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Immunomodulatory and Hepatoprotective Effects of Extract from Coffee-Like Product Derived from *Rhizophora mucronata* Fruit of Sangihe Islands in LPS-Treated Mice

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ABSTRACT

The mangrove fruit *Rhizophora mucronata*, native to Indonesia's Sangihe Islands, represents a promising functional food ingredient. This study evaluated the immunomodulatory and hepatoprotective potential of a coffee-like extract derived from this fruit in a lipopolysaccharide (LPS)-induced systemic inflammation mouse model. Thirty male BALB/c mice were randomly allocated into six groups (n=5/group): normal control, LPS control, LPS+vitamin E (500 mg/kg), and LPS+extract (500, 1000, or 1500 mg/kg). Extracts were administered orally for 14 consecutive days prior to LPS challenge (5 mg/kg). Phytochemical screening identified steroids (10.84 mg/kg), terpenoids (2.50 mg/kg), and saponins (0.52 mg/kg), alongside moderate antioxidant activity (IC_{50}=113.66 µg/mL). Proximate analysis revealed moisture (5.31%), ash (5.25%), protein (13.07%), fat (11.17%), carbohydrate (65.21%), energy value (413.61 kcal), and caffeine content (0.96%). LPS administration significantly reduced serum immunoglobulin G (IgG) concentrations from 2700 ± 29.42 µg/mL (normal) to 1890 ± 12.83 µg/mL. One-way ANOVA ($F_{(5,24)}$ = 1368.34, $p < 0.001$) with Tukey's HSD post-hoc test demonstrated that the 500 mg/kg dose partially restored IgG levels (2600 ± 26.90 µg/mL, $p < 0.05$ vs. normal), while the 1000 and 1500 mg/kg doses achieved full restoration (2739 ± 18.53 and 2737 ± 2.55 µg/mL, respectively), statistically comparable to both normal and vitamin E groups ($p > 0.05$). At the higher doses, the extract also attenuated hepatic injury, evidenced by increased healthy hepatocyte counts and reduced nuclear abnormalities (pyknosis, karyorrhexis, karyolysis). These preliminary findings indicate that *R. mucronata* coffee-like extract exhibits notable immunomodulatory and hepatoprotective properties, warranting further translational investigation.

INTRODUCTION

Mangrove ecosystems, which thrive along tropical and subtropical coastlines, are increasingly recognized as rich sources of bioactive secondary metabolites with therapeutic and nutritional potential[1]. Among the mangrove species dominating the shores of Indonesia's Sangihe Islands is *Rhizophora mucronata*[2], a species widely distributed in the Indo-Pacific region[3] A growing body of literature documents that mangroves synthesize various phytochemicals—tannins, flavonoids,

saponins, steroids, and terpenoids[4]—that exhibit antioxidant[5], antimicrobial[6,7], and anti-inflammatory activities[8]. Sungkar

Beyond their pharmacological potential, *R. mucronata* fruits and leaves have demonstrated significant nutritional value, including dietary fiber, protein, lipids, and essential micronutrients such as ascorbic acid, thiamine, riboflavin, tocopherol, sodium, iron, and calcium, which could help address micronutrient malnutrition[9,10]. This chemical and nutritional wealth has sparked interest in transforming mangrove fruits into functional food products, such as an innovative coffee-like beverage with antioxidant properties[11,12].

Earlier work on *R. mucronata* fruit from the Sangihe Islands demonstrated that processing steps including soaking, drying, and roasting can preserve bioactive compounds such as *cis*-9-hexadecenal and 9-octadecenal, and that the resulting coffee-like product possesses moderate antioxidant capacity (IC_{50} = 131.28 ± 0.44 µg/mL) and contains steroids, terpenoids, and saponins as major bioactive constituents[13]. Subsequent research revealed that oral administration of this product at doses of 500, 1000, and 1500 mg/kg body weight for 14 days significantly increased the relative number of $CD4^+CD62L^+$ and $CD8^+CD62L^+$ T lymphocyte cells and protected against renal necrosis Skrzypczak-Wierciocin LPS-induced mice[14]. These findings suggested that the antioxidant and anti-inflammatory properties of the coffee-like product, mediated by its bioactive constituents—particularly steroids, terpenoids, and saponins—could modulate cellular immunity and provide organ protection[15].

During infection, immune cells—neutrophils and macrophages—generate substantial amounts of reactive oxygen species (ROS) to eliminate pathogens. However, excessive ROS can damage host tissues and impair immune function[16]. Hence, adequate antioxidant defenses are crucial to keep ROS in check, enabling immune cells to work efficiently without succumbing to oxidative stress[17]. In the present study, we used lipopolysaccharide (LPS) from Gram-negative bacterial cell walls to induce oxidative stress and systemic inflammation in mice, a well-established model[18]. LPS triggers a cascade of events: it suppresses both cellular and humoral immunity[19], boosts free radical production[20], and inflicts damage on organs such as the liver and kidneys[21,22]. These effects highlight the need for safe compounds that can modulate immunity and protect vital organs. Plant-derived bioactive compounds, including those from mangroves, have been shown to influence T-lymphocyte activity and shield cells from necrosis caused by oxidative stress[23,24].

Although earlier investigations have pointed to the antioxidant potential of processed *R. mucronata* fruit, important gaps remain. Cahyono et al. demonstrated that the coffee-like product from this mangrove fruit increases the numbers of certain T-lymphocyte and improves kidney histology in LPS-treated mice[14]. However, its impact on humoral immunity—specifically on serum immunoglobulin G (IgG) levels—has not been examined. IgG is a cornerstone of adaptive immunity, responsible for neutralizing pathogens and establishing immunological memory[25,26]. Moreover, data on the product's

protective effects on the liver, the primary metabolic organ and a major target of LPS toxicity[27], are still scarce. In addition, this study will evaluate food safety factors by examining the effects of administering a coffee-like product extract on the livers of mice. This study complements the research by Cahyono et al., which examined the effects of coffee-like products on the kidneys of mice. This is crucial for demonstrating its potential as a functional food.

The selection of extract doses (500, 1000, and 1500 mg/kg body weight) was based on the effective dose range identified in our previous study, which demonstrated significant immunomodulatory and renoprotective effects at these doses without observed mortality or overt toxicity[14]. Furthermore, *in silico* toxicity predictions for the major compounds identified in the coffee-like product indicated an oral LD_{50} of 5000 mg/kg (toxicity class V), suggesting a favorable preliminary safety profile[13], although formal toxicological evaluation according to OECD guidelines remains necessary. A dose of 500–1,500 mg/kg was also used as a high limit, to evaluate the hepatoprotective and nephroprotective effects of coffee-like product extract[28]. The liver and kidneys are the primary focus because they are key organs in metabolism and toxin elimination, making them the most vulnerable to damage from exposure to natural substances[29].

Therefore, this study was designed to test the hypothesis that oral administration of an extract from a coffee-like extract prepared from *R. mucronata* fruit can restore serum IgG concentrations and prevent liver damage in mice challenged with LPS. The specific aims were (i) to characterize the bioactive components of the coffee-like extract using qualitative and quantitative phytochemical methods, (ii) to evaluate its effect on serum IgG levels, and (iii) to assess its influence on liver histopathology in LPS-induced BALB/c mice. By addressing these questions, this study not only extends our previous findings on cellular immunity and kidney protection to humoral immunity and hepatoprotection but also provides a scientific basis for promoting this coffee-like beverage as a safe, economically viable, and sustainable functional food product derived from Indonesia's unique local biodiversity, particularly from the Sangihe Islands.

MATERIALS AND METHODS

Preparation of Coffee-like Powder and Extraction

Fruits of *Rhizophora mucronata* were collected from the coastal area of Miulu Village, Sangihe Islands, North Sulawesi, Indonesia. Only the pericarp (the brown fleshy portion of the fruit) was used, while the seeds and the hypocotyl base were discarded (Figure 1). The procedure for making coffee-like powder followed previously described methods[12,13] with modifications. Briefly, fruits were peeled, seeds were discarded, and the pulp was soaked in clean water for 36 hours, with water replacement every 6 hours. This was followed by a 24-hour soak in a 6% lime solution ($Ca(OH)_2$) at room temperature. After soaking, the pulp was cut into small pieces (2–3 cm) and dried in an oven

(Memmert UM500) at 60–65 °C for 36 hours. The dried material was then roasted in a stainless-steel pan at 116–118 °C for 30–40 minutes, ground, and sieved to a particle size of 80–100 mesh.

For phytochemical profiling and antioxidant assays, the coffee-like powder was macerated in 96% ethanol (MERCK) at a ratio of 1:10 (w/v) for 3 × 24 hours with occasional stirring. The filtrate was concentrated using a rotary evaporator (Buchi R-300) at 40 °C until a thick extract remained. This concentrate was freeze-dried (Christ Alpha 1-4 LDplus) to yield a dry ethanolic extract, which was stored at –4 °C until further use.

For *in vivo* administration, the coffee-like powder was brewed in hot distilled water at 90 °C at a concentration of 50 mg/mL (adjusted to achieve the desired doses of 500, 1000, and 1500 mg/kg body weight) for 10 minutes, filtered, and the aqueous filtrate (coffee brew) was freshly prepared daily and administered orally to mice within 30 minutes of preparation. This brewing method was chosen to mimic the actual human consumption pattern of the coffee-like product as a functional beverage. The use of aqueous extract for in vivo studies is justified by safety considerations (ethanol is toxic for oral administration) and relevance to the intended food application[30].

Figure 1. *Rhizophora mucronata* Mangrove Fruit Originating from the Sangihe Islands (left) and The Coffee-like Products made from it (right). Only the pericarp (the brown fleshy portion of the fruit) was used.

Qualitative Screening of Bioactive Components in Coffee-Like Extracts from Mangrove Fruit

The presence of seven major bioactive compound classes—alkaloids, flavonoids, steroids, terpenoids, phenols, saponins, and tannins—was assessed by thin-layer chromatography (TLC) following modified standard protocols Harborne; Wagner & Bladt[31,32]. Silica gel 60 F_{254} plates (MERCK) served as the stationary phase. For each test, 100 mg of extract was mixed with 1 mL of 96% ethanol, vortexed for 2 minutes, sonicated (Branson 2800) for 60 minutes, and then left to macerate for 24 hours at room temperature. After centrifugation at 3000 rpm for 5 minutes, the supernatant was spotted onto TLC plates. Development conditions and detection reagents were specific to each compound class:

- Alkaloids: Mobile phase chloroform:methanol:ammonia (90:10:1); detection with Dragendorff's reagent (orange or red spots)[33].

- Flavonoids: Mobile phase n-butanol:acetic acid:water (4:1:5); detection with 1% $AlCl_3$ in ethanol (yellow-green fluorescence under 365 nm UV)[34].
- Steroids and Terpenoids: Mobile phase toluene:ethyl acetate (80:20); detection with Lieberman-Burchard reagent (blue-green) for steroids; and detection with vanillin-sulfuric acid reagent (red-purple) for terpenoids[35,36].
- Phenols and Tannins: Mobile phase n-butanol:acetic acid:water (4:1:5); detection with 1% $FeCl_3$ (green, blue, or black) for phenols; and for tannins, after spraying with $FeCl_3$, detection was performed as described[37].
- Saponins: Mobile phase chloroform:glacial acetic acid:methanol:water (60:32:12:8); detection with anisaldehyde-sulfuric acid reagent (purple or reddish-brown after heating)[38].

