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Liver Protection Efficacy of Lemon Pepper Fruit's Methanol Extract Against Cadmium-Induced Rats

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Abstract: ***Studies had been performed to investigate various natural sources to look for liver protection effects against cadmium toxicity when the usage of cadmium in everyday life is high. Thus, this study was performed to investigate liver protection effects from a lemon pepper fruit against cadmium toxicity. Twenty-five male Wistar rats were grouped into five groups. Control (0.5% Na-CMC), standard, Lemon Pepper methanol extract (LPME)- I, II, and III were given a milliliter of 0.5% Na-CMC, 25 mg/kg BW quercetin, 300, 600, and 1, 200 mg/kg BW of LPME, respectively. The intervention was performed for 14 days, and cadmium solution was given at the last seven days. After 14 days, all rats were sacrificed for liver function tests and liver histology study. The best liver protection effect was shown at the highest dose than other doses; the highest dose of lemon pepper methanol extract decreased 41.10% ALT level and 59.39% AST level compared to control groups. The results were also supported by improvement of liver structure with minimal ballooning degeneration at the highest dose group. Overall, it can be concluded that the lemon pepper methanol extract at dose of 1, 200 mg/ kg BW had liver protection effects against cadmium toxicity.***

Keywords: ALT, AST, Liver, Cadmium, Lemon Pepper

1. Introduction

Cadmium is a commonly used toxic heavy metal which serves mostly as corrosion-resistant material. Cadmium occurs so extensively that exposure includes occupational such as battery manufacturing, zinc mining, welding, and daily environmental exposure such as smoking cigarettes, kids' second hand painted toys and consuming contaminated food or beverages [1]. While this metal is vastly exposed, it has long biological half-life period in human body of up to several decades (approximately 20-30years)[2]. The long-term effects of this metal are tumor formation, lung, liver, or kidney injury, and chronic toxicity like nephrotoxicity, immunotoxicity, and osteotoxicity [3, 4].

Omar Hyder et al analysed urinary cadmium in 12, 732 US general population adults found significant association between cadmium exposure and hepatic necroinflammation, non-alcoholic fatty liver disease, and non-alcoholic steatohepatitis. In addition, the top quartile urinary cadmium individuals showed threefold increased risk of liver disease mortality [5]. The debilitating effect of cadmium on kidney is well documented, however, few studies have focused on the liver.

Studies on mice by Li X et al (2021) showed chronic oral exposure to cadmium is significantly linked to liver inflammation by NLRP3 inflammasome activation and Xu Y et al (2021) found that a mere exposure at environmental-relevant level accelerated the development of hepatotoxicity to hepatocarcinogenesis [6, 7]. Based on this information, cadmium was as obvious cause of liver damage and it becomes important to look for an alternative drug to neutralize the toxicity of the cadmium that is so widely present.

As a tropical region, Indonesia has numerous herbs that can be used as traditional medicine. Around 15.5% of world plant species can be found in Indonesia. One of these herbs is lemon pepper fruit famously used in Batak Community. The utility of lemon pepper extract as herbs are based on the culture's knowledge with minimal scientific evidence, and this herbs' specific usage and safety hasn't been fully uncovered [8-10]. Hence this study was performed to explore the pharmacological effects of the lemon pepper fruit as a local natural product and its protection effect on liver tissue.

Lemon pepper fruit is reported to contain phytochemicals such as phenols, saponins, flavonoids, triterpenoids, and alkaloids [11]. Moreover, Winarti et al. (2018) reported that lemon pepper ethyl acetate and n-hexane extract contained 2-methoxy-4-vinilfenol responsible for the antioxidant effect; lemon pepper ethyl acetate and n-hexane extract have antioxidant effects by DPPH Scavenging activity with IC50 Value 66.91 ppm and 135.58 ppm, respectively. Negi et al. (2012) also reported a similar result; lemon pepper essential oil has antioxidant activity with IC_{50} Value 27.0 ± 0.1 µg/ml and moderate antibacterial activity. Other pharmacological effects reported from lemon pepper fruits are anti-inflammatory and antidiabetic. Yanti et al. (2011) reported that the lemon pepper ethanol extract could significantly inhibit the expression of several types of

inflammatory biomarkers at the level of protein synthesis (TNF-α, COX-2 protein, and MMP-9) and genes (TNF-α, IL-6, iNOS, COX-2, and MMP-9) in lipopolysaccharide-induced macrophages by in vitro study. Chiuman et al. (2021) also reported that the lemon pepper ethanol extract had a liver protection effect against Non-Alcoholic Fatty Liver Disease (NADLF) in diabetic conditions [12-18].

