia officinalis* L. both in vivo and in vitro. Moreover, plants of the Avicennia family are known to contain high levels of polyphenols, which have a wide range of biological potentials due to their antioxidant effects. This study was designed to assess the antioxidant and hepatoprotective properties of the ethanolic and aqueous extracts from five parts (leaves, flowers, fruits, roots, and bark) of *Avicennia officinalis* L., native to Vietnam, against paracetamol-induced liver toxicity in a mouse model. Additionally, a standardization process using quantitative high-performance liquid chromatography combined with a photodiode array detector (HPLC-PDA) was established to determine the quantity of bioactive compounds in AOL.

2. EXPERIMENTAL SECTION

2.1 Chemical and Reagents

Methanol (CH_3OH, HPLC grade), acetonitrile (CH_3CN, HPLC grade), 2,2- diphenyl-1- picrylhydrazyl (DPPH), potassium chloride (KCl), thiobarbituric acid (TBA), quercetin, silymarin, paracetamol, and trypan blue were purchased from Sigma-Aldrich (St. Louis, USA); Ammonium acetate ($NH_4CH_3CO_2$, HPLC grade), formic acid (HCOOH, HPLC grade), isopropanol (C_3H_8O), hydrochloride acid (HCl) from Merck (Germany); Sodium dihydrogen phosphate (NaH2PO4), sodium hydrogen phosphate (Na_2HPO_4), trichloroacetic acid (TCA), carbon tetrachloride (CCl_4) from Xilong Scientific (China); phosphate buffered saline (PBS) and bovine serum albumin (BSA) from Gibco (Carlsbad, CA, USA); Eagle's minimal essential medium (MEM), bovine fetal serum (BFS), phosphate buffered saline (PBS), L-glutamine, Penicillin-Streptomycin, Trypsin-EDTA from Gibco (Carlsbad, CA, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) from Invitrogen (Carlsbad, CA, USA); Doxorubicin "Ebewe" from Ebewe Pharma (Austria).

2.2 Preparation of *Avicennia officinalis* L. Extract

Five components of *Avicennia officinalis* L., namely leaves, flowers, fruits, roots, and barks, were gathered in Ca Mau province, located in the Mekong Delta region of Vietnam. The dried sample had a moisture level of less than 13 percent. The samples were kept at room temperature and kept in opaque glass containers. The Department of Biology at Can Tho University in Vietnam used polymerase chain reaction (PCR) methods to find the plants.

Five parts of *Avicennia officinalis* L. including leaves, flowers, fruit, root and bark were extracted with five solvents (Ethanol absolute, Ethanol 80%, Ethanol 60%, Citric acid aqueous, Tartaric acid aqueous). The dried powdered herbals (100g) were extracted reflux with 1000 mL surveyed solvent three times for 1 hour each. The resulting extract was evaporated under vacuum, at a temperature not exceeding 50°C, until almost free from solvent.

2.3 Phytochemical Qualitative Analysis

The extracts of five parts of *Avicennia officinalis* including leaves, flowers, fruit, root and bark were assessed for the existence of the phytochemical analysis by using the standard methods (Sofowora, 1993).

Quality control and chemical characterization of *A. officinalis* using High Performance Liquid Chromatography (HPLC)

2.3.1 Sample Preparation

A 20 mL volumetric flask was filled with 0.5g of precisely weighed *A. officinalis* sample (leaf, flower, fruit, root, and bark). MeOH (w/v) was used to modify the volume after the sample was sonicated. Prior to HPLC analysis, all sample solutions were degassed and filtered via a 0.45 lm membrane filter into sample vials.

2.3.2 Construction of the Standard Calibration Curves

Accurate weighing was used to create standard stock solutions of luteolin-7-O-glucoside and cinnamic acid, which were then dissolved in MeOH to final concentrations of 0.05 and 20 μg/mL, respectively. Six concentrations were used to create standard calibration curves. Every sample was run three times.

2.3.3 HPLC Analysis

The study was performed using an Ultrafast Liquid Chromatography connected to Diode Array Detector (UFLC-DAD) equipment (Shimadzu LC-20AD; Tokyo, Japan; includes a quaternary pump). The LabSolution software was used to control the system. The samples were placed on a pre-column (C18, 4.6 mm), injected (20 mL) into a Phenomenex (Torrance, California, USA) C18 5 μm (4.6 × 250 mm), and detected at 280 nm at 30°C. Using a gradient of acetonitrile (A):methanol (B): water containing 0.2% ammonium acetate and 0.1% formic acid (C), the mobile phase (1 mL/min) was composed of the following compositions: 1:9:90 (32 min), 7:10:83 (41 min), 7:11:82 (48 min), 11:15:74 (72 min), 9:18:73 (86 min), 0:20:80 (90 min), and 7:3:90 (95 min), maintaining this composition until 95 minutes.

In order to do quantification, the measured integration area must be reported in the associated standard's calibration equation. Total phenolic acid content (TPC) was determined

using acid cinamic, whereas total flavonoid content (TFC) was determined using luteolin-7-O-glucoside. As determined by HPLC, the total amounts of the relevant components were total phenolic acid and total flavonoid.

2.4 Antioxidant Assay of *Avicennia officinalis* L. Extract

DPPH radical scavenging activity Using the stable radical DPPH, the free radical-scavenging activity of each extract was quantified in terms of its capacity to donate hydrogen or scavenge radicals (Blois, 1958). To create a sample solution, methanol was added to the plant extract at different concentrations. Each 960 μl solution was put into a test tube and combined with 40 μl of DPPH (1000 μg/mL) to make a total of 1 mL with methanol. After that, the reaction solutions were allowed to stand at room temperature for 30 minutes in the dark, and their absorbance at 517 nm was measured. Ascorbic acid, or vitamin C, was utilized as the standard material in this investigation; blanks were run in each experiment. EC_{50} (μg/mL) was used to represent the DPPH radical ability, and the following formula was used to get the inhibition percentage (Equation (1))

$$\%\,\text{Inhibition} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{blank}}} \times 100 \quad (1)$$

where A_{sample} is the absorbance of a sample and A_{blank} is the absorbance of the control sample. Every test was run at least three times, and the graphs were plotted using the average of the three results.

