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Anti-liver fibrosis of green materials *Moringa oleifera* seed oils from Madura Island against hepatocellular carcinoma development

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ABSTRACT. Hepatocellular carcinoma (HCC) is a global health problem, primarily in the Asia Pacific continent. The initial incident of HCC can be triggered by diet, viral infection, and chemical exposure to induce chronic inflammation and fibrosis. The presence of free radical effects from chemical exposure may result in oxidative stress and generate chronic liver injury. The active compounds within *Moringa oleifera* seed oil can restraint hepatotoxicity and obstruct the risk factors of HCC by having a role as an anti-fibrosis agent through the mechanism of antioxidative responses. This animal study and *in silico* model aims to explore the essential property of green material *M. oleifera* seed oil from Madura Island for liver fibrosis drug development. The study was conducted to evaluate the alteration of circulating levels of liver injury marker-linked liver fibrosis development, histological alteration, and liver morphology. Also, the molecular interaction between *M. oleifera* seed oil bioactive compound and liver injury marker was conducted through molecular docking method. The Balb-c mice aged 6-8 weeks were treated by intraperitoneal injection of carbon tetrachloride (CCl_4) + corn oil dose 1 µl/1 g of BW for 8 weeks. Moreover, the experimental groups received green materials MOSEIL treatment by intraperitoneal injection with the same dosage. Fascinatingly, the baseline data of our animal model shows that the administration of green materials *M. oleifera* seed oil can alleviate the higher level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Furthermore, the *in silico* analysis shows that epicatechin bond on the essential ALT residue is Tyr302 and Lys341. It's observed on the PLP binding site through the hydrophobic contact and Lys258 and Trp140 as the essential AST residue bond on the PLP binding site through hydrophobic contact with ellagic acid and catechin. To sum up, *Moringa oleifera* seed oil treatment can prevent significant changes in liver weight, morphology, and even histological feature-associated liver injury and fibrosis. Thus, Moringa seed oil from Madura Island may become the future green materials source for liver fibrosis prevention-related HCC development.

Keywords: green materials; hepatocellular carcinoma; liver injury; molecular docking; *Moringa* seed oils

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the primary causes of death and is ranked as the third most common cancer, with 906,000 cases and an 830,000 mortality rate (Sung *et al*., 2021). The average HCC case mortality rate in Asia is 72.4% in all gender groups (Goodarzi *et al*., 2019). The incidence and death rate of HCC in East Asia, Southeast Asia, North America, and the United States of America is observed to increase by 2.4 times higher in men (Deng *et al*., 2015; Sung *et al*., 2021). Meanwhile, HCC is ranked as cancer in Indonesia with the fourth most incidence and highest mortality rate with 21,392 new cases and 8.9% death rate (WHO: International Agency for Research on Cancer, 2021).

A number of risk factors have been observed causing high HCC prevalence in an area, such as hepatitis B virus (HBV) infection and hepatitis C (HCV), autoimmune hepatitis, obesity, diabetes mellitus, chemical exposure, and alcohol consumption that induces inflammation and fibrosis (Geh *et al*., 2021; Singh *et al*., 2018). From those risk factors, chemical exposure, such as carbon tetrachloride (CCl_4) can worsen anisonucleosis and generate chronic liver injury, marked by various hepatocyte nucleus sizes and activation of liver stellate cells (Guzman *et al*., 2011; Liu *et al*., 2021). CCl_4 is generally used for the model of heart injury on the animal as it induces free radicals and prompts a chain of peroxide reactions (Chen *et al*., 2016). The presence of free radicals is caused by CCl_4 and may result in oxidative stress due to the accumulated reactive oxygen species (ROS) (Lin *et al*., 2019). The CCl_4, triacylglycerol, and phospholipid bond also induce subcellular fraction that leads to lipid peroxidation on liver parenchyma cells (Baliga *et al*., 2013).

The free radicals effects can be reduced using antioxidant compounds obtained from a natural material, such as drumstick tree (*Moringa oleifera)* (Xu *et al*., 2019). Traditionally, the drumstick tree is mostly used in the treatment of hyperglycemia, inflammation, as well as infection of bacteria, viruses, and cancer (Tiloke *et al*., 2019; Tumer *et al*., 2015). The seed of the drumstick tree has abundant antioxidant content (Jahan *et al*., 2018). The active compounds in the *M.oleifera* seed oil are glycosidic glucosinolates (GLs), isothiocyanates (ITCs), nitrile, carbamate, chlorogenic acid (CGA), 2,4-diphenyl-4-methyl-2(E)-pentene and thiocarbamates (Jaja-Chimedza *et al*., 2017; Kayode & Afolayan, 2015; Stohs & Hartman, 2015). It also contains ascorbic acid and phenol (catechins, epicatechins, ferulic acid, ellagic acid, quercetin and myricetin) (Govardhan Singh *et al*., 2013; Sulastri *et al*., 2018). High antioxidant compounds within *M.oleifera* can also restraint hepatotoxicity caused by cadmium induction on rats through the mechanism of increasing alkaline phosphatase (ALP) and superoxide dismutase (SOD) (Kou *et al*., 2018; Vergara-Jimenez *et al*., 2017). Additionally, the active compounds within the drumstick tree also obstruct the HBV development, one of the risk factors of HCC, as anti-fibrosis and anti-virus agent, and also play a role in the antioxidative response on HBV initiation (Feustel *et al*., 2017).