These previous studies were performed in search for lemon pepper fruit's antioxidant and anti-inflammatory effects. However, none of these studies investigates liver protection effect of lemon pepper extract against hazardous substances exposed in daily life. Hence, the objective of this study is to investigate the effectiveness of different dosages of lemon pepper methanol extract in protecting the liver tissue from cadmium-induced liver injury wistar rats and as well comparing this local natural product's effectivity with the standard quercetin.

2. Methods

A. Study Design

This experimental study used Post Test Only Control Group Design in Pharmacology Laboratory, Universitas Prima Indonesia from May-July 2021. The Health Research Ethics Committee approved this study protocol from Universitas Prima Indonesia with Letter No. 006/KEPK/UNPRI/V/2021.

B. Materials

Materials used in this study included: Lemon peppers, distilled water, phytochemicals screening reagents, 10% formalin buffer, sodium Carboxyl Methyl Cellulose (Na-CMC) powder, chloroform, methanol, Cadmium, Dialab® ALT, and AST kit reagent, toluene, dye powder (hematoxylin and eosin).

C. Extraction Process

Lemon peppers were obtained from a traditional market in Medan city. After that, these lemon peppers were identified at the Herbarium Medanense (MEDA) in the Faculty of Sciences and Mathematics, Universitas Sumatera Utara. They are then dried in a drying cabinet and weighed. After that, these dry lemon peppers were ground to form a simplicia powder.

Maceration methods extracted all simplicial powder. This simplicial powder was soaked into 98% methanol with a ratio of 1:10, and it was stirred regularly. After that, it was filtered, and this process was repeated two times. Then the obtained filtrate was evaporated by a rotary evaporator at 50^{o}C to form a concentrated extract [19].

D. Phytochemical Screening

The obtained concentrated extract underwent phytochemical screening based on Fansworth methods with some modifications. The phytochemical screening identified phenols, flavonoids, alkaloids, steroids/triterpenoids, terpenoids, saponins, and tannins [20-22].

E. Formulation of Cadmium Solution

The cadmium dose in this study was based on the cadmium chloride dose that has been described by Prabu et al. (2012). The Median Lethal Dose (LD_{50}) value from cadmium chloride was 75 mg/ kg BW. Fifteenth of LD_{50} value was used as the induction dose that was 5 mg/ kg BW. Due to unavailability, this study used cadmium sulphate instead of cadmium chloride, as described in the previous study. Based on the molecular structure and relative molecular weight, 5 mg/ kg BW of cadmium chloride was equal to 7 mg/ kg BW of cadmium sulphate [23]. According to the cadmium dose, the Cadmium solution (5 mg/ ml) was made of 0.5 grams hydrate $3CdSO_4.8H_2O$ (cadmium sulphate) in 100 ml distilled water.

F. Formulation of Oral Suspension

This study also formulated 0.5% Na-CMC as a dispersion medium other than cadmium solution. Half grams of Na-CMC were spread into a surface of one-tenth from total hot distilled water for a quarter-hour to make a transparent mass. Then, it had been grounded to make a gel and diluted by the remaining (one-ninth) distilled water; it had been used as a vehiculum for either extract or standard drug (quercetin). Moreover, 1.2 grams of lemon pepper methanol extract and 250 mg quercetin were ground into 10 ml of 0.5% Na-CMC suspension to make lemon pepper methanol extract and quercetin suspension, respectively [24, 25].