2.5 Thiobarbituric Acid Eeactive Substances Assay (TBARS)

Lipid peroxidation was measured as described before (Buege and Aust, 1978) determining thiobarbituric acid reactive substances (TBARS). At 0–5°C, each liver was homogenized in a solution containing 1.15% w/v KCl and 50 mM Na_2HPO_4. Following an hour at 37°C and a 10-minute centrifugation at 3000 rpm in a MiniSpin Centrifuge, the homogenate was separated into 0.5 mL and aliquots were taken out of the incubation mixture. Then, 0.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) and 10% (w/v) trichloroacetic acid (TCA) were added and vortexed. There was a 5-minute chilling interval on ice after a 15-minute boil at 100°C. The control samples were set up in the same way, except one extract sample was left out. For the blank samples, the same protocols were followed, with the exception that liver homogenate was not added. At 532 nm, the color produced was measured. By contrasting the oxidized samples with a blank and control, the percentage suppression of TBARS generation was ascertained (Equation (2)).

$$\%\,\text{Inhibition} = \left(\frac{OD_{\substack{\text{group treated} \\ \text{with AOL/Silymarin}}} - OD_{\substack{CCl_4 \\ \text{treated group}}}}{OD_{\substack{\text{control} \\ \text{group}}} - OD_{\substack{CCl_4 \\ \text{treated group}}}} \right) \times 100 \quad (2)$$

The result of extract with strongest antioxidant and hepatoprotective activity in vitro was standardized following the standard pulished in our previous study (Duong et al., 2023). The standardized AOL extract was conducted in vivo assay by against paracetamol-induced liver toxicity model in mice.

2.6 Hepatoprotective Assay of Standardized *Avicennia officinalis* L. Extracts by Against Paracetamol-Induced Liver Toxicity Model in Mice

2.6.1 Experimental Animals

Swiss albino mice (20 × 2 g, 6-8 weeks old) were obtained from the Pasteur Institute in Ho Chi Minh City. These mice were housed in groups of 12 and provided with ad libitum food and water. A seven-day acclimation period to the laboratory environment at Can Tho University of Medicine and Pharmacy preceded the experiment. The mice were maintained on a standard pellet diet throughout the study.

2.6.2 Experimental Design

Thirty-five mice in total were split into seven groups of five animals each at random. Group I got distilled water once a day and operated the vehicle. As the paracetamol control group, Group II received 400 mg/kg body weight of paracetamol (PCM) in distilled water. A dose of silymarin (50 mg/kg, b. wt. op) was given to Group III. AOL standardized extract (AOLS) was administered as a pretreatment to groups IV and V at dosages of 200 mg/kg and 400 mg/kg (b. wt. op), respectively. AOL total extract (AOLT) was given to Groups VI and VII according to the same dosage and schedule. Every group received daily treatment for seven days in a row. Every group, with the exception of the mice in group I, received a single 400 mg/kg dosage of paracetamol on the eighth day. Blood samples were taken by direct heart puncture under mild ether anesthesia 48 hours after taking paracetamol. Centrifugation at 3000 rpm for 10 minutes at 4°C produced serum, which was then frozen at -20°C for further examination.

2.6.3 Biochemical Parameters Examination

Serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) were among the biochemical markers measured. To quantify oxidative stress, glutathione (GSH) activity was assessed using the technique published by Rahman et al. (2006). The Ohkawa et al. (1979) technique was used to quantify malondialdehyde (MDA), a marker of lipid p. All enzymatic assessments were conducted using a UV spectrophotometer (Model Synergy HT BIOTEK INSTRUMENTS, INC. - USA) in accordance with standard kit set procedures.

2.6.4 Histopathological Studies

The livers were removed right away and kept in 10% formalin. Tissues were embedded in paraffin after being dehydrated in an increasing ethanol series and cleared in xylene. Hematoxylin and eosin-stained sections of 4-5 μm thickness were prepared, and liver histology in both treated and control animals was assessed under a microscope.

2.7 Statistical Analysis

Statistical analyses were performed using Microsoft Excel and GraphPad Prism 8. The biochemical data have been expressed as mean ± SEM (standard error of mean). One-way ANOVA was used to analyze differences among groups, followed by Dunnett's test for multiple comparisons. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 27.0.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Qualitative Analysis

The *Avicennia officinalis* L. extract was subjected to a preliminary phytochemical screening, which included the analysis of five different parts: leaves, flowers, fruit, root, and bark. Three solvents with varying polarity (aqueous, methanol, and diethyl ether) were utilized, and the resulting outcomes are presented in Table 1. Tannins, coumarin, and fatty oil were the primary compounds found in the diethyl ether extract of the leaves. No bioactive chemicals were detected in the diethyl ether extracts of the flower, fruit, bark, and root. The methanol extract of five parts of *Avicennia officinalis* L. was shown to include an abundance of glycosides, flavonoids, coumarin, and tannins in the leaves. The fruit extract and flower extract also contained tannins. The aqueous extract of the leaves contains a significant amount of glycosides, tannins, and flavonoids components. The aqueous extract of the fruit and flower had a significant amount of tannin compounds. In summary, the methanol and aqueous leaf extracts include several components including glycosides, saponins, flavonoids, tanins, and triterpenes. The results of phytochemical qualitative analysis were the preliminary data to identify potential compounds in five parts of AOL and choose the suitable method for extracting and analyzing compounds by HPLC/PDA.