A high level of HCC prevalence can be the basis of early cancer drug exploration. Challenges in managing cancer progression in its early development have placed cancer as a silent killer for many people. This research aims to determine the effect of *M. oleifera* seed oil on the inhibition of HCC progression through circulating ALT and AST levels on in vivo models, liver histological profile, and *in silico* test. These procedures are used to analyze the efficacy of *M. oleifera* seed oil as a potential drug during early stage of HCC. Therefore, exploration of the natural material-based drug, such as the antioxidant from *M. oleifera* seed oil through the mice HCC progressive model can serve as an effort to prevent HCC progression.

MATERIALS AND METHODS

Animal. This *in vivo* model has obtained proper permission from the Institutional Review Board (IRB) of Universitas Brawijaya with ethics approved number 1184-KEP-UB 2019. The animal was male *Mus musculus* strains Balb/C that were purchased at the Fatma Veterinary Center (PUSVETMA) Surabaya, aged 2-3 months, and with a similar bodyweight (BW) 25 ± 2g. The animals were acclimatized for approximately two weeks before treatment. Food and water were given with procedure ad libitum. The animal was then divided into 3 groups. Each group contained 8-10 male animals. The placebo (K-), positive control group (K+) with CCl_4+ corn oil, and treatment group (P) with CCl_4+ *M. oleifera* seed oil treatment.

M. oleifera **seed oil preparation**. *Moringa* seed oil from Madura islands was prepared using a simple pressing method at CV Nurul Jannah, Madura. The dried seed of Moringa was prepared at room temperature for 3-4 days. Dried Moringa seed was blended and pressed to obtain the crude oil. Moringa crude seed oil was then filtered for the purification of the oil.

M. oleifera **seed oil and CCl_4 treatment**. The stock solutions were prepared by dissolving CCl_4 (Sigma Aldrich, USA) in corn oil in a ratio 1: 3 (1 mL of CCl_4 and 3 mL of corn oil). In addition, the

M. oleifera seed oil material was then prepared by mixing the *M. oleifera* seed oil and CCl_4 with the same ratio. For the intraperitoneal injection, the recommended dosage was considered (1 µl/1 g BW per mice for both solution). The placebo (K-) received the intraperitoneal injection of Phosphate Buffered Saline (PBS) solution (Sigma Aldrich, USA) while the positive control group (K+) was treated by intraperitoneal injection of 100µL CCl_4+ corn oil. For the *M. oleifera* seed oil group, the mice were treated with 100µL CCl_4+ *M. oleifera* seed oil L by the same injection method. The treatment duration was carried out in eight weeks by giving treatment three times a week. Post the treatment period, the intraorbital blood collection was done followed by anesthetized step. Furthermore, the mice were dissected and their liver was taken. The liver samples were then fixed with 10% formalin for 7 hours before the next step (paraffin block process for the histological examination).

Baseline characteristics. After the mice were given treatment, they were then dissected to be analyzed according to the parameters used. We measured some baseline parameters including body weight, liver weight, and serological levels of AST-ALT (liver injury parameters) in each group. Also, for the histological analysis, the percentage of the number of cells experiencing necrosis or steatosis was categorized into normal stage (0-5%), mild (6-33%), moderate (33-66%), and massive stage (>67%) (Maulina, 2018).

Protein and ligand preparation. We used ALT and AST as the protein, obtained from RCSB PDB (https://www.rcsb.org/) in Protein Data Bank (PDB) format. The protein 3D structure was prepared using PyMOL and *cleaning* protein was completed by eliminating the *native ligand* and water molecule in the targeted protein structure. The bioactive compounds of *M.oleifera* were obtained from data mining. The 2D structure of the bioactive compounds was attained from PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF format. Further, drug-likeness screening was carried out based on the Lipinski rule of five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp. Among 82 *M. oleifera* seed oil active bio compounds, 30 compounds matched the drug similarity measure. Vigabatrin (CID: 5665) and Hydrazinosuccinic acid (CID: 124897) were used as the control drug, in this study (Antti & Sellstedt, 2018; Ohtsuka, 2018) .