G. Intervention

Twenty-five male Wistar rats were grouped into five different groups: control (1ml NaCMC 0.5%), standard (quercetin 25mg/kg BW), lemon pepper methanol extract-I (300 mg/ kg BW), II (600 mg/ kg BW), and III (1, 200 mg/ kg BW). After diluting into suspension, the control received 1ml of 0.5% Na CMC, standard received 1 ml/ kg BW quercetin, LPME-I received 2.5 ml/ kg BW, LPME-II received 5 ml/ kg BW, and LPME-III received 10 ml/ kg BW of lemon pepper methanol extract suspension once each day for seven days. After these 7 consecutive days, the study continues for another 7 days where all rats were still given the same ingredients as their initial 7 days but with additional intervention of 1.4 ml/ kg BW of cadmium solution. This procedure was based on the protection assay described by Chiuman et al. that also looked for the protection effect against other heavy metal substances. This study was given the extract at the first week to form liver protection in the body, and the next week the formed liver protection will prevent the cadmium toxicity, and the additional extract is still given to prevent the decreased liver protection effects against the cadmium toxicity [19].

After fourteen days, all rats were sacrificed with chloroform inhalation at the end of the intervention. Then, these rats were fixed into paraffin block and vertically

incised from thorax to abdomen region for blood collection and liver dissection.

Blood was directly drawn from the heart by an intracardiac puncture using a 5 ml syringe with a 25-G Needle. After that, this blood was collected into a red-colored blood tube. Then, all blood tubes were centrifuged at 3000 rpm for a quarter-hour to obtain serum at the upper layer. Meanwhile, the liver tissue was obtained by direct dissection of liver tissue in the right-upper quadrant abdomen, and this tissue was reserved into a 10% buffer solution.

The obtained serum was used for the liver function test. This test was used to determine ALT and AST levels by Enzymatic reaction methods, and this test was performed based on its manufacture instruction (Dyasis ®). Meanwhile, the obtained liver tissue was sliced with a 4-6 mm thickness. After that, these slices were stained by hematoxylin and eosin (H&E) and observed under a microscope. The liver histology evaluated ballooning degeneration, inflammation, apoptotic cells, and fibrosis. The evaluation of each of these parameters was described in Table I.

Table 1: Liver Tissue Histology Scoring System (24)

Parameter	Description	Score
Ballooning degeneration	Not found	0
	Minimal enlargement of some hepatocytes	1
	Mild enlargement of many hepatocytes.	2
	Moderate enlargement of most hepatocytes	3
	Severe enlargement of most hepatocytes	4
Inflammation	No foci of inflammation	0
	An inflammatory focus per 200 large visual fields	1
	2-4 inflammatory foci per 200 large visual fields	2
	> 4 foci of inflammation per 200 large visual fields	3
Apoptotic cells	None	0
	Some apoptotic cells	1
Fibrosis	No fibrosis	0
	Minimal portal/sinusoidal	1
	Mild portal/sinusoidal	2
	Bridging fibrosis	3
	Cirrhosis	4

H. Data Analysis

All data were analyzed by a descriptive statistic, Central tendency, and dispersion. After that, One-Way Anova analyzed ALT and AST, followed by Post Hoc Tukey HSD.

3. Results

Lemon pepper fruit was obtained and identified as Zanthoxylum acanthopodium. Based on the identification from Herbarium Medanense (MEDA), lemon pepper division, class, ordo, and family are spermatophyte, dicotyledonous, Sapindales, and Rutaceae, respectively. After that, 500 grams of lemon pepper fruit was dried into 212 grams of simplicia powder, and this simplicial powder was soaked in 2120 millilitres methanol as the solvent. At the end of the extraction process, it formed 15.55 grams of a concentrated extract. Hence, the yield extract was 7.33%. This study also performed a phytochemical screening, and the screening showed that lemon pepper methanol extract had some phytochemicals. The phytochemicals found were Alkaloids (Mayer's test, Wagner's test), Flavonoids (FeCl3 5% test), Tannins (FeCl3 1%), Steroids, and Terpenoids (Liberman Bouchard test).

The liver function test and liver histology study evaluated liver protection effects from lemon pepper methanol extract. The liver function test was analyzed the ALT and AST levels from all rats, and the liver function test result was described in Table II.