Quantitative Evaluation of Bioactive Coffee-Like Extracts from Mangrove Fruit

Based on qualitative phytochemical screening, three bioactive components were detected in the ethanolic extract of the coffee-like powder. Furthermore, these bioactive components were quantified using the following procedures:

- Total Steroids (β-Sitosterol Equivalent): The concentration was determined by TLC-densitometry using a CAMAG TLC scanner equipped with winCATS software, following the method described by Thatipelli et al. (2023)[39]. Briefly, β-sitosterol standard solution (1000 µg/mL, 60% purity) at concentrations of 0.24, 0.48, 0.72, 0.96, 1.20, and 1.50 µg was spotted on a TLC plate along with the extract samples. After elution with toluene:ethyl acetate (80:20) and drying, the plate was sprayed with Lieberman-Burchard. The plate was then scanned at λ 340 nm. A linear calibration curve ($y = 6243.2x + 1082.8$; $r^2 = 0.9969$) was created from the peak areas of the standards. The total steroid content in the sample was calculated from the total peak area with steroid Rf characteristics (0.52-0.56) and expressed in mg/kg β-sitosterol equivalent.
- Total Saponins (Quillaja Equivalent): Total saponin content was determined spectrophotometrically using a colorimetric method modified from Hossain (2019)[37]. The sample (50 mg) was hydrolyzed with 2 mL of 25% H_2SO_4 in an autoclave at 110°C for 120 minutes. The neutralized extract was then reacted with 50 µL of anisaldehyde and 2 mL of 50% H_2SO_4, incubated at 60°C for 10 minutes. The solution was diluted to 10 mL, and the absorbance was read at λ 435 nm (Shimadzu UV-1800). A calibration curve was prepared using saponin standards from Quillaja bark (Sigma-Aldrich) with a concentration range of 3.125–400 ppm ($y = 0.0009445x - 0.003321$; $r^2 = 0.99974$). Total saponin content was expressed in mg/kg Quillaja equivalent.
- Total Terpenoids (Terpineol Equivalent): Total terpenoid content was determined using a modified Thin Layer Chromatography-Densitometry (TLC-Densitometry) method from Barwant et al. (2025)[40]. The extract sample (100 mg) was extracted with 1 mL of 96% ethanol through sonication for 60 minutes. The clear supernatant from centrifugation (5000 rpm, 10 minutes) was

used for analysis. A total of 10 µL of the sample, together with a terpineol standard solution (0.1–1.6 µg), was spotted on a Silicagel 60 F254 TLC plate. Elution was performed with a mobile phase of toluene:ethyl acetate (93:7) in a saturated chamber. After drying, the plate was sprayed with Vanillin-Sulfuric Acid reagent and heated at 110°C for 2 minutes until characteristic purple-red spots appeared. The plate was then scanned at 550 nm using a CAMAG TLC Scanner. The calibration curve was constructed from the peak area of the terpineol standard. The total terpenoid content was calculated from the total peak area, with Rf patterns (0.24, 0.41, and 0.65) corresponding to the terpenoid profile, and expressed in mg/kg terpineol equivalent.

Evaluation of the Antioxidant Capacity of Coffee-Like Extracts from Mangrove Fruit

The radical-scavenging capacity of the ethanolic extract was evaluated using the DPPH method[7,41]. A stock solution (200 µg/mL) was prepared and serially diluted to final concentrations of 20, 40, 60, 80, and 100 µg/mL in 96% ethanol. Each dilution (2 mL) was mixed with 2 mL of 50 µM DPPH solution (prepared in ethanol) and incubated in the dark at room temperature (25–27 °C) for 30 minutes. The final concentrations after mixing with DPPH solution were 10, 20, 30, 40, and 50 µg/mL. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Blank absorbance (DPPH without sample) was used to calculate percent inhibition:

$$\text{Antioxidant capacity (\%)} = \frac{(\text{Blank Absorbance} - \text{Sample Absorbance})}{\text{Blank Absorbance}} \times 100\%$$

The IC_{50} (concentration required to inhibit 50% of DPPH radicals) was derived from the linear regression of concentration versus percent inhibition. Activity was classified according to Blois[41]: very strong ($IC_{50} \leq 50$ µg/mL), strong ($50 < IC_{50} \leq 100$), moderate ($100 < IC_{50} \leq 150$), weak ($150 < IC_{50} \leq 200$), and very weak ($IC_{50} > 200$). Assays were performed in triplicate; data are expressed as mean ± SD.

Animals and Experimental Design

The sample size of five animals per group (n = 5) was determined based on the resource equation method[42] and previous studies using the same LPS-induced mouse model, which demonstrated significant immunological and histopathological differences with n = 5 per group[14]. With a total of 30 animals (6 groups × 5 animals), this sample size provides adequate statistical power (≥ 80%) to detect a large effect size (Cohen's f ≥ 0.40) at α = 0.05, based on one-way ANOVA with six groups[43].

Thirty male BALB/c mice (age 6–7 weeks, weight 23–26 g) were obtained from the Animal Research Laboratory, Faculty of Medicine, Brawijaya University. They were housed under standard conditions (12 hours light/dark cycle, 22 ± 2 °C, 55 ± 5% humidity) with free access to commercial

feed and water. After a 7-day acclimatization, the mice were randomly assigned to six groups (n = 5 per group). The six groups were:

KN (normal): received distilled water only (no LPS, no treatment)

KL (negative control): LPS induction (5 mg/kg body weight) without any treatment

KP (positive control): LPS + vitamin E (500 mg/kg BW)

D500: LPS + coffee-like extract (500 mg/kg BW)

D1000: LPS + coffee-like extract (1000 mg/kg BW)

D1500: LPS + coffee-like extract (1500 mg/kg BW)

The normal control group (KN) received distilled water via oral gavage for 14 days (identical volume and handling to the treatment groups) to control for handling stress, followed by an intraperitoneal injection of sterile saline on day 15. This ensures that any observed effects are due to the treatment rather than the stress of gavage administration[44].

Extracts (prepared as aqueous brews) and vitamin E (prepared in corn oil) were administered orally (gavage) once daily for 14 consecutive days. The selection of doses (500, 1000, and 1500 mg/kg BW) was based on the effective dose range identified in our previous study[14], which demonstrated significant immunomodulatory and renoprotective effects at these doses without observed mortality or overt toxicity. On day 15, 30 minutes after the final administration, all groups except KN received an intraperitoneal injection of LPS (*Escherichia coli* O127:B8, Sigma-Aldrich) at 5 mg/kg. The KN group received an equivalent volume of sterile saline.

Mice were euthanized 6 hours after LPS challenge, as this time point corresponds to the peak inflammatory response and organ damage in the LPS-induced systemic inflammation model[19]. Euthanasia was performed under ketamine/xylazine anesthesia at a dose of 80 mg/kg ketamine and 10 mg/kg xylazine administered intraperitoneally (3:1 ratio, v/v)[45]. To minimize bias, the investigator responsible for histopathological evaluation was blinded to the treatment groups[46]. The animal care technician who administered treatments was not blinded due to logistical constraints, but all outcome assessments (IgG ELISA and histopathology) were performed by investigators unaware of group allocation. The study protocol was approved by the Brawijaya University Health Research Ethics Committee (No. 098-KEP-UB-2022) and followed the guidelines for laboratory animal care[45].

Serum IgG Measurement

Six hours after LPS challenge, mice were euthanized under ketamine/xylazine anesthesia (80 mg/kg ketamine and 10 mg/kg xylazine, i.p.; 3:1 ratio, v/v). Blood was collected via cardiac puncture into microtubes without anticoagulant. Serum was separated by centrifugation (3000 rpm, 15 minutes, 4 °C) and stored at –80 °C until analysis. Total IgG levels were quantified using a commercial ELISA kit (Mouse IgG Total ELISA Kit, ELK Biotechnology, cat. ELK1253) following the manufacturer's

protocol. Absorbance was read at 450 nm on a microplate reader (BioTek Epoch 2). All samples were analyzed in triplicate, and the mean values were used for statistical analysis.

Preparation and Evaluation of Liver Histopathology

Immediately after euthanasia, livers were excised, washed in cold phosphate-buffered saline, and fixed in 10% neutral formalin for 24 hours. Tissues were routinely processed for paraffin embedding (Leica ASP300S), sectioned at 5 µm thickness (Leica RM2245), and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope (Olympus BX51) at 400× magnification by a pathologist blinded to group assignment. For each sample, 500 hepatocytes from three different fields were categorized as: healthy (round nucleus, homogeneous cytoplasm), pyknotic (shrunken, dense nucleus), karyorrhectic (fragmented nucleus), or karyolytic (loss of nuclear staining)[47]. The percentage of cells in each category was calculated.

Statistical Analysis

All data are expressed as mean ± SD. Normality was checked with the Shapiro–Wilk test and homogeneity of variances with Levene's test. Normally distributed, homogeneous data were analyzed by one-way ANOVA followed by Tukey's post-hoc test. Differences were considered significant at $p < 0.05$. Statistical analyses were performed using SPSS version 20 (IBM Corp., USA).

RESULTS

Proximate Composition and Physicochemical Properties of the Coffee-Like Product

The proximate composition of the *R. mucronata* coffee-like powder is presented in Table 1.

Table 1. Proximate Composition and Physicochemical Properties of *R. mucronata* Coffee-Like Powder

Parameter	Result	Ground Coffee Standard (SNI No. 01-3542-2004)
Moisture (%)	5.31 ± 0.18	Max. 7%
Ash (%)	5.25 ± 0.21	Max. 5%
Protein (%)	13.07 ± 0.13	–
Fat (%)	11.17 ± 0.21	–
Carbohydrate (%)	65.21 ± 0.34	–
Energy (kcal/100g)	413.61 ± 0.29	–
Caffeine (%)	0.96 ± 0.05	0.45 – 2%
pH (brew, 90°C)	5.37 ± 0.05	–

Phytochemical Profile and Antioxidant Activity

Qualitative thin-layer chromatography (TLC) screening of the coffee-like extract revealed the presence of three bioactive compound classes: steroids, terpenoids, and saponins. Alkaloids, flavonoids, phenols, and tannins were not detected (Table 2). Quantitative analysis demonstrated that steroids were the most abundant component, followed by terpenoids, and saponins (Table 3). Quantitative analysis was performed using the equivalence method: steroids were equivalent to β-sitosterol, terpenoids were equivalent to terpineol, and saponins were equivalent to Quillaja.