Molecular docking and visualization. Specific protein-ligand docking was carried out using Autodock Vina integrated into PyRx 8.0. As this present study was explorative, our docking covered all structures of the targeted protein. The coverage area of the ALT molecule in the center is X:-17.461, Y:58.6851, Z: 10.5858, while in the dimension (Angstrom) is X: 60.6939, Y: 69.9459, and Z: 69.3006. The coverage area of the AST molecule in the center is X: 20.60871, Y: 113521, Z: 18.1328, while in the dimension (Angstrom) is X: 61.3767, Y: 59.8086 dan Z: 72.5523. Analysis on *binding site* and formed chemical interaction between protein and ligand was carried out using PyMOL software for the 3D visualization, and LigPlot$^+$ v.2.2.4 for 2D visualization (Hidayatullah et al., 2021).

Microscopic analysis. The staining slides with Hematoxylin-Eosin **(**HE) were then observed under a light microscope with 400x magnification. The observation was focused on the fibrogenesis profile linked to inflammatory cells, fibrotic cells, and so forth.

Data Analysis. We normalized data based on the Kolmogorov-Smirnov test. Parametric analysis using One-Way Analysis of Variance (ANOVA) at the 95% confidence level ($\alpha = 0.05$) was chosen for the advanced analysis.

RESULTS AND DISCUSSION

Bodyweight and liver mass of the samples in different groups. CCl_4 carries a role as a liver toxin that causes deterioration on hepatocytes, prompting fibrosis, cirrhosis, and carcinoma (Marques *et al*., 2012). Damages on the liver are marked by decreasing body mass and liver mass, followed by an increase of AST and ALT levels (Zhang *et al*., 2020). It is linear with a previous study that induces CCl_4 on mice samples, showing that initially, both control and placebo (K^-) groups have a bodyweight of 26.4 g and a normal liver weight of 1.37 g. However, once they were given (intraperitoneal

injection) CCl_4 (K^+), both body and liver weight decreased to 26.2 g and 1.34 g, respectively. In this present study, the body and liver mass of the group treated with CCl_4 + *M. oleifera* seed oil (P) is 24 g and 1.31 g (Figure 1).

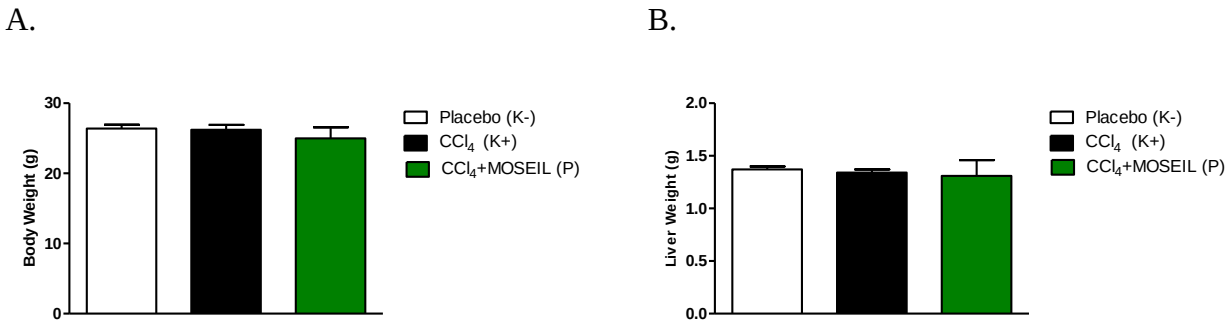

Fig. 1. Bodyweight and liver mass of the samples in different groups. A. Weight at the end of treatment; B. Weight of liver.

The effect of *M. oleifera* seed oil on circulating ALT and AST levels. The level of AST and ALT increase two times after the CCl_4 injection. The AST and ALT level, as the marker liver injury, increase after K^+ and decrease after being injected with *M. oleifera* seed oil (P) (Figure 2). In the K^- group, initially the AST and ALT level was (1,7 U/L & 1,5 U/L), and increased two times to (3 U/L & 2,4 U/L) on K^+ group. However, it decreased into (1,8 U/L & 1,5 U/L) once it received *M. oleifera* seed oil (P).

