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Hepatoprotective effects of ethanol extract of mobe leaves (*Artocarpus lacucha buch-ham.*) on liver enzymes and bilirubin levels in rats induced by carbon tetrachloride

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ABSTRACT

Liver diseases caused by toxic substances like CCl_4 (carbon tetrachloride) pose significant health risks, with limited effective therapies available. This study evaluates the hepatoprotective effects of ethanol extract of *Artocarpus lacucha* Buch.-Ham. (mobe leaves), known for their antioxidant properties. Mobe leaf extract was prepared using 96% ethanol. Thirty male rats were divided into six groups and treated with varying doses of the extract (50, 100, 200, and 400 mg/kg BW (body weight) or a control, followed by CCl_4 induction. Liver enzyme levels—ALT (alanine aminotransferase) and AST (aspartate aminotransferase) along with bilirubin levels, were measured to assess hepatotoxicity. Phytochemical analysis identified the bioactive compounds in the extract. Administering 400 mg/kg BW of the extract significantly reduced TB (total bilirubin), ALT, and AST levels in CCl_4-induced rats compared to controls. Phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, triterpenoids, and glycosides, which are potential hepatoprotective agents. The ethanol extract of *Artocarpus lacucha* (mobe leaves) shows significant hepatoprotective effects in rats, likely due to its antioxidant properties. This suggests its potential as a natural therapeutic agent for liver protection. Further research and clinical trials are recommended to confirm these findings.

Keyword: Hepatoprotective, Ethanol Extract of Mobe Leaves, Carbon Tetrachloride

ABSTRAK

Penyakit hati yang disebabkan oleh zat-zat toksik seperti CCl_4 (karbon tetraklorida) menimbulkan risiko kesehatan yang signifikan, dengan terapi efektif yang terbatas tersedia. Studi ini mengevaluasi efek hepatoprotektif dari ekstrak etanol daun Artocarpus lacucha Buch.-Ham. (daun mobe), yang dikenal dengan sifat antioksidannya. Ekstrak daun mobe disiapkan menggunakan etanol 96%. Tiga puluh tikus jantan dibagi menjadi enam kelompok dan diberi perlakuan dengan berbagai dosis ekstrak (50, 100, 200, dan 400 mg/kg BB (berat badan) atau kontrol, diikuti dengan induksi CCl_4. Kadar enzim hati—ALT (alanin aminotransferase) dan AST (aspartat aminotransferase) serta kadar bilirubin diukur untuk menilai hepatotoksisitas. Pemberian ekstrak 400 mg/kg BB secara signifikan mengurangi kadar TB (bilirubin total), ALT, dan AST pada tikus yang diinduksi CCl_4 dibandingkan dengan kontrol. Skrining fitokimia mengungkapkan adanya flavonoid, alkaloid, tanin, saponin, triterpenoid, dan glikosida yang merupakan agen potensial hepatoprotektif. Ekstrak etanol daun *Artocarpus lacucha* (daun mobe) menunjukkan efek hepatoprotektif yang signifikan pada tikus, kemungkinan besar disebabkan oleh sifat antioksidannya. Ini menunjukkan potensinya sebagai agen terapeutik alami untuk perlindungan hati. Penelitian lebih lanjut dan uji klinis direkomendasikan untuk mengonfirmasi temuan ini. Kata kunci: Hepatoprotektif, Ekstrak Etanol Daun Mobe, Karbon Tetraklorida.

Keyword: Hepatoprotektif, Ekstrak Etanol Daun Mobe, Karbon Tetraklorida

1. Introduction

The liver is vital for numerous metabolic processes, including the detoxification of biological and xenobiotic compounds. As a result, it is frequently exposed to harmful substances that can cause damage. Oxidative stress and inflammation are significant contributors to various liver diseases[1] [2]. Oxidative stress activates macrophages and triggers the release of pro-inflammatory cytokines, such as Nuclear Factor Kappa Beta (NF-κB), which regulates inflammatory mediators like interleukins (IL-1β, IL-6) and Tumor Necrosis Factor (TNF-α). This process promotes cell apoptosis through increased expression of Caspase 3 and 7, worsening liver disease [3].

Xenobiotic compounds, which are foreign to the body [[4], are metabolized in the liver, producing hydroxyl radicals as by-products [5] CCl_4, a commonly used xenobiotic, induces lipid peroxidation and liver cell damage. In the liver, CCl_4 undergoes biotransformation via the endoplasmic reticulum (cytochrome P450), producing CCl_3- (trichloromethyl) and Cl- radicals. These radicals cause lipid peroxidation, membrane damage, and increased leakage of liver enzymes such as ALT and AST, indicating liver damage ([6];[7]).