3.2 Standardization of *Avicennia officinalis* L. Extract by HPLC

The methanol extract contains a variety of chemical components, including flavonoids and tannins, as determined by the result of the phytochemical qualitative analysis. Further research is needed to investigate the phenolic chemicals found in five different parts of AOL using HPLC-PDA methods to analyze polar extracts. This is necessary because hydroalcoholic preparations of AOL are widely used. The validity of the quantitative method was confirmed in our earlier study (Van Nguyen et al., 2024). Overall, the analysis revealed that the extract derived from AOL leaves included a greater amount of phenolics and flavonoids compared to the extract obtained from the bark, fruit, and root (Table 2). The total phenolic content, measured in cinnamic acid equivalents (CAE), was 16.59 ± 0.030 mg/g extract. The total flavonoid content, measured in luteolin equivalents (LE), was 52.55 ± 0.070 mg/g extract. The flower extract exhibited the lowest levels of phenolic acid components and no detectable levels of flavonoid compounds.

Based on the outcome of fingerprint chromatography analysis of all parts, it was revealed that the leaf extract contains the highest concentration of bioactive compounds (Figure 1a). We detected a minimum of seven prominent peaks in the leaf extract, which exhibited the characteristic UV absorption pattern of flavonoids as determined by HPLC-UV (Figure 1b). Consistent with our research, a previous investigation reported the existence of luteolin-7-O-glucoside (peak 3) and cinnamic acid (peak 8) in the methanolic extract derived from AOL leaves. The bark extract contains four primary compounds that exhibit characteristic UV absorption profiles of phenolic acids and flavonoids, as determined by HPLC-UV analysis. The chromatography analysis of fruit, root, and flower extracts revealed either the absence or low levels of phenolic compounds. Therefore, the leaves and bark of the AOL tree were considered a promising source of raw material in Vietnam (Figure 1).

According to the results of the phytochemical qualitative analysis, the methanol extract has a high concentration of chemical substances such as flavonoids, tannins, and other compounds. The phenolic compounds present in the five portions of AOL were analyzed using HPLC-PDA techniques to determine their primary components, as well as the overall phenolic acid and flavonoid content. The total flavonoid content, measured in luteolin equivalents (LE), was 52.55 ± 0.070 mg/g extract. The total phenolic content, measured in cinnamic acid equivalents (CAE), was 16.59 ± 0.030 mg/g extract. Swagat Kumar Das's research found that the total flavonoid concentration of AOL leaves collected in India was 0.051 ± 0.002 mg/g extract (Das et al., 2018). The floral extract exhibited the lowest concentration of phenolic acid constituents and no flavonoid compounds were detected. Considering the antioxidant properties of flavonoids and acid phenolics, it is reasonable to suggest that these compounds enhance the liver-protective powers of the plant. Phenolic compounds are considered antioxidants because they have the ability to donate hydrogen to free radicals, hence disrupting the chain reaction responsible for lipid peroxidation in tissues (Flora, 2009). The hepatoprotective effect of this extract was evaluated in HepG2 cells and mice, considering the antioxidant potential of phenolic compounds and the significant impact of oxidative stress on the liver (Cichoz-Lach and Michalak, 2014).

3.3 Antioxidant Assay of *Avicennia officinalis* L. Extract

Despite significant progress in the field of medicine, hepatic diseases remain a prevalent global health issue. There is a wide range of herbal formulations that have been proven to have hepatoprotective properties, as demonstrated in a study conducted by Zhang et al. (2013). Plant extracts high in phenolic components have been associated with hepatoprotective advantages (Shehab et al., 2015). The presence of antioxidants in medicinal plants is frequently associated with both hepatotoxicity and protection against oxidative stress (Meng et al., 2020). Hence, we hypothesized that *Avicennia officinalis* L., a medicinal plant found in Vietnam, exhibits antioxidant properties, suggesting its potential as a hepatoprotectant (Thirunavukkarasu et al., 2011; Das et al., 2019; Assaw et al., 2020; Bui et al., 2021). We evaluated the hepatoprotective characteristics of

Table 1. Phytochemical Qualitative Analysis of Five Part of *A. officinalis* L.

Compounds	Leaves			Fruits			Flowers			Bark			Root		
	AE	ME	DE	AE	ME	DE	AE	ME	DE	AE	ME	DE	AE	ME	DE
Alkaloids	+	+	-	+	+	-	-	+	-	-	++	-	-	+	-
Glycosides	+++	+++	X	+	+	X	-	+	X	+	+	X	+	+	X
Saponins	++	++	X	+	+	X	+	-	X	+++	+		++	-	X
Flavonoids	+++	+++	+	+	-	-	++	-	-	+	-	-	+	-	-
Coumarin	X	+++	+++		-	-	X	-	-	X	-	-	X		-
Anthraquinons	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
Tanins	+++	+++	X	+++	+++	X	+++	+++	X	-	++	X	-	-	X
Reducing sugars	+	++	X	-	-	X	-	-	X	+	-	X	-	+	X
Carotenoids	X	X	-	X	X	-	X	X	-	X	X	-	X	X	-
Fatty acid	X	X	+++	X	X	-	X	X	-	X	X	+	X	X	+
Oil	X	X	-	X	X	-	X	X	-	X	X	-	X	X	-
Triterpene	++	++	X	-	-	X	-	-	X	-	-	X	-	-	X

Notes:
AE, Aqueous extract; ME, Methanol extract; DE, Diethyl eter extract
(+): Positive; (++): Clearly positive; (+++): Strong positive; (-): Negative; (X): not conduct

Table 2. Total Phenolic and Total Flavonoid Content of Five Part of *A. officinalis* L. Extract

Sample	Total Phenolic Content as Cinnamic Acid Equivalents (CAE) (mg/g Extract)	Total Flavonoid Content as Luteolin Equivalents (LE) (mg/g Extract)
Leave	16.59 ± 0.030	52.55 ±0.070
Bark	6.59 ± 0.012	34.97 ± 0.060
Fruit	1.30 ± 0.015	15.62 ± 0.082
Root	0.89 ± 0.023	4.48 ± 0.034
Flower	0.30 ± 0.068	nd*

*nd, not detect

the extract from A. officinalis, as well as its role in antioxidant processes.