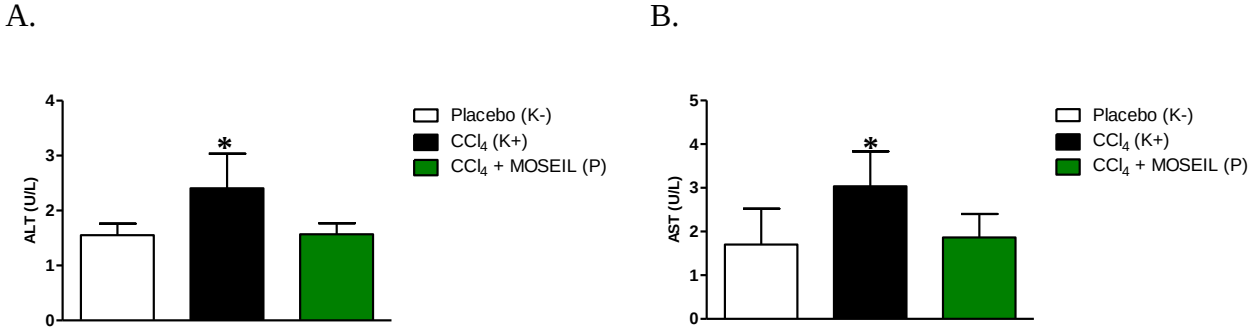

Fig. 2. The effect of *M. oleifera* seed oil on circulating ALT (A) and AST levels (B). * *p*-value < 0.05 vs placebo

In the mice samples that accepted CCl_4 injection, the level of AST and ALT increased within 24 to 48 hours after the injection (Endig *et al*., 2019). A high level of AST and ALT is affected by centrilobular hepatic necrosis due to the exposure of CCl_4 causing the formation of toxic metabolites through cytochrome P450 2E1 (Cyp2e1) (Ghafoory *et al*., 2013). Cyp2e1 changes CCl_4 into trichloroethane radical (CCl_3) that will be transformed again into trichloromethyl peroxyl radical (CCl_3O_2), using molecule O (Chen *et al*., 2016). CCl_3O_2 induces chain reaction and lipid peroxidation in the structures that are rich in a phospholipid, such as the endoplasmic reticulum and mitochondria, resulting in Ca homeostasis in the disrupted cells (Hafez *et al*., 2014). All of these domino effects lead to cells damage and proinflammatory cytokines will respond through activating neutrophil respiratory and produce free radicals, in the form of O molecule (Lin *et al*., 2019). It worsens oxidative stress and liver damage (Tsai *et al*., 2013). TNF-α, IL-1β, and MCP-1 hold an essential role during the liver pathological process induced by CCl_4 (Li *et al*., 2021; Shin *et al*., 2013).

The results also suggest an increase of body and liver mass, following the decrease of AST and ALT levels after the *M. oleifera* seed oil injection on the samples that previously had been exposed to CCl_4. The decrease of AST and ALT levels is correlated with protection effects from *M. oleifera* seed oil which acts as a free radical scavenger (Albrahim & Binobead, 2018). High antioxidant content on MO may hinder 89.7-92% of peroxidation activity from linoleic acid, while also obstructing TNF-α, IL-6, and IL-8 activity (Kooltheat *et al*., 2014; Vergara-Jimenez *et al*., 2017).

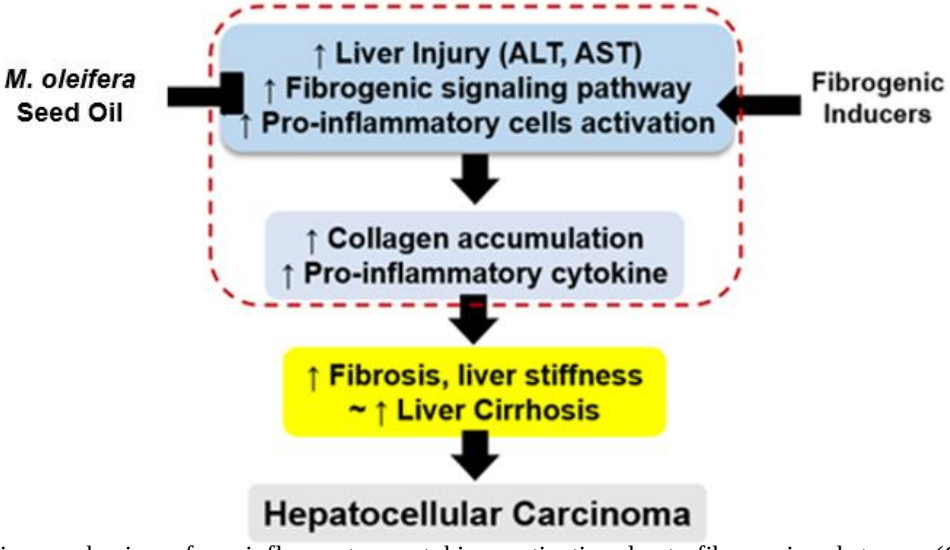

Fig 3. Inhibition mechanism of pro-inflammatory cytokines activation due to fibrogenic substances (CCl_4) induction in HCC (Jung *et al*., 2015; Supriono *et al*., 2020a).