Despite advances in modern medicine, effective therapeutic approaches to protect liver function or enhance liver cell regeneration are lacking [8] Alternative therapies using medicinal plants play a significant role in human health through their preventive, curative, and rehabilitative properties [9]. Many medicinal plants are accessible, cost-effective, and environmentally friendly. Flavonoid compounds are known for their hepatoprotective activity [10], enhancing antioxidant defences, such as increased Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Glutathione (GSH) levels [11]These compounds combat free radicals and induce cellular stress defence systems, making plants with antioxidant and anti-inflammatory activities potential therapeutic strategies for liver damage [12]

Mobe leaves contain flavonoids, tannins, saponins, phenolics, steroids, and triterpenoids with anti-inflammatory, antidiabetic, antioxidant, antibacterial, and antifungal effects[13]. This plant is known for its phenolic derivatives, specifically di- or tri-oxygenated and isoprenylated flavones at the C3 position [14] The Artocarpus genus, which includes mobe, contains flavonoids that inhibit chemical mediators released from mast cells, neutrophils, and macrophages. The Artocarpus family also contains secondary metabolites beneficial as hepatoprotective agents [15]

Previous research tested the antioxidant activity of ethanol, ethyl acetate, and n-hexane extracts of mobe leaves using the ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] method. The antioxidant activity was measured with a spectrophotometer at 734 nm, showing IC_{50} values of 87.547 µg/ml for the ethanol extract (strong effect), 138.767 µg/ml for the ethyl acetate extract (moderate effect), and 558.094 µg/ml for the n-hexane extract (very weak effect).

This study aims to determine the hepatoprotective activity of ethanol extract of mobe leaves in rats induced with CCl_4. Oxidative stress is the main impact affecting hepatocytes exposed to CCl_4. SOD-1 is an antioxidant that inhibits free radicals causing lipid peroxidation in cell membranes, followed by the release of liver enzymes (ALT, AST) and a decrease in TB levels, biomarkers of liver damage. Liver damage due to CCl_4 can be confirmed through histological examination of the liver. This research enhances understanding of mobe leaves as medicinal plants with hepatoprotective potential, offering a safer, easily accessible, and affordable alternative treatment. For researchers, these findings open opportunities for further studies to develop standardized herbal preparations based on mobe leaf extract, contributing to pharmacology and herbal medicine development.

2. Method

2.1 Research Location

This study was conducted in several laboratories at the University of North Sumatra, including the Laboratory of Pharmaceutical Biology, University Hospital Laboratory, and the Histology Laboratory at the Faculty of Medicine, Universitas Sumatera Utara.

2.2 Equipment and Materials

Equipment used included laboratory glassware, analytical balance, drying oven, rotary evaporator, centrifuge, syringes, and surgical tools. Materials included mobe leaves (*Artocarpus lacucha* Buch-Ham.), silymarin, CCl_4, corn oil, CMC-Na (Sodium Carboxymethyl Cellulose), and various reagents for phytochemical screening and analysis.

2.3 Plant Material Collection and Identification

Mobe leaves were purposively collected from Laguboti District, North Sumatra Province, and identified at the MEDA (Herbarium Medanese), Universitas Sumatera Utara.

2.4 Preparation of Mobe Leaf Simplicia and Ethanol Extract
Fresh mobe leaves were cleaned, air-dried, and dried in an oven at ±40°C. The dried leaves were ground into a powder and stored. The ethanol extract was prepared by soaking the powdered leaves in 96% ethanol for 24 hours with occasional stirring [16]. The filtrate was concentrated using a rotary evaporator to obtain a thick extract.

2.5 Experimental Animals and Treatment
Thirty male rats, averaging 200 grams, were divided into six groups:

1. EEML (Ethanol Extract Mobe Leaves) 400 mg/kg Body Weight (BW) + CCl_4
2. EEML 200 mg/kg BW + CCl_4
3. EEML 100 mg/kg BW + CCl_4
4. EEML 50 mg/kg BW + CCl_4
5. Silymarin 100 mg/kg BW + CCl_4 (positive control)
6. Na CMC 1% + CCl_4 (negative control)

The rats were acclimatized for one week before treatment. After 14 days, the rats were sacrificed, and blood was drawn for liver biomarker analysis.