The methanol and aqueous extracts were found to contain a high concentration of bioactive components, including glycosides, saponins, flavonoids, tannins, and triterpenes, as shown by the preliminary phytochemical screening and HPLC results. Therefore, ethanol and aqueous solutions were used to assess their antioxidant activity and hepatoprotective effects. Due to its green and safe solvent properties for in-vivo experiments on mice, ethanol was selected for the survey instead of methanol, which has the same polarity as ethanol. Figure 2 displays the impact of different concentrations of ethanol absolute, ethanol 80%, ethanol 60%, citric acid aqueous, and tartaric acid aqueous from *A. officinalis* on the DPPH radical scavenging activity and thiobarbituric acid reactive substances assay (TBARS).

The study utilized Vitamin C as a reference drug, and the ability of DPPH radicals was measured and expressed as EC_{50} μg/mL). The EC_{50} exhibited a decrease, indicating a higher level of antioxidant activity in the extract. The antioxidant activity in the DPPH assay result of five sections of *A. officinalis* L. exhibited a decreasing trend in the following order: Depart from the fruit, proceed to the bark, then to the root, and finally to the flower. Out of the extracts that were examined, the aqueous leaf extracts of *A. officinalis* containing citric acid showed the highest level of DPPH radical scavenging activity (EC_{50} = 43.52 μg/mL). The antioxidant activity, as determined by the thiobarbituric acid reactive substances assay (TBARS), exhibited a reduction in the following sequence. The sequence of plant development is as follows: Bark, Leave, Fruit, Root, Flower. The aqueous extract of the bark containing tartaric acid exhibited the highest level of inhibition, with a percentage of 30.69%. The DPPH technique revealed that the leaves had the highest level of antioxidant activity, but the TBRAS assay indicated that the bark yielded the best result. After conducting a survey on solvent extraction, it was shown that the aqueous solvent has a higher level of antioxidant activity compared to the ethanol extract (namely, the citric acid aqueous leaf extract and the tartaric acid aqueous bark extract).

The DPPH free radical approach is a convenient and efficient method for evaluating antioxidant compounds (Baliyan et al., 2022). Two limitations of the DPPH approach are the structural conformation of antioxidants and the polarity or pH of the reaction fluid (Martysiak-Żurowska and Wenta, 2012). The TBARS test, which is responsive to a wide range of antioxi-

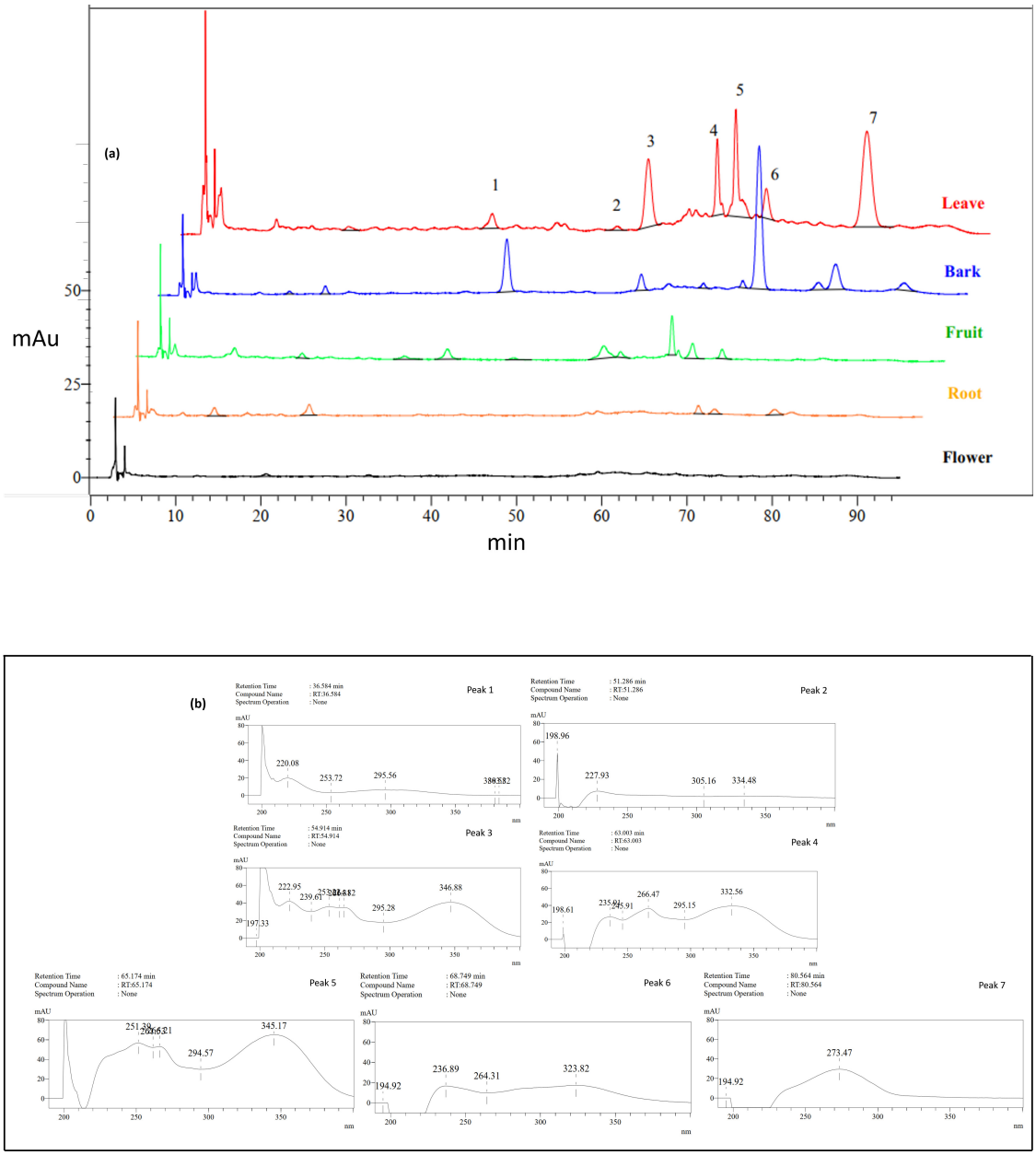

Figure 1. a) The Fingerprint Chromatography of Five Parts of *Avicennia officinalis* L. extract, (b) UV Absorption of 7 Major Compound of AOL Leave Extract (Peak 3: luteolin-7-O-glucoside; Peak 7: Cinnamic Acid)

dants and specifically designed to evaluate the protective effects on the liver, was also employed to measure the extract's antioxidant effectiveness in a laboratory setting. The AOL leaves exhibit the highest level of antioxidant activity in the DPPH method, however the TBRAS assay yielded the best result in the bark.

