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RESEARCH ARTICLE

Phytochemical Profiling and Pharmaceutical Properties of *Moringa oleifera* Leaves Powder and Seed Oil Against Hepatocellular Carcinoma

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Abstract. Hepatocellular carcinoma (HCC) is one of the deadliest types of cancer with a mortality rate of 8.9% of the total cancer deaths in Indonesia. This cancer can be caused by exposure to hepatitis B and C viruses, NAFLD, autoimmune, diabetes to sporadic genetic diseases. The development of chronic HCC is generally preceded by the occurrence of severe liver fibrosis and cirrhosis. One of the genes that play a role in fibrosis in the incidence of HCC is TGF-β1. As a pro-fibrotic cytokine, the presence of high levels of TGF-β1 may be due to oxidative stress activity early in cancer development. One of the natural ingredients with lots of phytochemical content in the form of antioxidants that can reduce this activity is Moringa plant (Moringa oleifera). In this study we used a computational approach using molecular docking on the results of the GC-MS and LC-HRMS tests on Moringa oleifera Seed Oil (MOSEIL) and Moringa oleifera Leaves Powder (MOLP) which are oil and flour products made from moringa. The results of the identification of phytochemical compounds through the GC-MS test showed that the dominant compound in MOSEIL was oleic acid (37.546%) and in MOLP was ester (8.802%) when using n-hexane as solvent. The percentage yield of the dominant compound from the LC-HRMS test in MOSEIL was nitro compound (72.55%) and at MOLP was alcohol (45.87%). These compounds are known to have effects as hepatoprotective agents through antioxidant, anti-inflammatory, and anti-fibrotic activities that can reduce hepatic oxidative stress as an early trigger of cancer development. Through molecular docking, MOSEIL and MOLP showed a lower level of binding affinity when compared to TGF-β1 control drugs such as metformin. This data implies MOSEIL and MOLP have a strong interaction to TGF-β1 than the control drug. The therapeutic potential of the hepatoprotective properties of MOSEIL and MOLP makes them one of the most-promising therapeutic agents in the initial step of renewable cancer treatment therapy.

Keywords: Bioactive characterization, Molecular docking, *Moringa oleifera*, Hepatocellular Carcinoma, Transforming Growth Factor β-1.

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Introduction

Hepatocellular carcinoma (HCC) is one type of cancer which is included in the fifth rank as the cancer that causes the highest death in the world (1). According to the World Health Organization (WHO), HCC is the second most common cause of cancer deaths (2). Based on GLOBOCAN 2020 data in Indonesia, HCC ranks fourth as the cause of death from cancer with 8.9% (20,920) deaths (3). HCC can be caused by infection with hepatitis B and hepatitis C viruses, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, diabetes mellitus, tobacco, and sporadic genetic diseases such as alpha-1 antitrypsin deficiency, hemochromatosis, tyrosinemia, and porphyria (4).

Of the many risk factors that can occur, most cases of HCC begin with fibrosis and severe liver cirrhosis can cause chronic damage to the liver which then progresses to cancer (5). HCC treatment generally uses a drug delivery strategy, namely by giving certain drugs to reduce the effects of cancer development and stimulation of immune cells such as macrophages and T cells (6). Treatment steps and preventive measures against HCC can also be done by utilizing herbal plants (7). Herbal plants are a source of phytochemical components that can be used in the health sector (8). Phytochemical groups in plants, such as xanthonoids, proteins, benzopyrans, coumarins, diarylheptanoids, indoles, polysaccharides, carotenoids, alkaloids, terpenes, flavonoids, tannins and saponins have antioxidant and anti-inflammatory activity. (9,10).

Moringa (*Moringa oleifera*) is a natural ingredient that contains many phytochemicals which have been reported to increase pharmacological properties with its role as anticancer, antiviral, anticonvulsant, and anticancer (11). Almost all parts of Moringa have medicinal benefits, including leaves, seeds, bark, and roots (12). Moringa leaves, seeds and pods contain several phytochemicals, including tannins, terpenoids, sterols, saponins, alkaloids, phenolics, and flavonoid groups such as quercitin, isoquercitin, kaemfericitin, isothiocyanates, and glycoside compounds (13). Its leaves have a high amount of protein and potassium compared to other kinds of plants which being consumed as food such as banana (*Musa sp.*) and spinach (*Spinacia oleracea*) (14,15). *Moringa oleifera* Seed Oil (MOSEIL) and *Moringa oleifera* Leaves Powder (MOLP) are one form of application for the use of Moringa plants. The content of phytochemical compounds in Moringa seed oil has low toxicity (16), and has a high fatty acid content (17). Moringa leaves powder contains more carbohydrates (38.2 g) than fresh Moringa (12.5 g), more protein (27.1 g) than fresh ones (6.7 g), and higher fat content (18).