2.6 Biomarker Measurement
Total bilirubin levels were measured using a photometric method with Vanadate Oxidating (VOX) reagent at 450 nm and 37ºC. ALT and AST levels were measured using a Portable Microlab 300 LX photometer.

2.7 Data Analysis
Data were analyzed using SPSS IBM 26 software with one-way Analysis of Variance (ANOVA). A p-value <0.05 was considered significant ($\alpha = 0.05$).

3. Results and Discussion

3.1 Plant Identification
The identification of the plant was conducted at the MEDA at the Universitas Sumatera Utara, confirming that the plant used was Mobe (*Artocarpus lacucha* Buch-Ham.). This identification supports the validity of the specimen used in the study.

3.2 Preparation of Ethanol Extract of Mobe Leaves
This study employed the maceration extraction technique using 96% ethanol as the solvent. The extraction process yielded a thick extract, which was weighed to calculate the yield percentage. The yield of the ethanol extract was 14.28%, indicating the efficiency of the extraction procedure.

3.3 Characterization of Simplicia
Characterization of simplicia determined the physical and chemical properties of the mobe leaf powder. The results showed a water content of 5.32%, water-soluble extract content of 12.82%, ethanol-soluble extract content of 12.27%, total ash content of 7.19%, and acid-insoluble ash content of 0.68%, all meeting the standards of the Indonesian Herbal Pharmacopoeia [17].

3.4 Phytochemical Screening of Simplicia and Extract
Phytochemical screening identified secondary metabolites in the simplicia and ethanol extract of mobe leaves. The results indicated the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids, and glycosides.

3.5 Health Research Ethics Approval
This study involved male white rats (200-240 grams) obtained from the Pharmacology Laboratory at the University of North Sumatra. The rats were housed in ventilated cages with controlled temperature and humidity were given pellet feed and water. They were acclimatized for seven days before the experiment. All experimental procedures were approved by the animal research ethics committee of FMIPA – University of North Sumatra (No. 0226/KEPH-F-MIPA/2023) according to the "Guide for the Care and Use of Laboratory Animals."

3.6 Hepatoprotective Activity Testing of Ethanol Extract of Mobe Leaves Measurement of Liver Damage Biomarkers

Liver damage biomarkers were evaluated by measuring TB, ALT, and AST in blood serum. Increased levels of TB, ALT, and AST in the blood indicate liver damage [18]. TB, ALT, and AST were measured on day 15 after the rats were given CCl_4 1 ml/kg BW. Blood was drawn from the heart and serum was separated for analysis using commercial kits. Data were analyzed using one-way ANOVA and Turkey Post-Hoc tests with SPSS software.

3.7 Total Bilirubin Measurement in Rat Serum

Elevated total bilirubin levels in the serum of rats given CCl_4 result from increased cell membrane permeability caused by hepatocyte damage [19]. One-way ANOVA analysis showed significant differences in total bilirubin levels among treatment groups ($p < 0.05$). The mean total bilirubin levels are shown in Table 1.

Table 1. Mean Total Bilirubin Levels

Group	Mean TB (µmol/L) ± SD
EEML (Ethanol Extract Mobe Leaves) 400 mg/kg BW + CCl_4 1 ml/kg BW (Body Weight)	0.15 ± 0.00ab
EEML 200 mg/kg BW + $CCl_4$1 ml/kg BW	0.18 ± 0.00ab
EEML 100 mg/kg BW + $CCl_4$1 ml/kg BW	0.19 ± 0.00ab
EEML 50 mg/kg BW + $CCl_4$1 ml/kg BW	0.22 ± 0.01ab
Silymarin 100 mg/kg BW + $CCl_4$1 ml/kg BW	0.14 ± 0.00a
Na CMC 1% + $CCl_4$1 ml/kg BW	0.27 ± 0.00b