Table 2. Phytochemical Screening of Bioactive Components in Coffee-Like Extracts

Bioactive Components	Results	Reagents
Alkaloids	Negative	Dragendorff
Flavonoids	Negative	$AlCl_3$
Steroids	Positive	Lieberman Bucard
Terpenoids	Positive	Vanillin Sulfuric Acid
Phenols	Negative	$FeCl_3$
Saponins	Positive	Anisaldehyde Sulfuric Acid
Tannins	Negative	$FeCl_3$

Table 3. Quantification of Bioactive Components in Coffee-Like Extracts

Bioactive Components	Total (mg/kg)
Total Steroids	10.84 ± 0.25
Total Terpenoids	2.5 ± 0.50
Total Saponins	0.52 ± 0.00

The antioxidant capacity of the ethanolic extract, assessed using the DPPH radical-scavenging assay, yielded an IC_{50} value of 113.66 ± 0.49 µg/mL (Figure 2). The DPPH radical-scavenging activity exhibited a concentration-dependent relationship, with the percentage of inhibition increasing from 7.81 ± 0.22% at 20 µg/mL to 42.97 ± 0.22% at 100 µg/mL in coffee-like extracts. For Vitamin E, the percentage of inhibition increased from 48.44 ± 0.64% at 20 µg/mL to 83.97 ± 0.39% at 100 µg/mL. The linear regression equation for the standard curve was y = 0,4452x - 0,5986 (R^2 = 0,9955) for coffe-like extracts; and y = 0,4527x + 38,574 (R^2 = 0,9973) for Vitamin E. According to the Blois classification criteria[41], this IC_{50} value categorizes the extract's antioxidant activity as moderate (100 < IC_{50} ≤ 150 µg/mL).

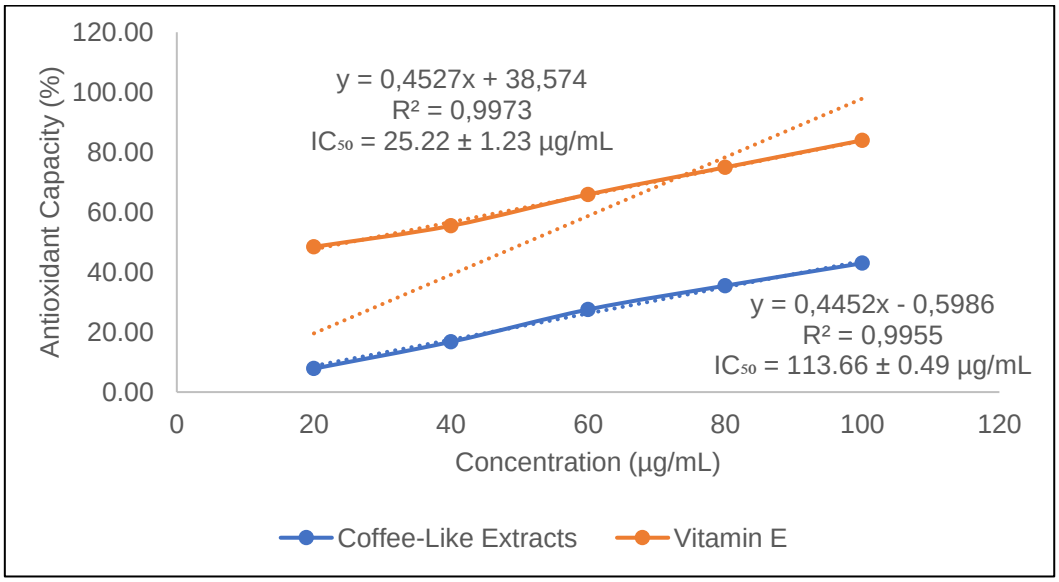


Figure 2. Antioxidant Capacity (DPPH) of Coffee-Like Extracts from *R. mucronata* Fruit

Serum Immunoglobulin G Levels

Serum IgG levels across all experimental groups are presented in Figure 3. One-way ANOVA revealed a significant effect of treatment on IgG concentrations ($F_{(5,24)}$ = 1368.34, p< 0.001, η^2_p = 0.9965, indicating a very large effect size). Lipopolysaccharide (LPS) challenge at 5 mg/kg body weight significantly reduced serum IgG levels in the negative control group (KL: 1890 ± 12.83 µg/mL) compared to the normal group (KN: 2700 ± 29.42 µg/mL), with a mean difference of –810 µg/mL (95% CI: –830.35 to –789.65, p< 0.001, Cohen's d = 36.52).

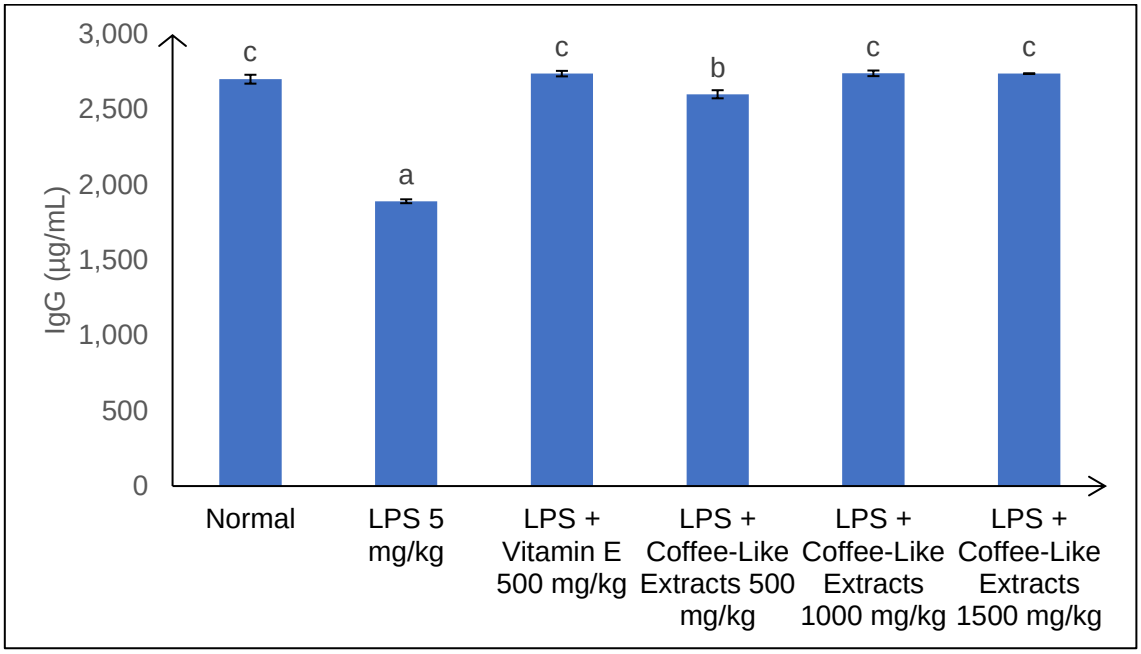


Figure 3. IgG Level in LPS-Induced BALB/c mice, with Administration of Coffee-Like Extracts from *Rhizophora mucronata* Fruit. The Same Letter Notation Indicates No Significant Difference (p > 0.05) Based on The Tukey Test. Groups with Different Letters Differ Significantly (p < 0.05).

Administration of the coffee-like extract at doses of 1000 and 1500 mg/kg restored serum IgG concentrations to levels comparable to those of the normal and vitamin E-treated groups. The mean IgG levels were:

- D500 (LPS + 500 mg/kg extract): 2600 ± 26.90 µg/mL (mean difference vs. KL = +710 µg/mL, 95% CI: 689.65 to 730.35, p< 0.001, Cohen's d = 32.00)
- D1000 (LPS + 1000 mg/kg extract): 2739 ± 18.53 µg/mL (mean difference vs. KL = +849 µg/mL, 95% CI: 828.65 to 869.35, p< 0.001, Cohen's d = 38.27)
- D1500 (LPS + 1500 mg/kg extract): 2737 ± 2.55 µg/mL (mean difference vs. KL = +847 µg/mL, 95% CI: 826.65 to 867.35, p< 0.001, Cohen's d = 38.16)

Post-hoc pairwise comparisons using Tukey's HSD test revealed the following patterns:

1. The LPS group (KL) showed significantly lower IgG levels compared to all other groups ($p < 0.001$ for all comparisons), confirming that LPS induction successfully suppressed humoral immunity.
2. The vitamin E group (KP) restored IgG levels to 2737 ± 18.27 µg/mL, which was statistically indistinguishable from the normal group (mean difference = +37 µg/mL, 95% CI: 16.65 to 57.35, $p = 0.071$, Cohen's $d = 1.66$).
3. The D1000 and D1500 groups achieved IgG levels that were not significantly different from the normal group ($p > 0.05$ for both comparisons):
   - D1000 vs. KN: mean difference = +39 µg/mL, 95% CI: 18.65 to 59.35, $p = 0.053$, Cohen's $d = 1.76$
   - D1500 vs. KN: mean difference = +37 µg/mL, 95% CI: 16.65 to 57.35, $p = 0.071$, Cohen's $d = 1.67$
4. The D500 group showed intermediate recovery (2600 ± 26.90 µg/mL), which was significantly higher than the LPS group ($p < 0.001$) but significantly lower than the normal, vitamin E, D1000, and D1500 groups ($p < 0.01$ for all comparisons).
5. No statistically significant differences were observed among the D1000, D1500, KP (vitamin E), and KN groups ($p > 0.05$ for all pairwise comparisons), indicating that the 1000 and 1500 mg/kg doses achieved complete restoration of serum IgG to normal levels.

Interestingly, the D500 group (2600 ± 26.90 µg/mL) showed a mean IgG level that was 139 µg/mL lower than the normal group (95% CI: 118.65 to 159.35, $p < 0.001$, Cohen's $d = 6.27$), suggesting that while the 500 mg/kg dose provided significant immunomodulation, it was insufficient to achieve full recovery. The absence of a significant difference between D1000 and D1500 ($p > 0.05$) indicates that the immunomodulatory effect reaches a plateau at 1000 mg/kg, with no additional benefit from further dose escalation.

The Tukey HSD post-hoc grouping ($\alpha = 0.05$) assigned the groups into three distinct categories:

- Group a: LPS (1890 µg/mL) — lowest IgG level
- Group b: D500 (2600 µg/mL) — intermediate recovery
- Group c: Normal (2700 µg/mL), Vitamin E (2737 µg/mL), D1000 (2739 µg/mL), D1500 (2737 µg/mL) — full recovery, statistically equivalent to normal

These findings demonstrate that the coffee-like extract from *R. mucronata* fruit exerts a potent immunomodulatory effect on humoral immunity in a dose-dependent manner up to 1000 mg/kg, with complete restoration of serum IgG achieved at 1000 and 1500 mg/kg, comparable to the standard antioxidant vitamin E.

Liver Histopathology

Histopathological examination of the liver revealed that LPS induction caused significant liver damage, characterized by a marked decrease in the proportion of healthy hepatocytes and substantial increases in nuclear abnormalities (pyknosis, karyorrhexis, and karyolysis) (Table 4). Representative photomicrographs of liver sections from each group are shown in Figure 4.