The quercetin content in *M. oleifera* seed oil is also involved in the inflammation process, by hampering the neutral factor kappa-beta (NF-kβ) activity (Das *et al*., 2012). In addition to obstructing the inflammation process, the drumstick tree also has the ability to reduce the TGF-β level, the primary fibrosis regulator (Fabregat & Caballero-Díaz, 2018; Susanto *et al*., 2021). The phenol in *M. oleifera* seed oil decreases liver fibrosis due to CCl_4 induction by suppressing hepatic stellate cell activation through obstructing the TGF-β/Smad3 signaling pathway (Wu *et al*., 2018). The general inhibition mechanism by the antioxidant compound in *M. oleifera* seed oil toward HCC development (Fig. 3).

Liver histological profile after *M. oleifera* seed oil treatment. The histology results of HE stain in every treatment group showed a significant different within the liver section. The K^- group is classified as normal, with a low number (4%) of cell necrosis from all of the hepatocytes. After the intraperitonealinjection of CCl_4 on the K^+ group, the necrosis cell significantly increase to 94%, considered massive (Maulina, 2018). Meanwhile, in the P group, the normal cell is 68% and the necrosis cell is 32%.

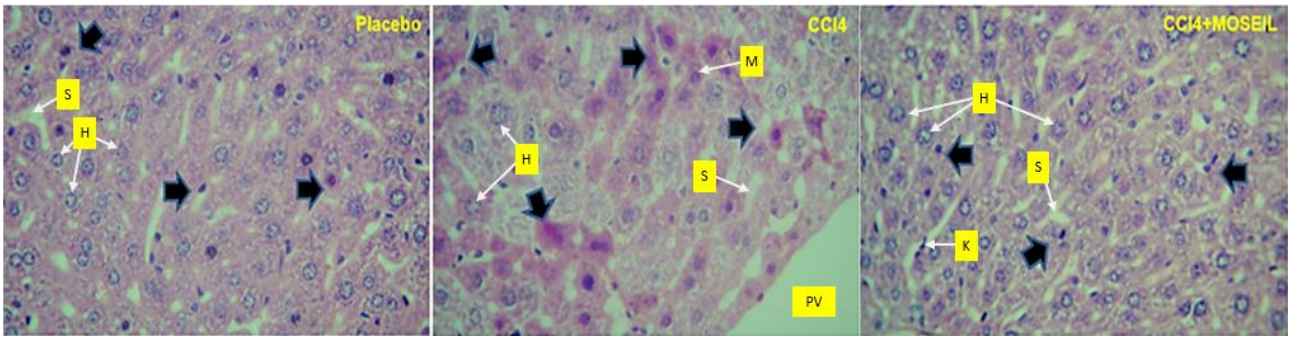

Fig 4. Liver histological profile after *M. oleifera* seed oil treatment. HE stains: Black arrow shows inflammatory and profibrogenic cells aggregation. The histology was observed using light microscope with 400x magnification. Portal Vein (PV), Sinusoid (S), Hepatocyte (H), Kupffer Cell (K), Monocyte (M).

The antithesis of *M. oleifera* seed oil toward liver fibrosis was histologically proven through HE stains (Fig. 4). In the P group, the number of the normal cell increased and being dominant than the necrosis cell, even if it had 32% necrosis cell. In contrast, the K^+ group had 94% of necrosis, classified as massive (Maulina, 2018). Effects of CCl_4 cause steatosis through inhibition of the triglyceride synthesis by very low-density lipoprotein (VLDL) secretion (Supriono et al., 2020b). Steatosis can be observed in the centrilobular, 15 hours after injection. The transformation into necrosis can be apparently observed after 20-30 hours (Abd-Rabou *et al*., 2016). The hepatic failure causes eosinophilic on the cytoplasm and is marked by the disappearing chromatin granules (Tiloke *et al.*, 2019).