3.4 Protective Activity of Extracts against CCl_4-Induced Hepatoxicity in HepG2 Cells

The findings indicated that HepG2 cells experienced a significant reduction in the percentage of viable cells, ranging from 76.34% to 83.49%, after being treated with CCl_4 for 24 hours. This decrease was observed when comparing the treated cells to control samples consisting of a culture medium containing 0.5% DMSO. The statistical analysis confirmed the significance of this decrease ($p < 0.01$), thus demonstrating the cytotoxicity of the 2 mM CCl_4 treatment on HepG2 cells. Following a 24-hour treatment of cells with 2 mM CCl_4, the citric acid aqueous leaf extract at a dosage of 100 μg/mL and silymarin at a concentration of 100 μM had a protective effect on HepG2 cells. The protection rates, compared to disease control samples, were found to be 17.08% and 18.72% respectively ($p < 0.05$).

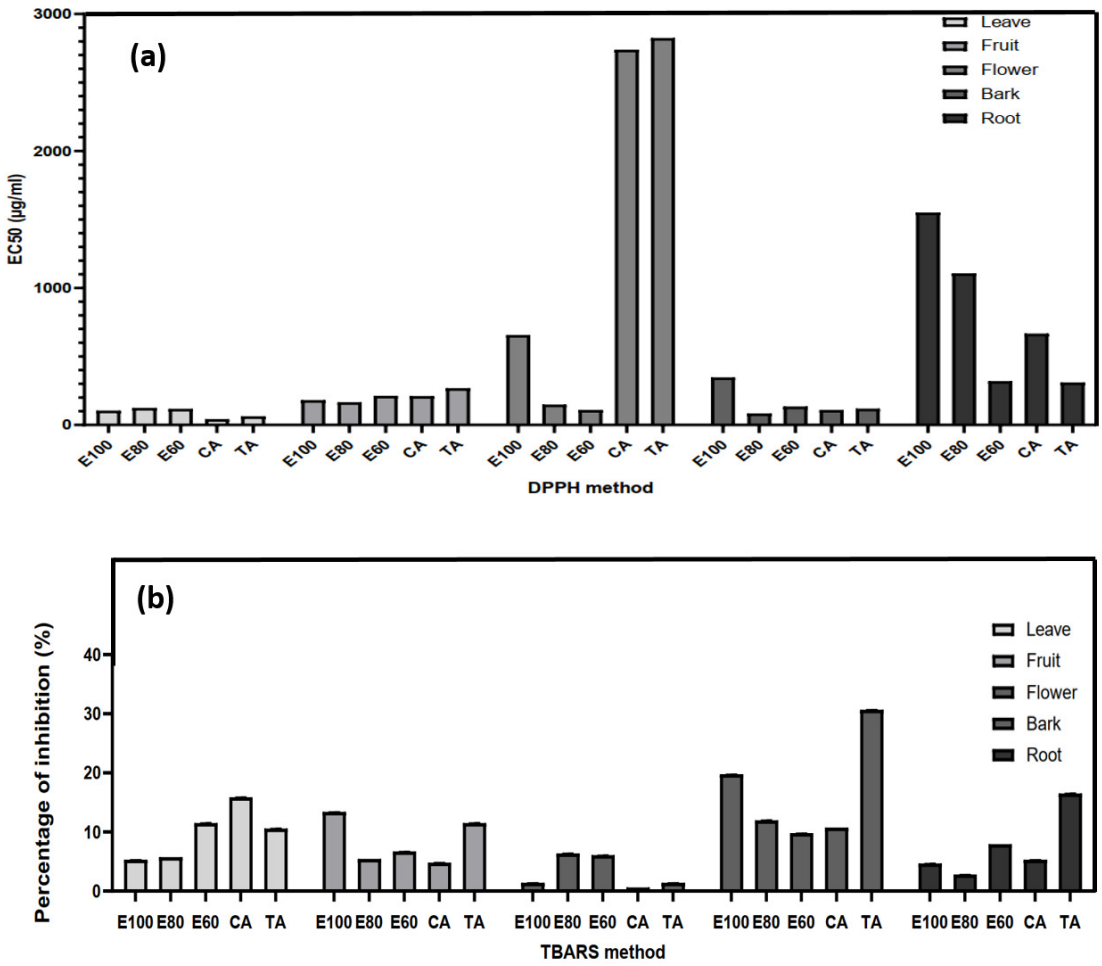

Figure 2. The Result of DPPH and TBARS of *A. officinalis* Extract (E100, Ethanol absolute; E80, Ethanol 80%, E60, Ethanol 60%, CA, Citric Acid Aqueous; TA, Tartaric acid Aqueous)