The one-way ANOVA test revealed significant differences among all treatment groups for all four histopathological parameters (healthy cells: $H = 22.14$, $p < 0.001$; pyknosis: $H = 18.76$, $p < 0.001$; karyorrhexis: $H = 20.53$, $p < 0.001$; karyolysis: $H = 21.87$, $p < 0.001$). LPS challenge significantly reduced the proportion of healthy hepatocytes from 85.2 ± 4.1% (normal group) to 65.8 ± 6.3% (LPS group), representing a mean reduction of –19.4% (95% CI: –27.8 to –11.0, $p < 0.001$, Cohen's $d = 2.87$). Concurrently, LPS increased pyknosis (from 7.1 ± 2.3% to 10.5 ± 4.2%; mean difference = +3.4%, 95% CI: +0.8 to +6.0, $p = 0.012$, Cohen's $d = 1.45$), karyorrhexis (from 5.5 ± 2.0% to 12.8 ± 5.1%; mean difference = +7.3%, 95% CI: +3.9 to +10.7, $p < 0.001$, Cohen's $d = 2.31$), and karyolysis (from 2.2 ± 1.1% to 10.9 ± 4.7%; mean difference = +8.7%, 95% CI: +5.4 to +12.0, $p < 0.001$, Cohen's $d = 2.67$).

Table 4. Histopathological Changes in The Liver of Mice Due to LPS Induction, with Treatment using Coffee-Like Extracts from *R. mucronata* Fruit.

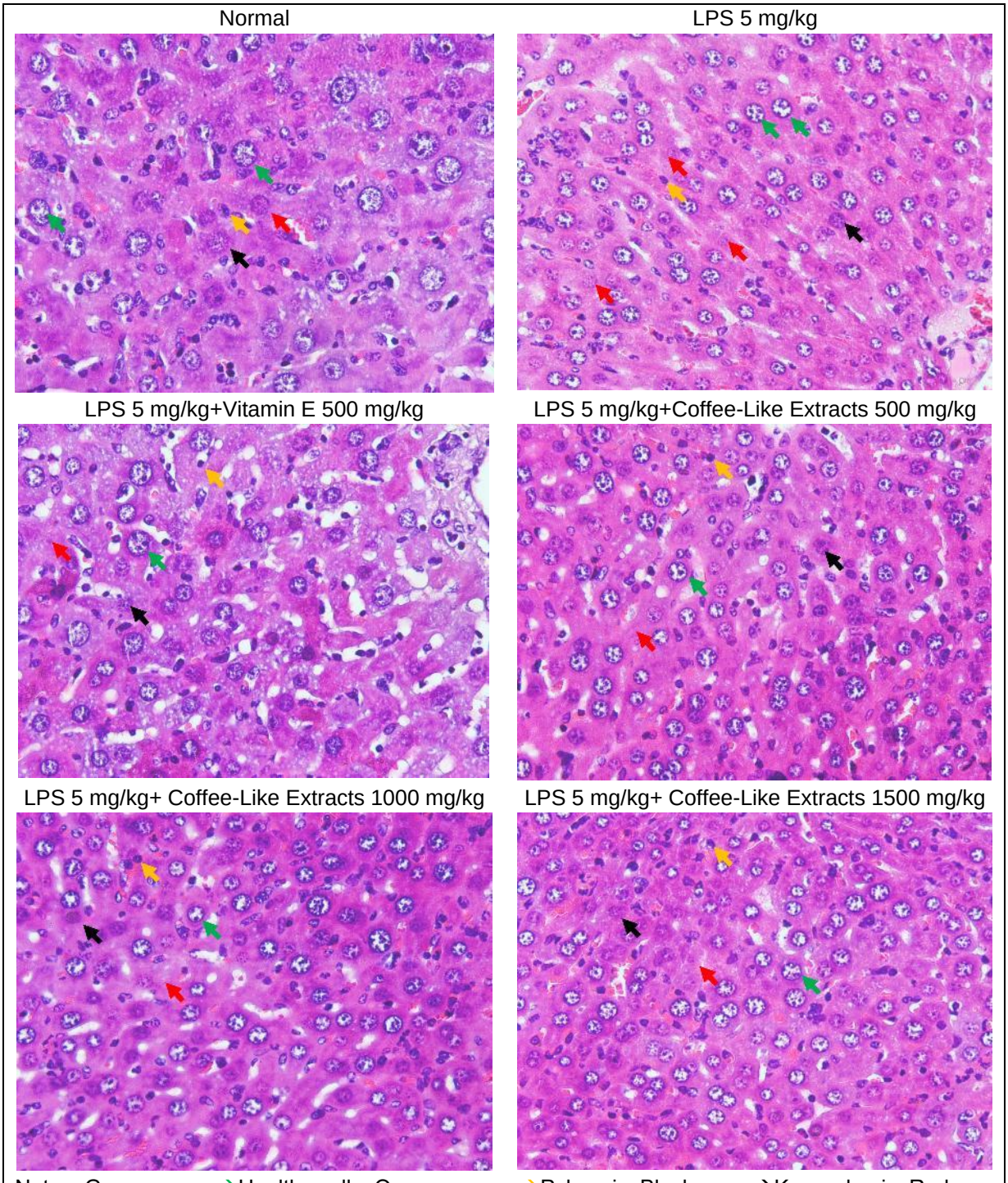
Treatments	BALB/c mice liver cells (%)			
	Healthy	Pyknosis	Karyorrhexis	Karyolysis
Normal	85.2 ± 4.1^{a}	7.1 ± 2.3^{d}	5.5 ± 2.0^{d}	2.2 ± 1.1^{d}
LPS 5 mg/kg	65.8 ± 6.3^{c}	10.5 ± 4.2^{a}	12.8 ± 5.1^{a}	10.9 ± 4.7^{a}
LPS + Vitamin E 500 mg/kg	78.4 ± 5.2^{b}	8.2 ± 2.8^{c}	7.9 ± 3.1^{c}	5.5 ± 2.4^{c}
LPS + Coffee-Like Extracts 500 mg/kg	72.1 ± 5.8^{bc}	9.3 ± 3.5^{ab}	9.8 ± 3.7^{b}	8.8 ± 3.5^{b}
LPS + Coffee-Like Extracts 1000 mg/kg	76.5 ± 4.9^{b}	8.5 ± 3.0^{bc}	8.3 ± 3.3^{bc}	6.7 ± 2.9^{bc}
LPS + Coffee-Like Extracts 1500 mg/kg	80.2 ± 4.7^{ab}	7.8 ± 2.7^{cd}	7.0 ± 2.8^{c}	5.0 ± 2.2^{c}

Notes: Values are mean ± SD (n=5). Different superscript letters within a column indicate significant differences ($p < 0.05$, Tukey's test).

Administration of the coffee-like extract demonstrated a dose-dependent hepatoprotective effect:

- D500 (500 mg/kg): Increased healthy hepatocytes to 72.1 ± 5.8% (mean difference vs. LPS = +6.3%, 95% CI: +1.5 to +11.1, $p = 0.008$, Cohen's $d = 1.12$), with corresponding reductions in pyknosis (9.3 ± 3.5%), karyorrhexis (9.8 ± 3.7%), and karyolysis (8.8 ± 3.5%).
- D1000 (1000 mg/kg): Increased healthy hepatocytes to 76.5 ± 4.9% (mean difference vs. LPS = +10.7%, 95% CI: +5.9 to +15.5, $p < 0.001$, Cohen's $d = 1.90$), with substantial reductions in karyorrhexis (8.3 ± 3.3%; mean reduction vs. LPS = –4.5%, 95% CI: –7.9 to –1.1, $p = 0.006$) and karyolysis (6.7 ± 2.9%; mean reduction vs. LPS = –4.2%, 95% CI: –7.4 to –1.0, $p = 0.008$).

- D1500 (1500 mg/kg): Achieved near-complete restoration of healthy hepatocytes to 80.2 ± 4.7% (mean difference vs. LPS = +14.4%, 95% CI: +9.6 to +19.2, $p < 0.001$, Cohen's d = 2.56), with pyknosis reduced to 7.8 ± 2.7%, karyorrhexis to 7.0 ± 2.8% (mean reduction vs. LPS = –5.8%, 95% CI: –9.2 to –2.4, $p < 0.001$), and karyolysis to 5.0 ± 2.2% (mean reduction vs. LPS = –5.9%, 95% CI: –9.1 to –2.7, $p < 0.001$).



Notes: Green arrows→Healthy cells; Orange arrows→Pyknosis; Black rrows→Karyorrhexis; Red arrows→Karyolysis. Magnification:400×

Figure 4. Histopathology of LPS-induced BALB/c mouse liver and treatment with coffee-like extracts made from *Rhizophora mucronata* fruit

The hepatoprotective effects of the coffee-like extract at 1500 mg/kg were statistically indistinguishable from those of vitamin E (500 mg/kg), which increased healthy hepatocytes to 78.4 ± 5.2% (mean difference vs. D1500 = –1.8%, 95% CI: –6.6 to +3.0, p = 0.462, Cohen's d = 0.32). These findings indicate that the highest dose of the coffee-like extract provides hepatoprotection comparable to the standard antioxidant vitamin E.

Post-hoc pairwise comparisons (Tukey's HSD test) revealed that the differences in healthy hepatocyte proportions among the three extract doses were statistically significant ($p < 0.05$) for D500 vs. D1500 (mean difference = –8.1%, 95% CI: –12.9 to –3.3, p = 0.001) and D500 vs. D1000 (mean difference = –4.4%, 95% CI: –9.2 to +0.4, p = 0.078, borderline), supporting the dose-dependent trend.

DISCUSSION

Proximate Composition and Its Nutritional Implications

The proximate analysis of the *R. mucronata* coffee-like powder revealed a composition of moisture (5.31%), ash (5.25%), protein (13.07%), fat (11.17%), carbohydrate (65.21%), and energy (413.61 kcal/100 g), with caffeine content of 0.96%. These values are generally consistent with previous reports on similar products from *R. mucronata* fruit processed with lower lime solution concentrations[13,14].

The moisture content met the Indonesian National Standard for ground coffee (SNI No. 01-3542-2004, maximum 7%), indicating good storage stability and resistance to microbial growth[48]. The caffeine content also fell within the SNI range (0.45–2%), confirming that the product possesses the characteristic stimulant properties of coffee beverages[49].

The ash content slightly exceeded the SNI maximum limit of 5%. This elevation is likely attributable to the prolonged soaking in 6% lime solution ($Ca(OH)_2$) during processing, which may have increased the mineral content, particularly calcium, in the final product. The increase in ash content in the final product is believed to be caused by two main mechanisms. First, the removal of protein and fat impurities by the alkaline solution increases the proportion of inorganic minerals (ash) in the product[50]. Second, Ca^{2+} ions from the $Ca(OH)_2$ solution precipitate onto the surface of the soaked material[51]. Similar increases in ash content have been observed in plant-based products subjected to alkaline soaking treatments[52]. While this exceeds the SNI standard for conventional ground coffee, it does not necessarily indicate a quality defect for a novel functional food product; rather, it may suggest a higher mineral content that could contribute to the product's nutritional value.

The pH of the aqueous brew (5.37 ± 0.05) falls within the typical range for brewed coffee (4.85–

5.13) and other coffee-like beverages[53]. This mildly acidic pH is characteristic of roasted plant-based beverages and is influenced by the roasting process, which generates organic acids and Maillard reaction products (MRPs)[54]. The pH value of 5.37 is comparable to that of Liberica coffee brews (5.40–5.55) and Arabica coffee[55], suggesting that the *R. mucronata* coffee-like product has an acidity profile similar to commercially available coffee beverages. This similarity in pH is important for consumer acceptance, as pH influences both flavor perception and gastrointestinal tolerability[56,57]. The moderate acidity may also contribute to the product's antioxidant stability, as certain phenolic compounds and MRPs are more stable at acidic pH[58].