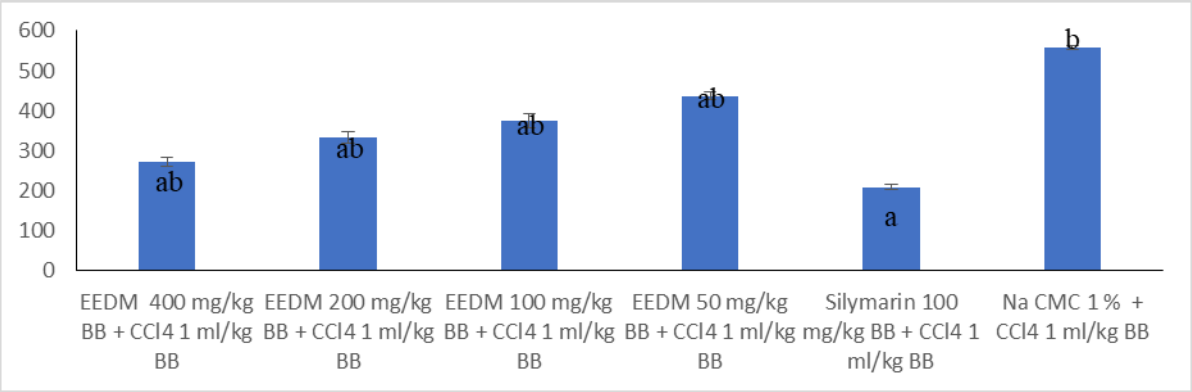

Figure 1. Figure 1 illustrates the effect of EEDM on total bilirubin levels. The Effect of EEDM on TB a. Sig (P) < 0.05 indicates a significant difference compared to the negative control group. b. Sig (P) < 0.05 indicates a significant difference compared to the Silymarin control group.

Groups with "ab": The ethanol extract of mobe leaves (EEML) at different doses (400, 200, 100, and 50 mg/kg BW) show similar effects and are not significantly different from each other or from the Silymarin group but may differ from the Na CMC group.

Group with "a" only: The Silymarin group is not significantly different from the EEML groups but is different from the Na CMC group.

Group with "b" only: The Na CMC group has significantly higher TB levels compared to the Silymarin group and potentially different from the EEML groups.

3.8 Alanine Aminotransferase Measurement in Rat Serum

Increased ALT levels in blood serum indicate liver damage [20]One-way ANOVA showed significant differences in ALT levels among treatment groups ($p < 0.05$). The mean ALT levels are shown in Table 2.

Table 2. Mean ALT Levels

Group	Mean ALT (U/L) ± SD
EEML 400 mg/kg BW + $CCl_4$1 ml/kg BW	255 ± 7.97ab
EEML 200 mg/kg BW + $CCl_4$1 ml/kg BW	286 ± 13.50ab

Group	Mean ALT (U/L) ± SD
EEML 100 mg/kg BW + $CCl_4$1 ml/kg BW	335.60 ± 13.94ab
EEML 50 mg/kg BW + $CCl_4$1 ml/kg BW	427.80 ± 7.46ab
Silymarin 100 mg/kg BW + CCl_4 1 ml/kg BW	224.60 ± 7.80a
Na CMC 1% + $CCl_4$1 ml/kg BW	505.20 ± 5.26b

3.9 Aspartate Aminotransferase Measurement in Rat Serum

Increased AST levels in serum indicate liver cell or tissue damage [20]One-way ANOVA showed significant differences in AST levels among treatment groups ($p < 0.05$). The mean AST levels are shown in Table 3.

Table 3. Mean AST Levels

Group	Mean AST (U/L) ± SD
EEML 400 mg/kg BW + $CCl_4$1 ml/kg BW	272.00 ± 12.04ab
EEML 200 mg/kg BW + $CCl_4$1 ml/kg BW	333.80 ± 13.48ab
EEML 100 mg/kg BW + $CCl_4$1 ml/kg BW	375.40 ± 17.01ab
EEML 50 mg/kg BW + $CCl_4$1 ml/kg BW	437.40 ± 9.81ab
Silymarin 100 mg/kg BW + $CCl_4$1 ml/kg BW	209.20 ± 5.67a
Na CMC 1% + $CCl_4$1 ml/kg BW	557.40 ± 5.67b

4. Discussion

CCl_4 is a toxic chemical metabolized in the liver by cytochrome P450 2E1 into trichloromethyl radicals, which react with oxygen to form highly reactive trichloromethylperoxy radicals, leading to increased ROS (reactive oxygen species) and decreased antioxidant defences. This causes lipid peroxidation, mitochondrial damage, DNA damage, and hepatocyte necrosis, indicated by changes in liver color and surface [21]

The hepatoprotective effects of the ethanol extract of mobe leaves (*Artocarpus lacucha* Buch-Ham.) were evaluated by measuring the levels of serum biomarkers: ALT, AST, and bilirubin. These biomarkers are commonly used to assess liver function and damage.