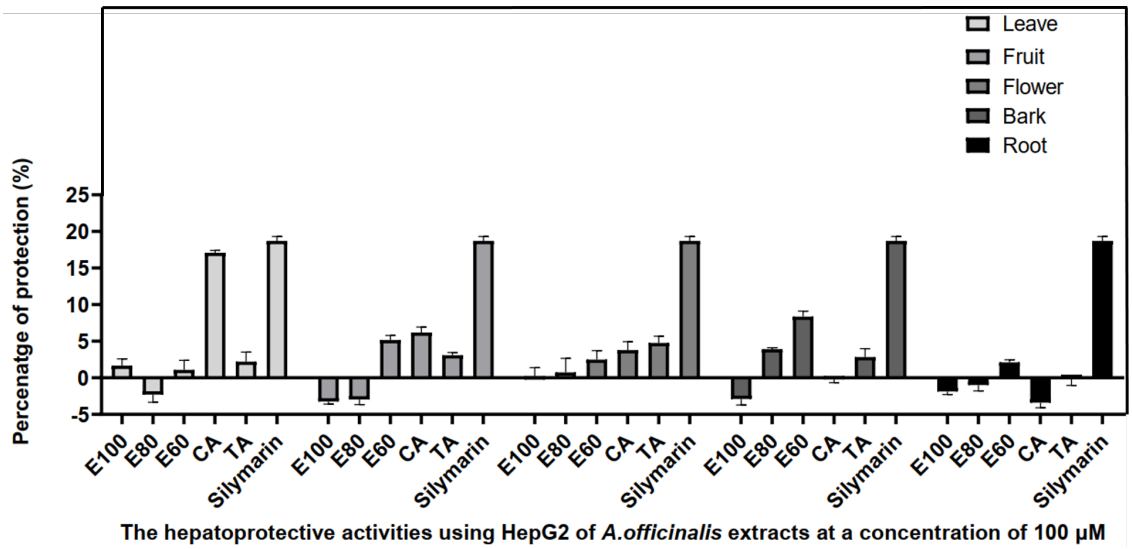

Figure 3. The Hepatoprotective Activities Using HepG2 of *A. officinalis* Extracts at a Concentration of 100 μM (E100, Ethanol absolute; E80, Ethanol 80%, E60, Ethanol 60%, CA, Citric Acid Aqueous; TA, Tartaric Acid Aqueous)

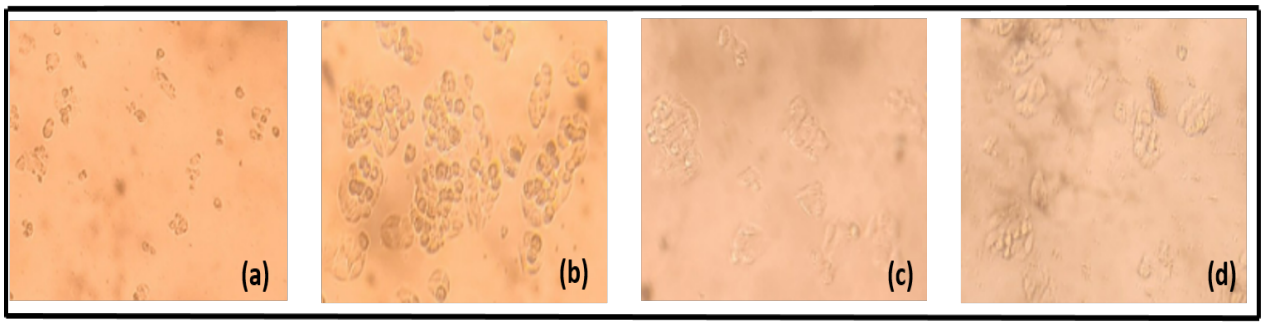

Figure 4. HepG2 Cells after 24 Hours of Treatment with Test Sample and CCl_4: (a) DMSO 0.5% + CCl_4 Sample; (b) DMSO 0.5% Sample; (c) Silymarin 100 μM + CCl_4 Sample; (d) Citric Acid Aqueous Leave Extract 100 μg/mL + CCl_4