The high carbohydrate content (65.21%) and corresponding energy value (413.61 kcal/100 g) indicate that the product is a substantial energy source, consistent with findings that mangrove fruits contain non-reducing sugars that impart a sweet taste[10,59]. The protein content (13.07%) is notable for a coffee-like beverage and may contribute to the product's nutritional profile. The protein content of mangrove fruits varies significantly among species, ranging from 0.31% to 15.6 mg/g fresh weight, with carbohydrates being the dominant nutrient[59,60]. The fruits of *Rhizophora apiculata* are reported to contain 14.4 mg/g of protein[61]. Processing mangrove fruits into flour, syrup, or other food products can affect protein levels[62].

Phytochemical Composition and Its Implications

The qualitative and quantitative phytochemical profiling of the coffee-like extract from *Rhizophora mucronata* fruit revealed the predominance of steroids, terpenoids, and saponins, while flavonoids, alkaloids, phenols, and tannins were absent. This compositional profile aligns with previous findings by Cahyono *et al*.[14], who similarly identified steroids and terpenoids in analogous preparations. However, it contrasts with studies on raw *R. mucronata* fruit extracts, which have reported the presence of alkaloids, flavonoids, and tannins using methanol-based extraction methods, which have reported *R.mucronata* fruit extract containing flavonoid 65.63 ppm; phenol 4.333 ppm; saponin 9.41%; alkaloid 21.11% and positive steroids[3,63,64]. Differences in the bioactive compound content of organisms from one location to another, even within the same species, can vary due to the interaction of genetic and geographic factors[65].

The absence of heat-sensitive phytochemicals in our preparation can be attributed to the multi-step processing protocol, particularly the roasting step at 116–118°C for 30–40 minutes. Thermal processing is known to degrade thermolabile compounds including alkaloids, flavonoids, and tannins through mechanisms such as oxidation, polymerization, and structural breakdown[66,67]. Conversely, steroids and terpenoids exhibit greater thermal stability, consistent with their retention in the final product. Supporting this, a recent GC-MS study on *R. mucronata* leaves from the same geographical origin identified fatty acid derivatives (e.g., n-hexadecanoic acid, methyl esters) as the dominant volatile compounds, which are known for their antioxidant and antimicrobial properties[68].

To our knowledge, no previous study has reported total steroid content in *R. mucronata* fruit; most have only provided qualitative data[63,69,70]. Sadeer *et al*.[71] quantified triterpenoids in methanolic extracts of this species (13.78 ± 0.47 mg/g oleanolic acid equivalent), a value considerably higher than our terpenoid measurement, likely due to differences in extraction solvent and processing. The soaking steps in water and lime solution likely contributed to the substantial reduction in saponin content (0.52 mg/kg), as aqueous leaching can remove up to 96% of saponins from plant materials[72], with additional degradation occurring during roasting[73]. Study previous reported that total saponin levels in unprocessed *R. mucronata* fruit have been reported as ~9.14%[64] or 8.05 ± 0.50 mg/g escin equivalent[74], far exceeding our values.

The moderate antioxidant activity (IC_{50} = 113.66 µg/mL) observed in this study is noteworthy given the absence of flavonoids and phenolics, which are typically considered primary antioxidant constituents. This activity is primarily attributable to the terpenoids and steroids present in the extract. Terpenoids are known to scavenge free radicals through hydrogen donation mechanisms[75], while certain steroid derivatives may contribute via radical quenching[76].

Furthermore, the roasting process during product preparation likely generated Maillard reaction products (MRPs), which are well-documented for their antioxidant properties. Studies have demonstrated that MRPs exhibit significantly enhanced DPPH radical scavenging activities compared to individually heated amino acids and sugars[77]. The Maillard reaction, which occurs during roasting at 116–118°C, produces melanoidins and other volatile compounds with potent radical scavenging capacity[78]. These MRPs are water-soluble and would be present in the aqueous brew used for *in vivo* administration, potentially contributing to the observed biological effects. The cumulative antioxidant capacity therefore likely results from synergistic interactions among steroids, terpenoids, and MRPs, compensating for the absence of conventional phenolic antioxidants[79].

The presence of steroids, terpenoids, and saponins provides a plausible molecular basis for the observed biological effects. Steroids and terpenoids are well-known for their anti-inflammatory and immunomodulatory activities[80]. Saponins, even in small amounts, can act as immune adjuvants by enhancing antigen presentation[81,82]. Thus, the phytochemical profile of the artificial coffee supports its potential as a source of bioactive compounds with antioxidant and immunomodulatory properties.

Immunomodulatory Effects on Humoral Immunity

The significant reduction in serum IgG levels following LPS challenge in this study reflects the well-characterized immunosuppressive effects of systemic inflammation. LPS induces a cascade of inflammatory responses that suppress both cellular and humoral immunity[19]. Mechanistically, LPS impairs B-cell responses and antibody production through multiple pathways, including the accumulation of extracellular ATP that compromises T-cell function, thereby indirectly affecting IgG synthesis[83]. The decline in IgG within 6–24 hours post-LPS administration is consistent with an acute

immunosuppressive phase, as previously documented in mice models[84].

The restoration of serum IgG levels by the coffee-like extract at doses of 1000 and 1500 mg/kg, and the partial restoration at 500 mg/kg, demonstrates potent immunomodulatory activity. Notably, the 500 mg/kg dose achieved only partial recovery (2600 ± 26.90 µg/mL), significantly lower than the normal group ($p < 0.001$), whereas the 1000 and 1500 mg/kg doses achieved complete restoration comparable to normal and vitamin E groups. This differential response indicates a dose threshold for full humoral immune recovery. This finding substantially extends our previous work, which focused on cellular immunity parameters ($CD4^+CD62L^+$ and $CD8^+CD62L^+$ T-lymphocyte subsets)[14], by establishing that the extract also benefits humoral immune responses.

The absence of a significant difference between the 1000 and 1500 mg/kg doses ($p > 0.05$) suggests a plateau effect for IgG restoration, with the immunomodulatory threshold reached at 1000 mg/kg. This pattern is consistent with saturable receptor-mediated mechanisms, where all available targets are fully occupied at the lower effective dose[85]. Interestingly, this plateau effect for IgG contrasts with the dose-dependent hepatoprotective effects observed in the liver histopathology, suggesting differential dose–response relationships for immune versus organ-protective outcomes. This differential sensitivity may reflect the distinct physiological thresholds for humoral immune restoration versus tissue protection, with the former requiring lower bioactive compound concentrations.

The proposed mechanism involves the protection of B lymphocytes and plasma cells from LPS-induced oxidative damage by the antioxidant constituents of the extract. Steroids and terpenoids are well-documented for their anti-inflammatory and immunomodulatory properties[80], while saponins, even at low concentrations, can act as immune adjuvants by enhancing antigen presentation and promoting antibody production[81,82]. By reducing reactive oxygen species (ROS) levels, these compounds may preserve the functional capacity of B cells to produce antibodies[86]. However, it is important to acknowledge that these proposed mechanisms are speculative, as oxidative stress biomarkers (e.g., MDA, SOD, CAT) and inflammatory cytokines (e.g., TNF-α, IL-6) were not directly measured in this study.

The comparable efficacy between the coffee-like extract and vitamin E suggests overlapping mechanisms of action. Vitamin E enhances adaptive immunity under oxidative stress by stabilizing cell membranes, reducing lipid peroxidation, and modulating signaling pathways in both T and B cells[87]. Similarly, the bioactive compounds in the mangrove extract likely protect immune cell membranes from oxidative damage, preserve cellular signaling integrity, and support the differentiation of B cells into antibody-secreting plasma cells[88–90].

Hepatoprotective Mechanisms and Dose-Dependent Effects

The severe hepatic damage observed in LPS-treated mice, characterized by reduced healthy

hepatocytes and increased nuclear abnormalities (pyknosis, karyorrhexis, and karyolysis), reflects the hepatotoxic consequences of systemic inflammation and oxidative stress. LPS triggers the release of pro-inflammatory cytokines (TNF-α, IL-6) and induces ROS production, leading to hepatocellular injury through apoptosis and necrosis pathways[21,27]. The nuclear morphological changes observed—pyknosis (nuclear shrinkage and condensation), karyorrhexis (nuclear fragmentation), and karyolysis (nuclear dissolution)—are hallmarks of irreversible cell death and indicate the severity of LPS-induced hepatotoxicity.

The coffee-like extract demonstrated dose-dependent hepatoprotective effects, with the highest dose (1500 mg/kg) achieving near-complete restoration of liver histology to levels comparable to the normal group and vitamin E control. This dose-response relationship underscores the extract's therapeutic potential and suggests that the protective effects are concentration-dependent. The 500 mg/kg dose provided moderate protection (72.1% healthy cells), while 1000 mg/kg produced substantial improvement (76.5% healthy cells), and 1500 mg/kg yielded optimal protection (80.2% healthy cells). Notably, the histopathological parameters at 1500 mg/kg were statistically indistinguishable from those of the vitamin E group (78.4% healthy cells), indicating comparable hepatoprotective efficacy.

The hepatoprotective mechanism is likely mediated by multiple pathways. First, the antioxidant constituents—steroids, terpenoids, and Maillard reaction products (MRPs)—may neutralize ROS within hepatocytes, preventing oxidative damage to cellular membranes and DNA[91]. Second, the anti-inflammatory properties of terpenoids and steroids may downregulate pro-inflammatory cytokine production in the liver, attenuating the inflammatory cascade that leads to hepatocellular injury[92]. This is supported by previous studies showing that *R. mucronata* bark extract reduced TNF-α expression and decreased oxidative stress in ethanol-induced liver injury models[93]. Third, the preservation of hepatocyte integrity supports the liver's metabolic and synthetic functions, including the production of complement proteins and acute-phase reactants essential for systemic immunity[94].

The integration of liver protection with IgG recovery is particularly noteworthy, but it is essential to clarify that the liver does not directly produce IgG. Rather, by preserving hepatocyte integrity, the coffee-like extract likely supports the liver's contribution to systemic immunity—including the synthesis of complement components and acute-phase proteins—which in turn creates a favorable microenvironment for secondary lymphoid organs and plasma B cells to produce IgG[27,94]. This dual action—protecting both the liver and immune cells—may explain the comprehensive immunomodulatory and hepatoprotective effects observed.

Integration with Previous Findings and Mechanistic Framework

Integrating the present findings with our earlier publications[13,14] establishes a coherent mechanistic framework for the biological activities of the *R. mucronata* coffee-like product. The extract

exerts immunomodulatory effects through two complementary pathways: (i) enhancement of cellular immunity, as evidenced by increased $CD4^+CD62L^+$ and $CD8^+CD62L^+$ T-lymphocyte subsets, and (ii) enhancement of humoral immunity, demonstrated by restoration of serum IgG levels in the present study. Simultaneously, the extract provides organ protection, attenuating LPS-induced damage to both the liver (present study) and renal.