Table 2: Comparison of Liver Function Test in All Rats

Group	ALT (IU/L)	AST (IU/L)
Control	730 (697-741)[a]	165 (129-168)[a]
Standard	395 (200-421)[b]	59 (57-61)[b]
Lemon Pepper Methanol Extract-I	680 (671-691)[c]	115 (108-120)[c]
Lemon Pepper Methanol Extract-II	585 (550-633)[d]	95 (70-98)[d]
Lemon Pepper Methanol Extract-III	430 (425-578)[e]	67 (62-68)[e]
P-Value	**< 0.05**	**< 0.05**

Data are expressed as Median (Min-Max), and P-Value was obtained from the analysis of Kruskal-Wallis; Different superscripts in the same column indicate a significant difference.

Based on Table II, there is a significant difference in ALT and AST levels of rats. The control groups showed the highest ALT level, 740 IU/L, and the lowest was the standard group, which was 395 IU/L. Meanwhile, the lemon pepper methanol extract groups showed a decrease in ALT level followed by the increase of lemon pepper

methanol extract dose. These results were supported by ALT and AST p-value lower than 0.05. However, these ALT levels were not lower than the standard group.

On the other hand, the AST level also showed a similar result with the ALT level. The control group showed the highest AST level, 165 IU/L, and the lowest was the standard group, 59 IU/L. Meanwhile, among the lemon pepper methanol extract, the highest AST level among lemon pepper was shown by the lowest dose, which was lemon pepper methanol extract-I (115 IU/L), followed by moderate dose, which was lemon pepper methanol extract-II (95 IU/L), and the lowest one was the highest dose which was lemon pepper methanol extract-III (67 IU/L). The liver histology study supported the liver function test result, and the result of histology was described in Table III and Fig 1.

Table 3: Comparison of Liver Histology Score in All Groups

Group	Histological Parameter				Total Score
	Ballooning Degeneration	Inflammatory	Apoptotic cells	Fibrosis	
Control	4 (4-4)	3 (2-3)	1 (0-1)	4 (3-4)	11 (11-12)
Standard	3 (2-3)	1 (1-1)	0 (0-0)	1 (0-1)	4 (2-5)
Lemon Pepper Methanol Extract-I	3 (3-3)	2 (2-3)	1 (1-1)	3 (2-3)	9 (8-10)
Lemon Pepper Methanol Extract-II	3 (3-3)	0 (0-1)	1 (1-1)	4 (3-4)	8 (7-9)
Lemon Pepper Methanol Extract-III	2 (2-2)	2 (1-2)	1 (0-1)	1 (1-1)	5 (4-6)

Based on Table II, the control groups showed the highest total score, and standard groups were the lowest. The higher total score indicates more severe liver tissue damage. The most severe liver tissue damage was found in the control groups, and the mildest liver tissue damage was found in the standard groups. The most common pathology damage in the control group was ballooning degeneration and fibrosis. On the other hand, the lemon pepper methanol extract showed to have corresponding result with liver function test. The lowest dose of lemon pepper extract showed the highest total score, followed by moderate and lowest dose. Microscopic view of liver tissue from all groups is described in Figure 1.

Based on Fig 1, the most severe damage can be seen in the control group, followed by lemon pepper methanol extract-I, II, III, and standard group. The control and lemon pepper methanol extract-I group showed some ballooning degenerations in the liver tissue that indicated severe liver tissue damage. These ballooning degenerations replace the normal liver tissue structure. Meanwhile, the other groups showed milder tissue damage with fewer ballooning degenerations; however, the other groups showed some other pathology changes like apoptotic cells and infiltration of inflamed cells that indicate various degrees of inflammation.