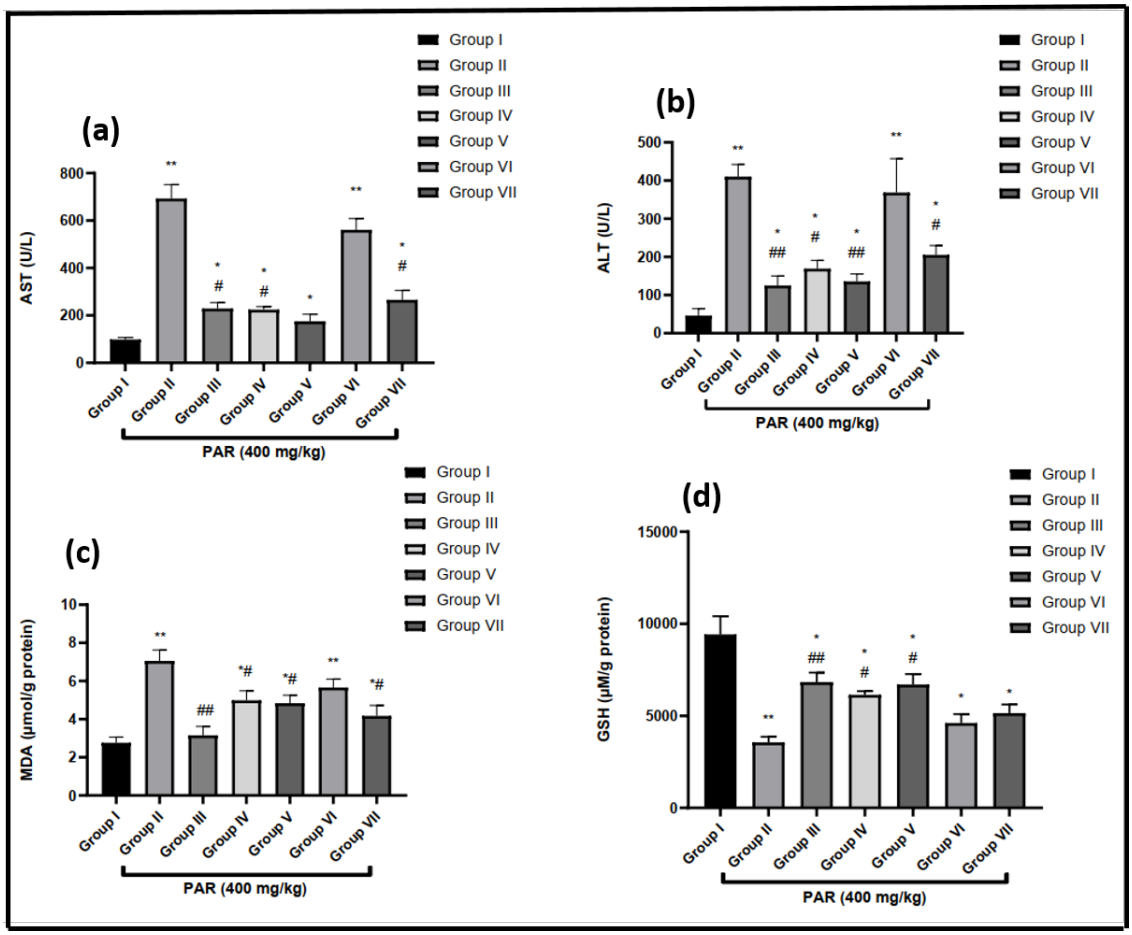

Figure 5. Effects of AOL Extract on AST (a) and ALT (b) in Serum, Liver MDA (c), and Liver GSH (d) Concentration. Values Are Expressed as Mean ± SEM of 5 Mice in Each Group. *Indicates $p < 0.05$ and ** Indicates $p < 0.01$ Compared to Respective Vehicle Control Group I, # Indicates $p < 0.05$ and Indicates $p < 0.01$ Compared to Respective Hepatotoxic Group II. Group I: Vehicle Control. Group II: PCM Control. Group III: Silymarin. Group IV: AOLS 200 Mg/Kg. Group V: AOLS 400 Mg/Kg. Group VI: AOLT 200 Mg/Kg. Group VII: AOLT 400 Mg/Kg.

The aqueous extract of citric acid exhibited a protective effect comparable to the positive control silymarin ($p > 0.05$). However, the protective impact of other samples was inferior to that of silymarin ($p < 0.01$) (Figure 3 and Figure 4). Based on the in-vitro results of antioxidant and hepatoprotective effects, we concluded that the aqueous extract of citric acid leaves exhibited the highest activity. Therefore, this extract underwent standardization as *Avicennia officinalis* L. standardized extract

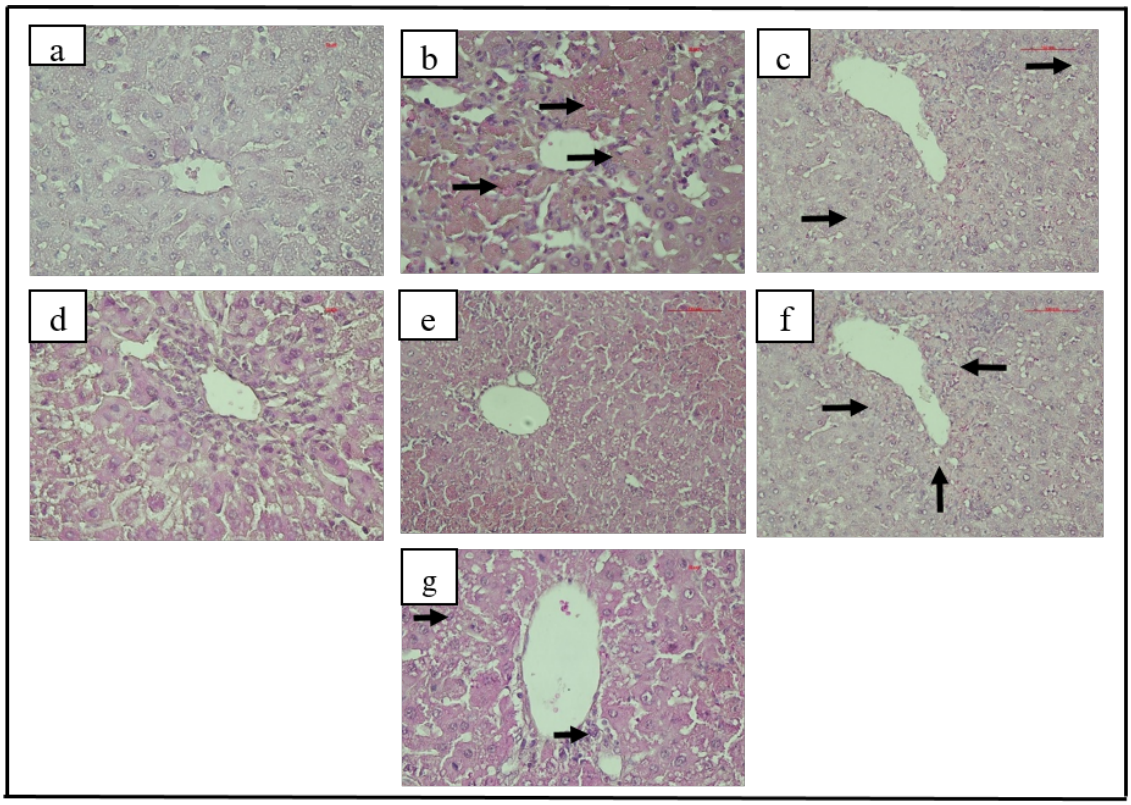

Figure 6. Effects of AOL Extract on Liver Histopathology in Mice. Stain: Haematoxylin–Eosin, Magnification: 100. (A) Vehicle Control Group: Normal Liver Architecture Observed. (B) PCM Control Group: Pronounced Hepatocellular Damage Characterized by Nuclear Alterations, Fatty Degeneration, and Necrosis (Arrows). (C) Silymarin (50 Mg/Kg) Group: Regenerating Hepatocytes (Arrows) Interspersed With Necrotic Areas, Indicative of Partial Hepatic Recovery. (D) AOLS (200 Mg/Kg) Group: Mild Hepatocellular Damage With a Low Incidence of Necrosis. (E) AOLS (400 Mg/Kg) Group: Predominantly Normal Hepatocytes With Rare Necrotic Foci. (F) AOLT (200 Mg/Kg) Group: Necrotic Hepatocytes (Arrows) Surrounding the Central Vein. (G) AOLT (400 Mg/Kg) Group: Scattered Necrotic Hepatocyte (Arrows) Around the Central Vein.