These diverse biological activities are likely mediated by the synergistic action of the extract's dominant bioactive constituents—steroids, terpenoids, and saponins—along with Maillard reaction products (MRPs) generated during roasting. The collective actions of these compounds include: (i) shielding immune cells from oxidative stress through radical scavenging and membrane protection, thereby preserving their functional capacity; (ii) curbing systemic and organ-specific inflammation via downregulation of pro-inflammatory cytokines; (iii) preventing hepatocellular necrosis through ROS neutralization and anti-inflammatory signaling; and (iv) supporting overall immunological and metabolic homeostasis through preservation of organ integrity.

However, it is important to acknowledge that the dose-response relationships differed between the two primary outcomes. For serum IgG, a plateau effect was observed at 1000 mg/kg, with no additional benefit at 1500 mg/kg. In contrast, for liver histopathology, a dose-dependent trend was observed, with the highest dose (1500 mg/kg) achieving near-complete restoration. This differential sensitivity may reflect the distinct physiological thresholds for immune restoration versus tissue protection, and suggests that the optimal dose for immunomodulation (1000 mg/kg) may differ from the optimal dose for maximal hepatoprotection (1500 mg/kg). This nuanced finding has important implications for product development, as it suggests that different health benefits may require different dosage regimens.

Significance and Future Directions

This study provides the first evidence that the artificially processed coffee-like product from *R. mucronata* fruit can restore humoral immune function and protect the liver in a mice model of systemic inflammation. These findings have significant implications for the development of functional foods from Indonesia's mangrove biodiversity, particularly from the Sangihe Islands, where *R. mucronata* is abundant.

The product's preliminary safety is supported by our previous *in silico* predictions indicating low oral toxicity (LD_{50} = 5000 mg/kg, toxicity class V) and absence of hepatotoxic potential for the major identified compounds[13]. However, it is crucial to emphasize that these *in silico* predictions and the absence of overt toxicity at the highest tested dose (1500 mg/kg) serve only as preliminary safety indicators and do not replace formal toxicological evaluation. Comprehensive toxicological studies according to OECD guidelines[95], including subacute and chronic toxicity assessments, are essential before any recommendation for human consumption can be made.

Furthermore, the distinction between the ethanolic extract used for phytochemical profiling and the aqueous brew used for *in vivo* administration must be considered when interpreting the results. While the ethanolic extract provided comprehensive characterization of the product's bioactive constituents, the aqueous brew more closely resembles the actual human consumption pattern. The presence of water-soluble MRPs in the brew, which are absent in the ethanolic extract, may have contributed to the observed biological effects. Future studies should characterize the phytochemical profile of the aqueous brew to better understand the active constituents responsible for the *in vivo* effects.

Future research should focus on: (i) isolation and characterization of individual bioactive compounds responsible for the observed effects; (ii) elucidation of specific molecular mechanisms, including receptor-mediated signaling pathways; (iii) evaluation of efficacy and safety in long-term administration protocols; (iv) assessment of bioavailability and pharmacokinetics of the active constituents; (v) formal toxicological evaluation according to OECD guidelines; and (vi) translational research and clinical trials to validate the functional food potential of this mangrove-based product.

The economic and sustainability implications are noteworthy. Transforming mangrove fruits into a value-added coffee-like beverage provides an alternative livelihood opportunity for coastal communities while promoting the sustainable utilization of mangrove resources. Moreover, the same mangrove leaves have been successfully incorporated into rice crackers, yielding a product that was sensorially well-accepted (ratings of "like" to "very like" for appearance, aroma, taste, and texture) and met the Indonesian National Standard for moisture content[68]. This demonstrates the versatility of *R. mucronata* as a functional ingredient across different food matrices and provides a model for community-based product diversification. The scientific validation of its immunomodulatory and hepatoprotective properties strengthens the evidence base for this innovative functional food and supports its commercialization as a health-promoting beverage derived from Indonesia's unique biodiversity.

Limitations of Study

Several limitations should be acknowledged. First, the study was conducted in a mouse model, and results may not directly translate to human applications. Second, the acute LPS challenge model differs from chronic inflammatory conditions, and the extract's efficacy in chronic disease models remains to be established. Third, the phytochemical analysis focused on major compound classes without identifying specific individual compounds; comprehensive metabolomic profiling, particularly of the aqueous brew used for *in vivo* administration, would provide deeper mechanistic insights. Fourth, oxidative stress biomarkers (e.g., MDA, SOD, CAT) and inflammatory cytokines (e.g., TNF-α, IL-6) were not measured, limiting our ability to confirm the proposed antioxidant and anti-inflammatory mechanisms. Fifth, the study did not evaluate potential interactions between the

extract and conventional therapeutic agents. Sixth, the use of different extraction solvents for *in vitro* (ethanol) and *in vivo* (aqueous brew) studies, while justified by safety and relevance considerations, limits direct comparability of the phytochemical profiles between the two sets of experiments. This study did not calculate the human equivalent dose (HED) for animal doses. The highest dose administered to test animals was used to evaluate liver and kidney effects, so formal toxicological evaluation and clinical trials are required before human consumption can be recommended.

CONCLUSION

This study demonstrates that the processed coffee-like product derived from *Rhizophora mucronata* fruit possesses immunomodulatory and hepatoprotective potential in a mouse model of LPS-induced systemic inflammation. Phytochemical characterization revealed the presence of steroids, terpenoids, and saponins, with moderate antioxidant activity. Proximate analysis confirmed that the product meets Indonesian National Standards for moisture and caffeine content, supporting its potential as a functional food ingredient.

Oral administration of the extract at 1000 and 1500 mg/kg fully restored serum IgG levels to normal, while the 500 mg/kg dose provided only partial recovery, indicating a dose threshold for complete humoral immune restoration. In contrast, the extract demonstrated dose-dependent hepatoprotective effects, with the highest dose (1500 mg/kg) achieving near-complete restoration of liver histology, comparable to vitamin E. These differential dose–response patterns suggest that the optimal dose for immunomodulation (1000 mg/kg) may differ from the optimal dose for maximal hepatoprotection (1500 mg/kg).

These preliminary findings provide a scientific rationale for further development of this coffee-like beverage as a functional food derived from Indonesia's local biodiversity. However, it is important to emphasize that these results are based on an acute animal model, and comprehensive toxicological evaluation according to OECD guidelines, as well as clinical trials, are essential before human consumption can be recommended. Future research should focus on isolating individual active compounds, elucidating specific molecular mechanisms, and conducting long-term safety and efficacy studies.

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BIBLIOGRAPHY

1. Dahibhate NL, Saddhe AA, Kumar K. Mangrove Plants as a Source of Bioactive Compounds: A Review. Nat Prod J. 2019;9(2):86-97. doi:10.2174/2210315508666180910125328
2. Arifin MZ, Mulalinda P, Kalesaran J, Tauladani SA, Asia. Study of the Level of Success of Mangrove Planting on the Coast of Dagho Village, Sangihe Islands Regency, Matahit Village, Talaud Islands Regency and Pasirpanjang Village, South Lembeh District, Bitung City. Frontiers (Boulder) [Internet]. 2019;2(1):21-33. Available from: https://garuda.kemdiktisaintek.go.id/documents/detail/1689806
3. Setyawan AD, Ragavan P, Basyuni M, Sarno S. Review: *Rhizophora mucronata* as Source of Foods and Medicines. Intl J Bonorowo Wetl. 2019;9(1):42-55. doi:10.13057/bonorowo/w090105
4. Hilmi E, Sari LK, Siregar AS, et al. Tannins in Mangrove Plants in Segara Anakan Lagoon, Central Java, Indonesia. Biodiversitas. 2021;22(8):3508-3516. doi:10.13057/biodiv/d220850
5. Rumengan AP, Mandiangan ES, Tanod WA, Paransa DSJ, Paruntu CP, Mantiri DMH. Identification of Pigment Profiles and Antioxidant Activity of *Rhizophora mucronata* Mangrove Leaves Origin Lembeh, North Sulawesi, Indonesia. Biodiversitas. 2021;22(7):2805-2816. doi:10.13057/biodiv/d220730
6. Das PS, Ashrafi S, Anjum J, Hossain MdE, Reza MdS, Ahsan M. Isolation of Terpenoids and Derivatives from a Bangladeshi Mangrove Plant, *Rhizophora mucronata* Lam., and Its Bioactivity Evaluation: *In Vitro* and *In Silico* Study. *J Chem*. 2024;2024(1):1-25. doi:10.1155/joch/8884812
7. Dewanto DK, Hermawan R, Muliadin, Riyadi PH, Aisiah S, Tanod WA. GC-MS Profile of *Rhizophora apiculata* Leaf Extract from The Coast of Tomini bay, Central Sulawesi with Antibacterial and Antioxidant Activity. J Kelautan: Indo J Mar Sci Technol. 2021;14(1):30-42. doi:10.21107/jk.v14i1.8904
8. Kalasuba K, Miranti M, Rahayuningsih SR, et al. Red Mangrove (*Rhizophora stylosa* Griff.)—A Review of Its Botany, Phytochemistry, Pharmacological Activities, and Prospects. Plants. 2023;12(11):2196. doi:10.3390/plants12112196
9. Suganthy N, Pandima Devi K. *In vitro* Antioxidant and Anti-cholinesterase Activities of *Rhizophora mucronata*. Pharm Biol. 2016;54(1):118-129. doi:10.3109/13880209.2015.1017886

10. Basyuni M, Yusraini E, Susilowati A, et al. Bioprospecting of Selected Mangrove Fruits Based-Nutritional, Antioxidant, and Element Properties to Support Functional Food Materials for Pulau Sembilan Coastal Communities, Indonesia. Int J Adv Sci Eng Inf Technol. 2021;11(4):1661-1667. doi:10.18517/ijaseit.11.4.13643
11. Miranti DI, Ichiura H, Ohtani Y. The Bioactive Compounds and Antioxidant Activity of Food Products of *Rhizophora stylosa* Fruit (Coffee and Tea Mangrove). Int J For Res. 2018;2018:1-6. doi:10.1155/2018/2315329
12. Sukma RN, Zahro M. Effect Utilization Mangrove *Rhizophora* sp. Fruit Extract in Production of Coffee Powder in Perspective of Water Content and Organoleptic Test. Aquasains [Internet]. 2020;9(1):881-886. Available from: https://jurnal.fp.unila.ac.id/index.php/JPBP/article/view/4747
13. Mandeno JA, Tanod WA, Cahyono E, et al. Characteristics of Analog Coffee from The Mangrove Fruit *Rhizophora mucronata* Origin Sangihe Islands. IOP Conf Ser Earth Environ Sci. 2023;1260(1):1-11. doi:10.1088/1755-1315/1260/1/012055
14. Cahyono E, Tanod WA, Ijong FG, et al. The Ameliorating Effect of Artificial Coffee from Mangrove Fruits (*Rhizophora mucronata*) on T Lymphocyte Cells and Renal Histopathology of BALB/c Mice Induced by Lipopolysaccharide. Squalen Bull Mar Fish Postharvest Biotechnol. 2024;19(1):55-69. doi:10.15578/squalen.835
15. Roşian ŞH, Boarescu I, Boarescu PM. Antioxidant and Anti-Inflammatory Effects of Bioactive Compounds in Atherosclerosis. Int J Mol Sci. 2025;26(3):1379. doi:10.3390/ijms26031379
16. Shekhova E. Mitochondrial Reactive Oxygen Species as Major Effectors of Antimicrobial Immunity. PLoS Pathog. 2020;16(5):e1008470. doi:10.1371/journal.ppat.1008470
17. Iddir M, Brito A, Dingeo G, et al. Strengthening the Immune System and Reducing Inflammation and Oxidative Stress through Diet and Nutrition: Considerations during the COVID-19 Crisis. Nutrients. 2020;12(6):1562. doi:10.3390/nu12061562
18. Skrzypczak-wiercioch A. Lipopolysaccharide-Induced Model of Neuroinflammation: Mechanisms of Action, Research Application and Future Directions for Its Use. Molecules. 2022;27:5481. doi:10.3390/molecules27175481
19. Yin R, Zhang K, Li Y, et al. Lipopolysaccharide-Induced Depression-Like Model in Mice: Meta-analysis and Systematic Evaluation. Front Immunol. 2023;14(1181973):1-15. doi:10.3389/fimmu.2023.1181973
20. Ozawa A, Nakano T, Tanaka T, Hozumi Y, Iseki K, Goto K. Diacylglycerol Kinase ε Depletion Suppresses LPS-Stimulated NF-κB Activation and Reduces Free Radical-Induced DNA Damage. FEBS Lett. 2025;599(24):3629-3641. doi:10.1002/1873-3468.70164