4. Discussions

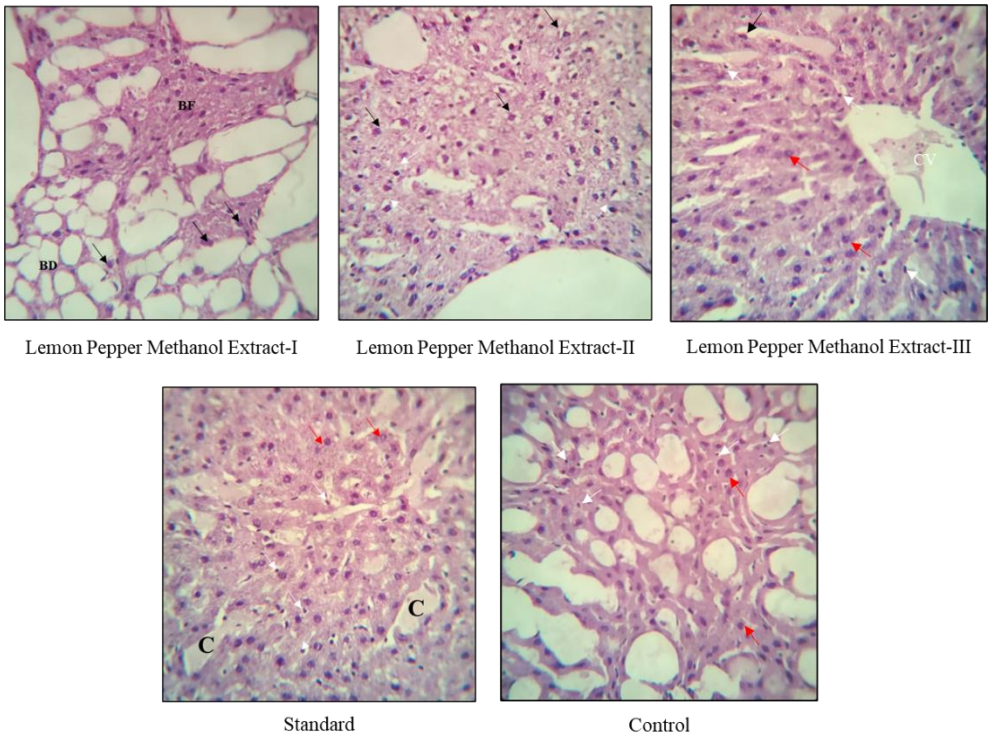

Figure 1: Histology View of Liver Tissues in All Groups. Stain: H&E. Magnification: 40x. BD: Ballooning degeneration; BF: Bridging Fibrosis; C: Congestion; Black Pointer: Apoptotic Cells; White Pointer: Inflammatory cells; Red pointer: Hepatocytes

The results of this showed that the highest lemon pepper methanol extract dose could significantly protect the liver tissue from cadmium toxicity; it was shown by the significant decrease of AST and ALT levels. The decrease of ALT and AST levels were in line with the improvement of the liver tissue structure. Moreover, this study also showed that the lemon pepper extract provides liver protection effect compared to the control group, which did not receive any interventions. Although the lemon pepper extract may give significant protection at the highest dose, this protection was exceeded by the standard group which received quercetin.

The administration of lemon pepper methanol extract significantly decreased the levels of ALT and AST. These decrease indicated liver protection effectiveness of the lemon pepper methanol extract. This protective effect is associated with the phytochemical content in lemon pepper methanol extract. The phytochemical screening showed that lemon pepper methanol extract contained alkaloids, flavonoids, tannins, steroids, and terpenoids. A flavonoid is a group of polyphenolic compounds. Thus, flavonoid has a hydroxyl group (OH) that can neutralize free radicals (antioxidants) in the body; cadmium is one of these free radicals. Zein et al. (2010) reported that Garcia mangostana contains flavonoids responsible for its antioxidant effect by neutralizing several heavy metals, one of which was cadmium. Moreover, Duan et al. (2018) also reported that Vitamin E and metallothionein could neutralize cadmium toxicity [26-28].

Although many studies have been performed to investigate the liver protection effect of various natural ingredients on cadmium toxicity, no studies specifically investigated the liver protection effect of lemon pepper fruit against cadmium toxicity or other free radicals by in vivo study. The liver protection effect against cadmium toxicity from lemon pepper extract came from the antioxidant activity of lemon pepper content. Phytochemicals in lemon pepper extract neutralize the unstable cadmium compound and decrease cadmium toxicity. In addition, lemon pepper extract also decreased the severity of inflammation caused by cadmium at the level of protein synthesis and genes.

5. Conclusion

Overall, it can be concluded that the lemon pepper methanol extract has liver protection effects against cadmium toxicity. The best liver protection effect was shown at the highest dose (1, 200 mg/ kg BW) than other doses; the highest dose of lemon pepper methanol extract decreased 41.10% of ALT level and 59.39% of AST level compared to control groups. The decrease of ALT and AST levels was also supported by the improvement of liver structure with minimal ballooning degeneration at the highest dose group.