(AOLS) in order to assess its effectiveness in a mouse model of paracetamol-induced liver injury.

3.5 Hepatoprotective Assay of Standardized *Avicennia officinalis* L. Extracts by Against Paracetamol-Induced Liver Toxicity Model in Mice

3.5.1 Biochemical Parameters Examination

The experimental and control groups' serum biochemical parameters were assessed (Figure 5). After 48 hours of intoxication, administering 400 mg/kg body weight of paracetamol resulted in a substantial ($p < 0.01$) rise of the hepatospecific blood markers (AST, ALT, and MDA) and a concurrent decrease in GSH concentration when compared to the control group. Group III treatment with silymarin (50 mg/kg) resulted in a substantial decrease in ALT, AST, and MDA levels ($p < 0.01$) as well as an increase in GSH concentration ($p < 0.01$). When AST enzyme activity and MDA concentration were pre-treated with AOLS (200 and 400 mg/kg), they were considerably reduced ($p < 0.05$) in comparison to the paracetamol control group. The 400 mg/kg dosage had a more noticeable impact on ALT reduction ($p < 0.01$), even though AOLS (200 mg/kg) considerably decreased ALT levels ($p < 0.05$). Furthermore, compared to the paracetamol control, pre-treatment with AOLS (200 and 400 mg/kg) markedly enhanced GSH concentration ($p < 0.05$). When compared to the paracetamol group, AOLW (400 mg/kg, Group VII) dramatically reduced blood levels of AST, ALT, and MDA ($p < 0.05$); however, GSH levels were unaffected. The hepatotoxicity caused by paracetamol was not reduced by the decreased AOLW dosage (200 mg/kg), and blood enzyme levels were still noticeably higher than in the control group.

3.5.2 Histopathological Studies

Figure 6 presents the impact of AOL and silymarin on the liver histopathology of mice treated with PCM. The histopathological examination of liver slices from the vehicle control group revealed the presence of normal liver cells that clustered together to form hepatocyte rafts (Figure 6a). Conversely, the group

that received PCM control showed the most extensive damage compared to all other groups. The liver sections in this group displayed hepatocellular damage, which was characterized by nuclear changes, fatty degeneration, and necrosis (Figure 6b). Pretreatment with silymarin led to partial restoration of the liver, as indicated by the presence of regenerated hepatocytes in areas of necrosis (Figure 6). Liver slices from mice treated with AOLS at a dosage of 200 mg/kg (Figure 6d) and 400 mg/kg (Figure 6e) showed a mostly intact lobular structure with small areas of necrosis. The hepatoprotective effect of the AOLS dose at 400 mg/kg was more evident than at the 200 mg/kg dose. The liver sections obtained from mice treated with AOLT at a dosage of 200 mg/kg (Figure 6e) exhibited signs of hepatocellular injury, characterized by the presence of necrotic hepatocytes distributed throughout the tissue. Liver slices obtained from animals with a dosage of 400 mg/kg AOLT (Figure 6g) exhibited hepatocyte regeneration, while necrotic cells were still present in the vicinity of the lobules.

In experimental animals, an overdose of the commonly used analgesic and antipyretic drug paracetamol (N-acetyl-para-aminophenol) can result in nephrotoxicity and hepatotoxicity. High doses of paracetamol can cause severe instances of centrilobular hepatic necrosis (Hosack et al., 2023) and tubular necrosis in the kidney (Blakely and McDonald, 1995) in both humans and lab animals. Liver damage is brought on by the poisonous metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is created by the cytochrome P-450 enzymes in the liver. AOL extract's hepatoprotective qualities were examined by the assessment of several enzyme activities, which function as markers of hepatocellular damage. It is well known that taking dangerously high doses of paracetamol causes a notable increase in serum MDA and GSH levels, as well as AST and ALT levels (Bhakuni et al., 2016). The results of biochemical markers suggestive of liver function are summarized in Figure 5. When compared to the 200 mg/kg AOLS and 400 mg/kg AOLT treatment groups, the administration of 400 mg/kg of AOLS demonstrated higher hepatoprotective effectiveness in terms of returning liver and antioxidant indicators to baseline values. 400 mg/kg AOLS had hepatoprotective efficacy equivalent to 50 mg/kg silymarin, the reference standard (Figure 6). Nevertheless, in this investigation, the 200 mg/kg AOLT dosage did not demonstrate any hepatoprotective effects. The hepatoprotective effectiveness of the examined AOL extracts may be rated in the following order based on the study's findings: > AOLT 400 mg/kg > AOLT 200 mg/kg < AOLS 400 mg/kg > AOLS 200 mg/kg.

The result shown that A. officinalis L. leave was potential herbal for liver protection with bioactive phenolic acid and flavonoid compounds. Our study aimed to provide a general assessment of the antioxidant and hepatoprotective activities of A. officinalis L. leaves. In the future, our recommendation will need to more study to research the mechanism of liver protective effects of this plant.

4. CONCLUSION

A thorough investigation on the phytochemical, total polyphenol content, and in vitro antioxidant activity of *Avicennia officinalis* L. has been carried out in Vietnam on all parts of the plant including leaves, flowers, fruit, roots, and bark. The findings indicated that AOL's leaves and bark may be a promising raw material source in Vietnam. The phytochemical components of AOL and their in vitro and in vivo hepatoprotections in mice and HepG2 were studied. *Avicennia officinalis* L. leaves that have been enriched with these robust antioxidants could serve as a promising natural source of hepatoprotective compounds. Further pharmacological and early placebo-controlled clinical trials should be presented in order to validate future implementation in medical practice.

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