Table 1. Docking results for top nine compounds and control with ALT

Compound	CID	Source	ΔG Average (kcal/mol)	Amino Acids Residue	Interaction
8-Dimethyl-2-isopropylphenanthrene	220273	*M. oleifera* seed oil	-9.4	Phe295, Lys262, Leu198, Lys293, His360, Glu332, Val263	Hydrophobic Contact
Strophantidine	6185	*M. oleifera* seed oil	-7.9	Cys311, Val306, Arg312, Phe313, Gln303, Arg170, Tyr166, Pro391, Tyr343, Asp304	Hydrophobic Contact
Kaempferol	5280863	*M. oleifera* seed oil	-7.6	Val409, Pro105, Gly342, Asn305, Tyr343, Met439, Leu413 *Leu498, Lys416, Asn412*	Hydrophobic Contact *Hydrogen Bond*
Epicatechin	72276	*M. oleifera* seed oil	-7.5	Ser340, Leu498, Tyr440, Thr496, Asn94, Met439, Val409, Tyr343, Gly342, Lys341, Tyr302	Hydrophobic Contact
Catechin	9064	*M. oleifera* seed oil	-7.5	Ser340, Gly342, Leu413, Tyr343, Val409, Met439, Leu498, Asn94 *Tyr440*	Hydrophobic Contact *Hydrogen Bond*
Quercetin	5380343	*M. oleifera* seed oil	-7.3	Thr490, Arg487, Glu247, Tyr234, Asn244 *Arg444, Glu238, Glu237, Asp236, Arg250*	Hydrophobic Contact *Hydrogen Bond*
Ellagic Acid	5281855	*M. oleifera* seed oil	-7.2	Arg487, Arg250, Glu254, Ala255, Val231 *Tyr234, Asn232, Ala251*	Hydrophobic Contact *Hydrogen Bond*
Glucosinalbin	9601115	*M. oleifera* seed oil	-6.8	His360, Val331, Lys293, Ala289, Trp290 *Asn330, Ser329, Leu294*	Hydrophobic Contact *Hydrogen Bond*
2,4-Diphenyl-4-methyl-2-(E)-pentene	5356300	*M. oleifera* seed oil	-6.8	Lys418, Lys415, Asp422, Leu419, Leu423, Lys505	Hydrophobic Contact
Vigabatrin	5665		-4.8	Trp290, Lys293, Val331, Ala289, Leu294 *Asn330*	Hydrophobic Contact *Hydrogen Bond*

Previous studies have extensively described the ability of *M. oleifera* leaf extract in suppressing the rate of hepatocyte injury since it contains antioxidants and phenolic compounds that reduce oxidative stress caused by carcinogenic substances (Sadek *et al*., 2017). *M. oleifera* tested on mice induced by acetaminophen (trigger of liver fibrosis) result in decreasing TNF-α and TGF-β levels (Aly *et al*., 2020). *M. oleifera* extract inhibited the increase in mRNA and protein levels of interleukin-6, tumor necrosis factor-alpha, inducible nitric oxide synthase, and cyclooxygenease-2 (Muangnoi *et*

al., 2012). In addition, *M. oleifera* was able to increase the expression of p53, p21, and Bax which are known as tumor suppressors (Abd-Rabou et al., 2017). The suppressive effect of M. oleifera was also mediated by inhibiting the phosphorylation of the kappa B inhibitor protein (Cirmi et al., 2019). These results suggest that the anti-inflammatory activity of the bioactive compounds present in the pod constituents of *M. oleifera* may contribute to ameliorating the pathogenesis of chronic inflammation-related diseases.

Docking result and visualization. The docking results signify that 9 out of 30 compounds have a lower binding affinity than the control. In the ALT, 8-Dimethyl-2-isopropylphenanthrene, strophantidine, kaempferol, epicatechin, catechin, quercetin, ellagic acid, glucosinalbin, and 2,4-Diphenyl-4-methyl-2-(E)-pentene have lower -6.8 to -9.4 kcal/mol binding affinity than control (Vigabatrin; -4.8 kcal/mol). The docking results for those nine compounds are presented in Table 1.

In Autodock Vina, a higher negative binding affinity value represents a more robust and stable bond between molecules (Xue et al., 2022). The epicatechin bond on the essential ALT residue is Tyr302 and Lys341, observed on the pyridoxal-phosphate (PLP) binding site through the hydrophobic contact. Meanwhile, glucosinalbin bond on the similar residue as the control, namely Val331, Ala289, Trp290, Lys293, Asn330, and Leu294 on the L-alanine substrate-binding site by blocking the attachment substrate (Williams et al., 1998). Those nine compounds and control are visualized in Fig. 5.