21. Chen S nan, Tan Y, Xiao X chan, et al. Deletion of TLR4 Attenuates Lipopolysaccharide-Induced Acute Liver Injury by Inhibiting Inflammation and Apoptosis. Acta Pharmacol Sin. 2021;42(10):1610-1619. doi:10.1038/s41401-020-00597-x
22. Kim K, Hong HL, Kim GM, Leem J, Kwon HH. Eupatilin Ameliorates Lipopolysaccharide-Induced Acute Kidney Injury by Inhibiting Inflammation, Oxidative Stress, and Apoptosis in Mice. Curr Issues Mol Biol. 2023;45(9):7027-7042. doi:10.3390/cimb45090444
23. Maheshwari S, Kumar V, Bhadauria G, Mishra A. Immunomodulatory Potential of Phytochemicals and Other Bioactive Compounds of Fruits: A Review. Food Front. 2022;3(2):221-238. doi:10.1002/fft2.129
24. Colomeu TC, de Figueiredo D, de Matos da Silva P, Fernandes LGR, Zollner R de L. Antiproliferative and Pro-Oxidant Effect of Polyphenols in Aqueous Leaf Extract of *Passiflora alata* Curtis on Activated T Lymphocytes from Non-Obese Diabetic (NOD SHILT/J) Mice. Antioxidants. 2022;11(8):1503. doi:10.3390/antiox11081503
25. Rodda LB, Netland J, Shehata L, et al. Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19. Cell. 2021;184(1):169-183. doi:10.1016/j.cell.2020.11.029
26. Hematianlarki M, Nimmerjahn F. Immunomodulatory and Anti-Inflammatory Properties of Immunoglobulin G Antibodies. Immunol Rev. 2024;328(1):372-386. doi:10.1111/imr.13404
27. Zhou Y, Hu X, Zhong S, et al. Effects of Continuous LPS Induction on Oxidative Stress and Liver Injury in Weaned Piglets. Vet Sci. 2022;10(1):22. doi:10.3390/vetsci10010022
28. Haruna HMS, Umar H, Zakari A, Baba G. Toxicological Assessment of Aqueous Leaf Extract of *Anisopus mannii*: Acute and Sub-Acute Studies in Albino Rats. Sahel J of Life Sci FUDMA. 2024;2(4):40-46. doi:10.33003/sajols-2024-0204-07
29. Olukanni AT, Omotosho D, Olalekan DT, et al. Hepatoprotective and Nephroprotective Effects of *Leea guineensis* Leaf Extract Against Paracetamol-Induced Toxicity: Combined Mouse Model-Integrated *in Silico* Evidence. Int J Mol Sci. 2025;26(13):6142. doi:10.3390/ijms26136142
30. Aliyu A, Shaari MR, Ahmad Sayuti NS, et al. Subacute Oral Administration of *Clinacanthus nutans* Ethanolic Leaf Extract Induced Liver and Kidney Toxicities in ICR Mice. Molecules. 2020;25(11):2631. doi:10.3390/molecules25112631
31. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Second. Springer Berlin Heidelberg; 1996. doi:10.1007/978-3-642-00574-9
32. Harborne JB. Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis. Vol 3. Chapman and Hall; 1998. doi:10.1017/CBO9781107415324.004
33. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. Afr J Tradit Complement Altern

Med [Internet]. 2011;8(1):1-10. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3218439/

34. Alara OR, Abdurahman NH, Ukaegbu CI, Kabbashi NA. Extraction and Characterization of Bioactive Compounds in *Vernonia amygdalina* Leaf Ethanolic Extract Comparing Soxhlet and Microwave-Assisted Extraction Techniques. J Taibah Univ Sci. 2019;13(1):414-422. doi:10.1080/16583655.2019.1582460
35. Shai LJ, Masoko P, Mokgotho MP, et al. Yeast Alpha Glucosidase Inhibitory and Antioxidant Activities of Six Medicinal Plants Collected in Phalaborwa, South Africa. S Afr J Bot. 2010;76(3):465-470. doi:10.1016/j.sajb.2010.03.002
36. Pillai JR, Wali AF, Al-Azzawi AM, Akhter R, El-Serehy HA, Akbar I. Phytochemical Analysis and Antimicrobial Activity of *Enicostemma littorale*. J King Saud Univ Sci. 2020;32(8):3279-3285. doi:10.1016/j.jksus.2020.09.011
37. Hossain MA. Biological and Phytochemicals Review of Omani Medicinal Plant *Dodonaea viscosa*. J King Saud Univ Sci. 2019;31(4):1089-1094. doi:10.1016/j.jksus.2018.09.012
38. Ravelliani A, Nisrina H, Komala Sari L, Marisah M, Riani R. Identification and Isolation of Saponin Glycosides from Several Plants in Indonesia. J sos sains. 2021;1(8):786-799. doi:10.59188/jurnalsosains.v1i8.176
39. Thatipelli S, Shanmugam M, Ramachandran S, Pushparathinam G. Screening and Validated Semi - Quantification High-Performance Thin Layer Chromatography Method Development for Lupeol, Lupeol Acetate, β-sitosterol, ρ-Coumaric Acid and Proto-Catechuic Acid in The Root Extracts of *Hemidesmus indicus* (L.) R.Br. & *Decalepis hamiltonii* Wight & Arn. J Appl Res Med Aromat Plants. 2023;36:100510. doi:10.1016/j.jarmap.2023.100510
40. Barwant MM, Satyannarayana B, Mahalaxmi SmtK. Phytochemical Insights: Techniques and Applications Volume I. First. Bhumi Publishing; 2025. doi:10.5281/zenodo.17876298
41. Blois M. Antioxidant Determinations by The Use of a Stable Free Radical. Nature. 1958;181(4617):1199-1200. doi:10.1038/1811199a0
42. Festing MFW, Altman DG. Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals. ILAR J. 2002;43(4):244-258. doi:10.1093/ilar.43.4.244
43. Kang H. Sample Size Determination and Power Analysis Using The G*Power Software. J Educ Eval Health Prof. 2021;18:17. doi:10.3352/jeehp.2021.18.17
44. Balcombe JP, Barnard ND, Sandusky C. Laboratory Routines Cause Animal Stress. Contemp Top Lab Anim Sci [Internet]. 2004;43(6):42-51. Available from: https://pubmed.ncbi.nlm.nih.gov/15669134/

45. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals, 8th Edition. 8th ed. National Academies Press; 2011. doi:10.17226/12910
46. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: Updated Guidelines for Reporting Animal Research. PLoS Biol. 2020;18(7):e3000410. doi:10.1371/journal.pbio.3000410
47. Muhammad-Azam F, Nur-Fazila SH, Ain-Fatin R, Noordin MM, Yimer N. Histopathological Changes of Acetaminophen-Induced Liver Injury and Subsequent Liver Regeneration in BALB/C and ICR Mice. Vet World. 2019;12(11):1682-1688. doi:10.14202/vetworld.2019.1682-1688
48. Zambrano MV, Dutta B, Mercer DG, MacLean HL, Touchie MF. Assessment of Moisture Content Measurement Methods of Dried Food Products in Small-Scale Operations in Developing Countries: A Review. Trends Food Sci Technol. 2019;88(April):484-496. doi:10.1016/j.tifs.2019.04.006
49. Ashihara H, Crozier A. Caffeine: A Well Known But Little Mentioned Compound in Plant Science. Trends Plant Sci. 2001;6(9):407-413. doi:10.1016/S1360-1385(01)02055-6
50. Gawborisut S, Ketkaew C, Buasook T. Production of Biocalcium from Fermented Fish Bone Residue for Fish Emulsion Sausage Fortification. Foods. 2024;13(6):882. doi:10.3390/foods13060882
51. Yamagishi K, Ike M, Guan D, Tokuyasu K. Washing Lime-Pretreated Rice Straw with Carbonated Water Facilitates Calcium Removal and Sugar Recovery in Subsequent Enzymatic Saccharification. J Appl Glycosci. 2019;66(1):11-19. doi:10.5458/jag.jag.JAG-2018_0003
52. Titisari PW, Elfis, Zen IS, et al. The Potential of Mangrove as a Food Source in Riau. Future Food: J Food Agric Soc. 2023;11(5):1-18. doi:10.17170/kobra-202307218420
53. About Coffee. Is coffee acidic? [Internet] 2024. Available from: https://www.aboutcoffee.org/health/faq/is-coffee-acidic/. 2024. Accessed July 31, 2026.
54. Lawan MZ, Muhammad IM, Ahmed IS, Olu OO, Yusuf AA, Maryam I. Kinetics and Thermodynamic Parameters of Coffee Senna Seed (*Senna occidentalis*) Using Degradation Data from Roasting. Mikailalsys J Adv Eng Int. 2025;2(2):238-261. doi:10.58578/mjaei.v2i2.6277
55. Azizah DR, Sunarharum WB, Mahatmanto T, Kartika AA, Hakim L. Exploring the Impact of Various Manual Brewing Techniques on the Physicochemical and Sensory Characteristics of Brewed Coffee. IOP Conf Ser Earth Environ Sci. 2024;1299(1):012011. doi:10.1088/1755-1315/1299/1/012011