ALT and AST Levels

ALT and AST are enzymes found predominantly in the liver. When the liver is damaged, these enzymes are released into the bloodstream, making their levels a reliable indicator of hepatocellular injury. In this study, rats treated with CCl_4 exhibited significantly elevated levels of ALT and AST, indicating severe liver damage induced by oxidative stress and inflammation.

Treatment with the ethanol extract of mobe leaves showed a significant reduction in both ALT and AST levels compared to the CCl4-treated control group. This reduction suggests that the extract effectively mitigated the hepatocellular damage caused by CCl4. The hepatoprotective effect observed can be attributed to the presence of bioactive compounds in the mobe leaves, which likely possess antioxidant and anti-inflammatory properties. These compounds may help stabilize cell membranes, reduce oxidative stress, and inhibit inflammatory pathways, thereby protecting hepatocytes from damage.

Bilirubin Levels

Bilirubin is a byproduct of the breakdown of red blood cells and is processed by the liver. Elevated levels of bilirubin in the blood indicate impaired liver function and the inability of the liver to properly process and excrete bilirubin. In this study, CCl4-induced hepatotoxicity led to a significant increase in serum bilirubin levels, reflecting severe liver dysfunction.

The administration of mobe leaf extract resulted in a notable decrease in bilirubin levels in treated rats. This decrease indicates an improvement in liver function and suggests that the extract helps restore the liver's ability to process and excrete bilirubin. The reduction in bilirubin levels further supports the hepatoprotective potential of the ethanol extract of mobe leaves, highlighting its role in enhancing liver function and preventing the accumulation of toxic bilirubin

5. Conclusion

This study shows that the ethanol extract of mobe leaves (*Artocarpus lacucha* Buch-Ham.) has significant hepatoprotective effects in rats induced with CCl_4. Administration of EEML (Ethanol Extract Mobe Leaves) at a dose of 400 mg/kg BW effectively reduced total bilirubin, ALT, and AST levels in blood serum and

improved the macropathological condition of the liver, approaching normal conditions like those in the positive control group with silymarin. These results indicate that the ethanol extract of mobe leaves has the potential as a therapeutic agent to protect the liver from damage caused by toxic substances like CCl_4.

Recommendations

Based on the results of this study, further research is recommended on the molecular mechanisms of the hepatoprotective effects of the ethanol extract of mobe leaves. Additionally, clinical trials on humans are needed to evaluate the safety and efficacy of this extract as herbal medicine. Developing herbal products based on the ethanol extract of mobe leaves could offer a natural, affordable, and environmentally friendly alternative for liver disease therapy.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper. All research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

[1] W. Dröge, “Free radicals in the physiological control of cell function,” 2002, *American Physiological Society*. doi: 10.1152/physrev.00018.2001.

[2] A. Allameh, R. Niayesh-Mehr, A. Aliarab, G. Sebastiani, and K. Pantopoulos, “Oxidative Stress in Liver Pathophysiology and Disease,” Sep. 01, 2023, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/antiox12091653.

[3] C. Chen *et al.*, “HbA1c may contribute to the development of non-alcoholic fatty liver disease even at normal-range levels,” *Biosci Rep*, vol. 40, no. 1, Jan. 2020, doi: 10.1042/BSR20193996.

[4] I. Putu *et al.*, “Metabolism of Xenobiotic Compounds Increases Free Radical Production and Reduces Endogenous Antioxidant Mechanisms,” 2023.

[5] S. Dalimartha, *Ramuan tradisional untuk pengobatan hepatitis*. Jakarta: Penebar Swadaya, 1999.

[6] Chodidjah, E. Widayati, and Utari, “Pengaruh pemberian air rebusan meniran (Phyllanthm niruriLinn) terhadap gambaran histopatologi hepar tikus wistar yang terinduksi CCL4,” *JurnalAnatomi Indonesia*, vol. 2, no. 1, pp. 8–12, 2007.

[7] M. B. Hariyanto, *Pengaruh pemberian buah melon (Cucumis melo L.) personde terhadap gambaran histopatologi zona sentrilobular hepar tikus putih (Rattus norvegicus) galur Wistar yang diinduksi CCl4 (karbon tetraklorida)*. Malang: Universitas Brawijaya, 2013.

[8] E. Madrigal-Santillán *et al.*, “Review of natural products with hepatoprotective effects,” Oct. 28, 2014, *WJG Press*. doi: 10.3748/wjg.v20.i40.14787.