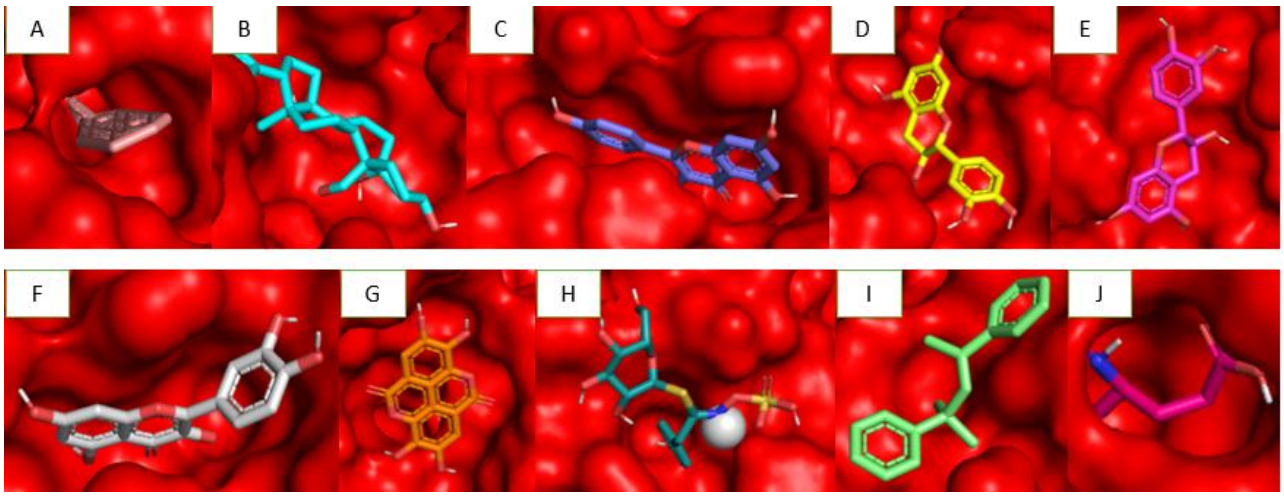

Fig. 5. Visualization of compounds with lower binding affinity compared to the control on ALT. A) 8-Dimethyl-2-Isopropylphenanthrene, B) Strophantidin, C) Kaempferol, D) Epicatechin, E) Catechin, F) Quercetin, G) Ellagic acid, H) Glucosinalbin, I) 2,4-Diphenyl-4-methyl-2-(E)-pentene, and J) Vigabatrin (Control).

The presence of epicatechin within *M. oleifera* seed oil in the liver which has experienced injury increases the ALT and AST levels, to the normal level (Shanmugam et al., 2017). Therefore, epicatechin can be the potential substance for the health promoter in the hepatitis situation, one of which is induced by CCl_4 (Uysal *et al*., 2016). Similar to epicatechin, hepatoprotective properties are also presented by glucosinalbin that contributes to the CCl_4 detoxification by reducing the ALT and AST level, while also increasing the serum albumin level, representing better liver synthesis function and reducing myeloperoxidase activity that decrease the infiltration of pro-inflammation cells (Hamza, 2010). The docking results of 8-Dimethyl-2-isopropylphenanthrene, ellagic acid, quercetin, catechin, strophantidine, kaempferol, 2,4-Diphenyl-4-methyl-2-(E)-pentene, epicatechin, and glucosinalbin with AST are presented in Table 2.

Table 2. Docking results of nine compounds and control with AST.

Compound	CID	Source	ΔG Average (kcal/mol)	Amino Acids Residue	Interaction
8-Dimethyl-2-isopropylphenanthrene	220273	*M. oleifera* seed oil	-10.1	Tyr123, Leu119, Phe218, Phe251, Val283, Trp122, Phe118	Hydrophobic Contact
Ellagic Acid	5281855	*M. oleifera* seed oil	-8.0	Lys258, Gly107, Ala224, Tyr225, Trp140 *Asn194, Gly108, Thr109, Arg266, Asp222*	Hydrophobic Contact *Hydrogen Bond*
Quercetin	5280343	*M. oleifera* seed oil	-7.8	Tyr123, Phe118, Phe218, Leu119, Glu249, Phe251, Gly274, Lys275, Glu278 *Trp122, Val273, Ile280, Glu276*	Hydrophobic Contact *Hydrogen Bond*
Catechin	9064	*M. oleifera* seed oil	-7.8	Ala224, Trp140, Ser255, Gly107, Ser257, Tyr262, Thr109 *Asn194, Tyr225, Asp222, Gly108, Arg266, Lys258*	Hydrophobic Contact *Hydrogen Bond*
Strophantidin	6185	*M. oleifera* seed oil	-7.7	Val128, Phe118, Trp122, Phe218, Tyr123 *Gln286, Glu249*	Hydrophobic Contact *Hydrogen Bond*
Kaempferol	5280863	*M. oleifera* seed oil	-7.5	Tyr123, Phe218, Leu119, Phe251, Glu249, Gly274, Lys275, Ile280, Glu278 *Glu276, Val273, Trp122*	Hydrophobic Contact *Hydrogen Bond*
2,4-Diphenyl-4-methyl-2-(E)-pentene	5356300	*M. oleifera* seed oil	-7.3	Val283, Phe118, Leu119, Glu249, Phe218, Leu217, Tyr123, Phe251, Trp122	Hydrophobic Contact
Epicatechin	72276	*M. oleifera* seed oil	-7.3	Ser279, Leu119, Phe118, Ile280, Tyr123, Val283, Glu249, Val272, Gly274 *Glu276, Trp122, Lys275*	Hydrophobic Contact *Hydrogen Bond*
Glucosinalbin	9601115	*M. oleifera* seed oil	-6.6	Phe216, Phe248, Phe118, Tyr123, Phe218 *Phe183, Leu217, Glu249, Asn124, Trp122*	Hydrophobic Contact *Hydrogen Bond*
Hydrazinosuccinic Acid	124897		-5.0	Gly107, Ala224, Thr109, Gly108, Trp140 *Arg266, Ser257, Ser255, Lys258*	Hydrophobic Contact *Hydrogen Bond*

The binding affinity of those compounds ranges between -6.6 to -10.1 kcal/mol lower than the control (Hydrazinosuccinic acid: -5.0 kcal/mol). The 3D visualization of those docking results showed in Fig. 6.