56. Iriondo-DeHond M, Iriondo-DeHond A, Herrera T, et al. Sensory Acceptance, Appetite Control and Gastrointestinal Tolerance of Yogurts Containing Coffee-Cascara Extract and Inulin. Nutrients. 2020;12(3):627. doi:10.3390/nu12030627
57. Batali ME, Cotter AR, Frost SC, Ristenpart WD, Guinard JX. Titratable Acidity, Perceived Sourness, and Liking of Acidity in Drip Brewed Coffee. ACS Food Sci Technol. 2021;1(4):559-569. doi:10.1021/acsfoodscitech.0c00078
58. Nooshkam M, Varidi M, Bashash M. The Maillard Reaction Products as Food-Born Antioxidant and Antibrowning Agents in Model and Real Food Systems. Food Chem. 2019;275:644-660. doi:10.1016/j.foodchem.2018.09.083
59. Rout P, Singh S, Kumar N, Basak UC. Nutritional and Antioxidant Potential of Some Selected Edible Mangrove Fruits of Odisha Coast. Int J Adv Sci Res. 2015;1(9):349-355. doi:10.7439/ijasr
60. Budiyanto F, Alhomaidi EA, Mohammed AE, et al. Exploring the Mangrove Fruit: From the Phytochemicals to Functional Food Development and the Current Progress in the Middle East. Mar Drugs. 2022;20(5):303. doi:10.3390/md20050303
61. Analuddin K, Septiana A, Nasaruddin, Sabilu Y, Sharma S. Mangrove Fruit Bioprospecting: Nutritional and Antioxidant Potential as a Food Source for Coastal Communities in the Rawa Aopa Watumohai National Park, Southeast Sulawesi, Indonesia. Int J Fruit Sci. 2019;19(4):423-436. doi:10.1080/15538362.2018.1555507
62. Ardiansyah PR, Wonggo D, Dotulong V, et al. Proximate Analysis of *Sonneratia alba* Mangrove Fruit Flour. Media Teknologi Hasil Perikanan. 2020;8(3):82. doi:10.35800/mthp.8.3.2020.27526
63. Ernawati, Suprayitno E, Hardoko, Yanuhar U. Extraction of Bioactive Compounds Fruit from *Rhizophora mucronata* Using Sonication Method. IOP Conf Ser Earth Environ Sci. 2019;236(1):012122. doi:10.1088/1755-1315/236/1/012122
64. Sungkar OF, Fahmi AS, Romadhon. Enrichment of Skin Lotion with Antioxidant from *Rhizophora mucronata* Fruit Extract. IOP Conf Ser Earth Environ Sci. 2019;246:012065. doi:10.1088/1755-1315/246/1/012065
65. Karimi A, Krähmer A, Herwig N, Schulz H, Hadian J, Meiners T. Variation of Secondary Metabolite Profile of *Zataria multiflora* Boiss. Populations Linked to Geographic, Climatic, and Edaphic Factors. Front Plant Sci. 2020;11:969. doi:10.3389/fpls.2020.00969
66. Chaaban H, Ioannou I, Chebil L, et al. Effect of Heat Processing on Thermal Stability and Antioxidant Activity of Six Flavonoids. J Food Process Preserv. 2017;41(5):e13203. doi:10.1111/jfpp.13203

67. Sebestyén Z, Jakab E, Badea E, Barta-Rajnai E, Şendrea C, Czégény Zs. Thermal Degradation Study of Vegetable Tannins and Vegetable Tanned Leathers. J Anal Appl Pyrolysis. 2019;138:178-187. doi:10.1016/j.jaap.2018.12.022
68. Tanod WA, Cahyono E, Rieuwpassa FJ, et al. Enhancing Functional Rice Crackers with *Rhizophora mucronata* Leaves Origin Sangihe Islands: A Novel Approach to Boost Nutritional and Antioxidant Properties. Adv Food Sci Sustain Agric Agroind Eng (AFSSAAE). 2025;8(4):362-373. doi:10.21776/ub.afssaae.2025.008.04.3
69. Sibero MT, Sabdono A, Pribadi R, et al. Study of Biomedical Properties of *Rhizophora mucronata* Fruit from Rembang, Central Java. IOP Conf Ser Earth Environ Sci. 2020;584(1):012001. doi:10.1088/1755-1315/584/1/012001
70. Hardoko, Suprayitno E, Puspitasari YE, Amalia R. Study of Ripe *Rhizophora mucronata* Fruit Flour as Functional Food for Antidiabetic. Int Food Res J [Internet]. 2015;22(3):953-959. Available from: https://www.ifrj.upm.edu.my/22%20(03)%202015/(11).pdf
71. Sadeer NB, Rocchetti G, Senizza B, et al. Untargeted Metabolomic Profiling, Multivariate Analysis and Biological Evaluation of the True Mangrove (*Rhizophora mucronata* Lam.). Antioxidants. 2019;8(10):489. doi:10.3390/antiox8100489
72. Mhada M, Metougui ML, El Hazzam K, El Kacimi K, Yasri A. Variations of Saponins, Minerals and Total Phenolic Compounds Due to Processing and Cooking of Quinoa (*Chenopodium quinoa* Willd.) Seeds. Foods. 2020;9(5):660. doi:10.3390/foods9050660
73. Yang M, Hou CY, Hsu HY, et al. Enhancing Bioactive Saponin Content of *Raphanus sativus* Extract by Thermal Processing at Various Conditions. Molecules. 2022;27(23):8125. doi:10.3390/molecules27238125
74. Vittaya L, Charoendat U, Janyong S, Ui-eng J, Leesakul N. Comparative Analyses of Saponin, Phenolic, and Flavonoid Contents in Various Parts of *Rhizophora mucronata* and *Rhizophora apiculata* and Their Growth Inhibition of Aquatic Pathogenic Bacteria. J Appl Pharm Sci. 2022;12(11):111-121. doi:10.7324/JAPS.2022.121113
75. Vo Q V., Tam NM, Hieu LT, et al. The Antioxidant Activity of Natural Diterpenes: Theoretical Insights. RSC Adv. 2020;10(25):14937-14943. doi:10.1039/D0RA02681F
76. Hunyadi A. The Mechanism(s) of Action of Antioxidants: From Scavenging Reactive Oxygen/Nitrogen Species to Redox Signaling and The Generation of Bioactive Secondary Metabolites. Med Res Rev. 2019;39(6):2505-2533. doi:10.1002/med.21592
77. Somjai C, Siriwoharn T, Kulprachakarn K, Chaipoot S, Phongphisutthinant R, Wiriyacharee P. Utilization of Maillard Reaction in Moist-Dry-Heating System to Enhance Physicochemical and Antioxidative Properties of Dried Whole Longan Fruit. Heliyon. 2021;7(5):e07094. doi:10.1016/j.heliyon.2021.e07094

78. Langner E, Rzeski W. Biological Properties of Melanoidins: A Review. Int J Food Prop. 2014;17(2):344-353. doi:10.1080/10942912.2011.631253
79. Molina-Cortés A, Quimbaya M, Toro-Gomez A, Tobar-Tosse F. Bioactive Compounds as an Alternative for The Sugarcane Industry: Towards an Integrative Approach. Heliyon. 2023;9(2):e13276. doi:10.1016/j.heliyon.2023.e13276
80. Sadeer NB, Zengin G, Mahomoodally MF. Biotechnological Applications of Mangrove Plants and Their Isolated Compounds in Medicine-A Mechanistic Overview. Crit Rev Biotechnol. 2023;43(3):393-414. doi:10.1080/07388551.2022.2033682
81. den Brok MH, Büll C, Wassink M, et al. Saponin-Based Adjuvants Induce Cross-Presentation in Dendritic Cells by Intracellular Lipid Body Formation. Nat Commun. 2016;7(1):13324. doi:10.1038/ncomms13324
82. Huis in 't Veld LG, Cornelissen LA, van den Bogaard L, Ansems M, Ho NI, Adema GJ. Saponin-Based Adjuvant Uptake and induction of Antigen Cross-Presentation by CD11b+ Dendritic Cells and Macrophages. NPJ Vaccines. 2025;10(1):15. doi:10.1038/s41541-024-01056-y
83. Sueyoshi K, Ledderose C, Shen Y, Lee AH, Shapiro NI, Junger WG. Lipopolysaccharide Suppresses T cells by Generating Extracellular ATP that Impairs their Mitochondrial Function via P2Y11 Receptors. J Biol Chem. 2019;294(16):6283-6293. doi:10.1074/jbc.RA118.007188
84. Ogikubo Y, Norimatsu M, Sasaki Y, Yasuda A, Saegusa J, Tamura Y. Effect of Lipopolysaccharide (LPS) Injection on the Immune Responses of LPS-Sensitive Mice. J Vet Med Sci. 2004;66(10):1189-1193. doi:10.1292/jvms.66.1189
85. Hematianlarki M, Nimmerjahn F. Immunomodulatory and Anti-inflammatory Properties of Immunoglobulin G Antibodies. Immunol Rev. 2024;328(1):372-386. doi:10.1111/imr.13404
86. Wheeler ML, DeFranco AL. Prolonged Production of Reactive Oxygen Species in Response to B Cell Receptor Stimulation Promotes B Cell Activation and Proliferation. J Immunol. 2012;189(9):4405-4416. doi:10.4049/jimmunol.1201433
87. Lewis ED, Meydani SN, Wu D. Regulatory Role of Vitamin E in The Immune System and Inflammation. IUBMB Life. 2019;71(4):487-494. doi:10.1002/iub.1976
88. Kilicarslan You D, Fuwad A, Lee KH, et al. Evaluation of the Protective Role of Vitamin E Against ROS-Driven Lipid Oxidation in Model Cell Membranes. Antioxidants. 2024;13(9):1135. doi:10.3390/antiox13091135
89. Câmara JS, Perestrelo R, Ferreira R, Berenguer C V., Pereira JAM, Castilho PC. Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. Molecules. 2024;29(16):3861. doi:10.3390/molecules29163861

90. Masyita A, Mustika Sari R, Dwi Astuti A, et al. Terpenes and Terpenoids as Main Bioactive Compounds of Essential Oils, Their Roles in Human Health and Potential Application as Natural Food Preservatives. Food Chem X. 2022;13:1-14. doi:10.1016/j.fochx.2022.100217
91. Jadon K, Padhi S. Comprehension of the Function of Antioxidants in Targeting Different Signaling Pathways to Cure Oxidative Stress-Induced Hepatotoxicity. Curr Top Med Chem. 2025;25. doi:10.2174/0115680266398364250811231140
92. Xu L, Yu Y, Sang R, Li J, Ge B, Zhang X. Protective Effects of Taraxasterol against Ethanol-Induced Liver Injury by Regulating CYP2E1/Nrf2/HO-1 and NF- *κ* B Signaling Pathways in Mice. Oxid Med Cell Longev. 2018;2018(1):8284107. doi:10.1155/2018/8284107
93. Jairaman C, Alehaideb ZI, Yacoob SAM, et al. *Rhizophora mucronata* Lam. (Mangrove) Bark Extract Reduces Ethanol-Induced Liver Cell Death and Oxidative Stress in Swiss Albino Mice: *In Vivo* and *In Silico* Studies. Metabolites. 2022;12(11):1021. doi:10.3390/metabo12111021
94. Thorgersen EB, Barratt-Due A, Haugaa H, et al. The Role of Complement in Liver Injury, Regeneration, and Transplantation. Hepatology. 2019;70(2):725-736. doi:10.1002/hep.30508
95. OECD. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Publishing; 2025. doi:10.1787/9789264070707-en