[9] A. Jamal, “International Journal of Multidisciplinary Sciences and Arts Embracing Nature’s Therapeutic Potential: Herbal Medicine,” vol. 2, no. 1, 2023, doi: 10.47709/ijmdsa.vxix.xxxx.

[10] M. Sahlan *et al.*, “Potential hepatoprotective effects of flavonoids contained in propolis from South Sulawesi against chemotherapy agents,” *Saudi J Biol Sci*, vol. 28, no. 10, pp. 5461–5468, Oct. 2021, doi: 10.1016/j.sjbs.2021.08.022.

[11] R. Domitrović, H. Jakovac, and G. Blagojević, “Hepatoprotective activity of berberine is mediated by inhibition of TNF-α, COX-2, and iNOS expression in CCl4-intoxicated mice,” *Toxicology*, vol. 280, no. 1–2, pp. 33–43, 2011, doi: https://doi.org/10.1016/j.tox.2010.11.005.

[12] F. Tacke, T. Luedde, and C. Trautwein, “Inflammatory pathways in liver homeostasis and liver injury,” *Clin Rev Allergy Immunol*, vol. 36, no. 1, pp. 4–12, 2009, doi: 10.1007/s12016-008-8091-0.

[13] O. A. Hanafiah *et al.*, “Quantity differences of secondary metabolites (Saponins, tannins, and flavonoids) from binahong plant extract (Anredera cordifolia (Ten.) Steenis) treated and untreated

with colchicines that play a role in wound healing," *World Journal of Dentistry*, vol. 8, no. 4, pp. 296–299, Jul. 2017, doi: 10.5005/jp-journals-10015-1453.

[14] I. Indarto, "Isolasi dan Identifikasi Senyawa Fenolik dari Kulit Akar Tumbuhan Artocarpus Dadah Miq 63-74," *Jurnal Ilmiah Pendidikan Fisika Al-Biruni*, vol. 4, no. 2, pp. 205–217, Oct. 2015, doi: 10.24042/jpifalbiruni.v4i2.93.

[15] A. Pandey and S. . P. Bhatnagar, "Preliminary phytochemical screening and antimicrobial studies on Artocarpus lookcha Roxb," *Journal Acient Science of Life*, vol. 28, no. 4, pp. 21–24, 2009.

[16] M. Yunita, M. Ohiwal, M. Z. Elfitrasya, and H. Rahawarin, "Antibacterial activity of Paederia foetida leaves using two different extraction procedures against pathogenic bacteria," *Biodiversitas*, vol. 24, no. 11, pp. 5920–5927, 2023, doi: 10.13057/biodiv/d241110.

[17] K. Kartini, N. I. E. Jayani, N. D. Octaviyanti, A. H. Krisnawan, and C. Avanti, "Standardization of Some Indonesian Medicinal Plants Used in 'Scientific Jamu,'" in *IOP Conference Series: Earth and Environmental Science*, Institute of Physics Publishing, Dec. 2019. doi: 10.1088/1755-1315/391/1/012042.

[18] W. Li *et al.*, "Associations of blood metals with liver function: Analysis of NHANES from 2011 to 2018," *Chemosphere*, vol. 317, Mar. 2023, doi: 10.1016/j.chemosphere.2023.137854.

[19] G. Shanmugam, M. Ayyavu, and D. M. Rao, "Hepatoprotective effect of Caralluma umbellate against acetaminophen induced oxidative stress and liver damage in rat," *J Pharm Res*, vol. 6, no. 3, pp. 342–345, 2013, doi: https://doi.org/10.1016/j.jopr.2013.03.009.

[20] S. Yadav, R. Jangra, B. R. Sharma, and M. Sharma, "Current Advancement in Biosensing techniques for determination of Alanine aminotransferase and Aspartate aminotransferase-a Mini Review," Mar. 01, 2022, *Elsevier Ltd*. doi: 10.1016/j.procbio.2022.01.010.

[21] W. Cahaya Widya Putri and H. Rahman, "Uji Aktivitas Hepatoprotektor Ekstrak Etanol Daun Rambutan (Nephelium lappaceum L.) pada Mencit Putih Jantan yang Diinduksi Parasetamol Hepatoprotective Activity of Rambutan Leaf (Nephelium lappaceum L.) Ethanol Extract in Male Mice Induced by Paracetamol," 2021. [Online]. Available: http://journals.ums.ac.id/index.php/pharmacon