Two out of nine selected *M. oleifera* seed oil compounds, ellagic acid and catechin bond on the different residue, compared to the other seven compounds. Ellagic acid and catechin bind AST on the same residue as the control (Hydrazinosuccinic acid), namely Lys258**,** Gly107, Ala224, Tyr225, Trp140, Asn194, Gly108, Thr109, Arg266, and Asp222, through hydrophobic contact and hydrogen bond. Both of them bond with Lys258 and Trp140, the essential AST residue bond on the PLP binding site through hydrophobic contact. The inhibition of ALT and AST, using epicatechin, ellagic acid, and catechin is predicted to obstruct the *first half-reaction* PLP complex in the transamination reaction (Zareei *et al*., 2017).

The ellagic acid content in MOSEIL carries a protective effect toward cirrhosis induced by CCl4 through obstruction of ROS and angiogenesis formation (Ding *et al*., 2017). Ellagic acid also presents protective effects on liver cirrhosis as marked by reducing the level of AST through significant inhibition of TNF-α level and obstruction on IκB-α and NF-κB phosphorylation (Gu *et al*., 2014). Ellagic acid is predicted to have a role in lowering AST levels by reducing the inflammation response and increasing the antioxidant defense system. Catechin is the second potential compound predicted to reduce HCC progression through AST inhibition. In a previous study, catechin is observed to carry potential in weakening cirrhosis by degrading the regulation of NF-κB activation, including the TNF-α and ROS (Bharrhan *et al*., 2012).

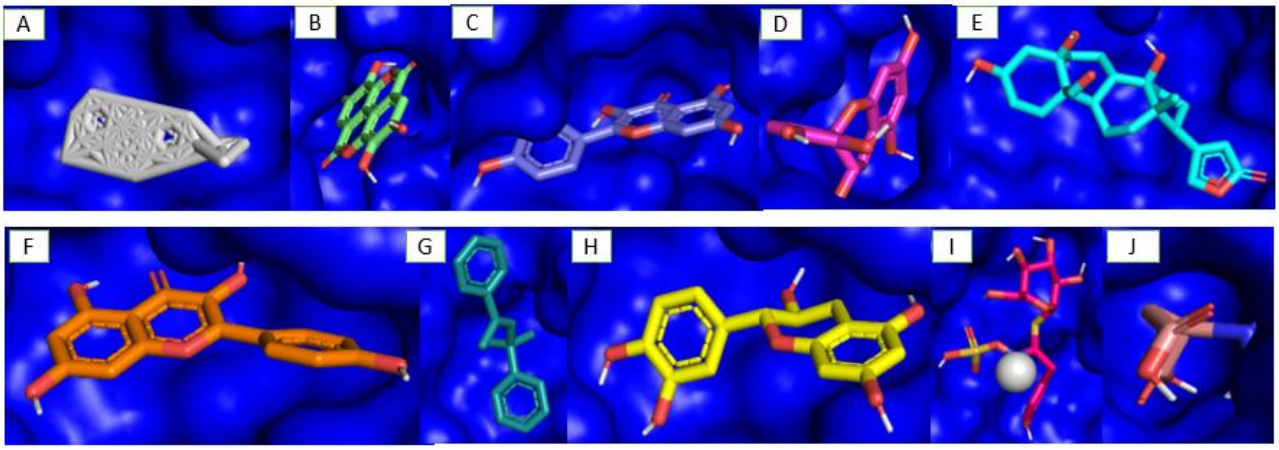

Fig 6. Visualization of compounds with lower binding affinity than control on AST. A) 8-Dimethyl-2-Isopropylphenanthrene, B) Ellagic acid, C) Quercetin, D) Catechin, E) Strophantidin, F) Kaempferol, G) 2,4-Diphenyl-4-methyl-2-(E)-pentene, H) Epicatechin, I) Glucosinalbin, and J) Hydrazinosuccinic acid (Control).

CONCLUSION

The findings suggest that the antioxidant content of *M. oleifera* seed oil has the potential to reduce HCC progression caused by fibrogenic substances induction. This mechanism was predicted through liver injury reduction, inflammation response, and inhibition of the fibrosis rate process. In addition, the *in silico* model improve the essential activities of this green material against the serological liver injury marker. Therefore, *M. oleifera* seed oil can be proposed as a potential preventive agent candidate to prevent HCC progression.

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