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References

[1] G. Munhwasa, “Korean Society for Preventive Medicine. Preventive Medicine and Public Health, ” 3rd ed., Seoul, 2019, pp.756-757 .
[2] J. J. V. Branca, G. Morucci, and A. Pacini, "Cadmium-induced Neurotoxicity: Still Much Ado, " Neural Regen Res 2018, 1879-1882, pp. 13(11).
[3] N. Kumar, V. Kumari, C. Ram, B. S. B. Kumar, and S. Verma, "Impact of oral cadmium intoxication on levels of different essential trace elements and oxidative stress measures in mice: a response to dose, " Environ Sci Pollut Res, 2018, pp. 25(6):5401–5411.
[4] G. Genchi, M. S. Sinicropi, G. Lauria, A. Carocci, and A. Catalano, "The Effects of Cadmium Toxicity, " Int J Environ Res Public Health, 2020, pp. 17(3782):1–24.
[5] H. Omar, M. Chung, D. Cosgrove, J. M. Herman, Z. Li, et al, "Cadmium Exposure and Liver Disease Among US Adults, " J Gastointest Surg, 2013, pp. 17(7): 1265-1273.
[6] X. Li, H. Li, D. Cai, P. Li, J. Jin, X. Jiang, et al., "Chronic oral exposure to cadmium causes liver inflammation by NLRP3 inflammasome activation in pubertal mice, " Food Chem Toxicol, 2021, pp. 148: 111944.
[7] Y. Xu, W. Mu, J. Li, Q. Ba, and H. Wang, "Chronic cadmium exposure at environmental-relevant level accelerates the development of hepatotoxicity to hepatocarcinogenesis, " Sci Total Environ, 2021, pp. 783: 146958.
[8] M. Rashrash, J. C. Schommer, and L. M. Brown, "Prevalence and Predictors of Herbal Medicine Use Among Adults in the United States, " J Patient Exp, 2017, pp. 4(3):108–113.
[9] D. D. L. Moreira, S. S. Teixeira, M. H. D. Monteiro, A. C. A. X. De-Oliveira, and F. J. R. Paumgartten, "Traditional Use and Safety of Herbal Medicines, " Rev Bras Farmacogn, 2014, pp. 24(2):248–257.
[10] D. Subositi and S. Wahyono, "Study of the Genus Curcuma in Indonesia Used as Traditional Herbal Medicines, " Biodiversitas, 2019, pp. 20(5):1356–1361.
[11] D. E. Saragih and E. V. Arsita, "The phytochemical content of Zanthoxylum acanthopodium and its potential as a medicinal plant in the regions of Toba Samosir and North Tapanuli North Sumatra, " Pros Semin Nas Masy Biodiversitas Indones, 2019, pp. 5(1):71–76.
[12] Yanti, T. E. Pramudito, N. Nuriasari, and K. Juliana, "Lemon Pepper Fruit Extract (Zanthoxylum acanthopodium DC.) Suppresses the Expression of Inflammatory Mediators in Lipopolysaccharide-Induced Macrophages In Vitro, " Am J Biochem Biotechnol, 2011 pp. 7(4):190–195.
[13] R. V. Worotikan, E. A. Tuju, and F. Kawuwung, "Analisa Efektivitas Antidiabetes Ekstrak Etanol Buah Andaliman (Zanthoxylum acanthopodium DC) pada Histopatologi Ginjal Tikus Putih (Rattus norvergicus) yang Diinduksi Alloksan [Analysis of Antidiabetic Effectiveness of Lemon Pepper Fruit, " Zanthoxylum aca. J Sains Mat Edukasi, 2017 pp. 5(1):29--37.

[14] W. Winarti, P. Simanjuntak, and M. F. Syahidin, "Identifikasi Senyawa Kimia Aktif Antioksidan Dari Ekstrak Etil Asetat Buah Andaliman (Zanthoxylum acanthopodium DC) [Identification of Antioxidant Active Chemical Compounds From Ethyl Acetate Extract of Andaliman Fruit (Zanthoxylum acanthopodium DC)], " In: Talenta Conference Series: Tropical Medicine (TM), Medan, Talenta Publisher, 2018, pp. 162–166.

[15] F. M. C. Sitanggang, A. S. Duniaji, and I. D. P. K. Pratiwi, "Daya Hambat Ekstrak Buah Andaliman (Zanthoxylum acanthopodium DC) dalam Etil Asetat terhadap Pertumbuhan Escherichia coli [Inhibitory Ability of Lemon Pepper Fruit (Zanthoxylum acanthopodium DC) Ethyl Acetate Extract on Escherichia coli Growth], " J Ilmu dan Teknol Pangan, 2019, pp. 8(3):257–266.

[16] D. Purnama, C. N. Ginting, L. Chiuman, and A. Khu, "Wound Healing Activity of Lemon Pepper Essential Oil in Burn Wound of Wistar Rat, " Asian J Pharm Clin Res, 2021, pp. 14(5):122–125.

[17] L. Chiuman, C. N. Ginting, O. Yulizal, Suhartomi, V. Chiuman, "Improvement of Liver Function from Lemon Pepper Fruit Ethanol Extract in Streptozotocin-Induced Wistar Rats, " In: 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce), Medan, Indonesia, IEEE, 2021.

[18] P. J. Salim, L. Chiuman, A. N. Nasution, and C. N. Ginting, "Exploration of Analgesic-Antipyretic Effect from Lemon Peppers Methanol Extract, " In: 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce), 2021, pp. 1–5.

[19] D. Vasanthakumar, D. Aruna, M. Mujeebunisa, S. Saravanan, and V. Divya, "Antibacterial activity of Rosa damascena petal extracts against the fish pathogen Aeromonas hydrophila, " Eur J Exp Biol [Internet], 2015, pp. 5(8):56–59. Available from: http://www.imedpub.com/articles/antibacterial-activity-of-irosa-damascenai-petal-extracts-against-the-fish-pathogen-iaeromonas-hydrophilai.pdf

[20] W. Widowati, B. W. Janeva, S. Nadya, A. Amalia, S. Arumwardana, H. S. W. Kusuma, et al., "Antioxidant and antiaging activities of Jasminum sambac extract, and its compounds, " J Reports Pharm Sci., 2018, pp. 7(3):270–285.

[21] W. Widowati, A. P. Rani, R. A. Hamzah, S. Arumwardana, E. Afifah, H. S. W. Kusuma, et al., "Antioxidant and antiaging assays of Hibiscus sabdariffa extract and its compounds, " Nat Prod Sci., 2017, 23(3):192–200.

[22] W. Widowati, N. Fauziah, H. Herdiman, M. Afni, E. Afifah, H. S. W. Kusuma, et al, "Antioxidant and Anti Aging Assays of Oryza sativa Extracts, Vanillin and Coumaric Acid, " J Nat Remedies, 2016, pp. 16(3):88–99.

[23] P. S. Milton, M. Muthumani, and K. Shagirtha, "Protective effect of Piper betle leaf extract against cadmium-induced oxidative stress and hepatic dysfunction in rats, " Saudi J Biol Sci [Internet], 2012, pp. 19(2):229–239. Available from: http://dx.doi.org/10.1016/j.sjbs.2012.01.005

[24] A. A. Fouad and J. Jresat, "Captopril and telmisartan treatments attenuate cadmium-induced testicular toxicity in rats, " Fundam Clin Pharmacol, 2013, pp. 27(2):152–160.

[25] B. A. Aldahmash and D. M. El-Nagar, "Antioxidant Effects of Captopril against Lead Acetate-Induced Hepatic and Splenic Tissue Toxicity in Swiss Albino Mice, " Saudi J Biol Sci., 2016 pp. 23(6):667–673.

[26] R. Zein, R. Suhaili, F. Earnestly, Indrawati, and E. Munaf, "Removal of Pb(II), Cd(II) and Co(II) from aqueous solution using Garcinia mangostana L. fruit shell, " J Hazard Mater, 2010.

[27] E. Munaf and R. Zein, "The use of rice husk for removal of toxic metals from waste water, " Environ Technol, United Kingdom, 2010.

[28] Y. Duan, J. Duan, Y. Feng, X. Huang, W. Fan, K. Wang, et al., "Hepatoprotective activity of Vitamin E and metallothionein in cadmium-induced liver injury in Ctenopharyngodon idellus, " Oxid Med Cell Longev, 2018.