The results of previous studies have shown that Moringa seed oil has an effect on healing therapy for colon cancer and breast cancer (19) . The content of MOLP can reduce the population of proinflammatory cells in the liver parenchyma area and reduce the expression of IL-6 and TNF-α (20). The hepatoprotective effect of the phytochemical content of Moringa can also reduce the expression of TGF-β1 as a gene that acts as a major regulator of fibrosis (21). Moringa phytochemical compounds are known to suppress the expression of type 1 collagen, fibronectin and PAI-1 which were previously induced by the TGF-β1 gene in the fibrosis process (22). In the process of HCC development, the TGF-β1 gene participates in an important phase of disease progression from early liver progression from inflammation to fibrosis, cirrhosis and cancer (23). TGF-β1 which produced by liver cells, plays a role in tumor progression via pro-fibrotic and pro-tumorigenic actions at late stages (24). Targeting TGF-β1 pathway which related to worst malignant features in HCC could be a promising and effective strategy in HCC treatment.

Phytochemical compounds contained in MOSEIL and MOLP can be identified using several analyses, including Gas Chromatography-High Mass Spectrometry (GC-MS) and Liquid Chromatography-High Resolution Mass spectrometry (LC-HRMS) analysis. GC-MS is a combined analytical method between GC and MS that can separate, identify and measure complex mixtures of a sample. LC-MS analysis is used for quantitative analysis, as well as identifying labile compounds in solution, such as flavonoids (25). GC-MS and LC-HRMS are often used together in order to obtain accurate, precise and complete results when detecting secondary metabolites in a sample. Phytochemical compounds that have been identified will then be developed as new drug designs in the future with molecular docking computational studies. Computer-aided drug design (CADD) is a method used to identify compounds that have the potential to develop drugs for several diseases (26). Based on this description, the potential of phytochemical compounds in Moringa oleifera Seed Oil (MOSEIL) and *Moringa oleifera* Leaves Powder (MOLP) will be investigated through LC-HRMS and GC-MS analysis. This study also aims to determine the characterization of bioactive compounds in MOSEIL and MOLP and explore their potential targets in suppressing HCC development through an in silico approach to TGF-β1.

Materials and Methods

This research employed a qualitative approach. The materials used include Moringa seeds, Moringa leaves, acetone, ethanol, distilled water and n-hexane. The tools used include a blander, 200 mess sieve, magnetic stirrer, centrifuge, microtube, analytical balance, beaker glass, tray, pipette, and Erlenmeyer. The programs used include Pyrx software, PyMol software, discovery studio software, RSCB PDB (www.rscb.org) which is a protein database, Pubchem which is a database of ligand compounds (https://pubchem.ncbi.nlm.nih.gov/), Uniprot yang merupakan database gen target (https://www.uniprot.org/), pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) and ADMET Predictors. Screening similarity of drug properties based on Lipinski rule of five (www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp).

Extraction of *Moringa oleifera* Seed Oil (MOSEIL) and *Moringa oleifera* Leaves Powder (MOLP)

Moringa oleifera Seed Oil (MOSEIL) was produced by drying the Moringa seeds and jump to the next step for peeling the skin so that the Moringa seed kernel is obtained. This part is then pressed and produced Moringa seed oil or MOSEIL. MOSEIL extraction was carried out using n-hexane as a solvent in a ratio of 1:1. The manufacture of *Moringa oleifera* Leaves Powder (MOLP) begins with washing the Moringa leaves with running water and separating the leaves from the stems. Then the Moringa leaves are dried at a temperature of 25-27 ˚C for 4-5 days within the drying oven. The dried Moringa leaves are then blended. Then separated using a sieve some materials that can not be destroyed when blended. Extraction using maceration method with two kinds of solvents, namely n-hexane and acetone solvents. The simple maceration process of Moringa leaves with n-hexane as solvent was carried out by soaking 4 grams of MOLP with 30 mL of ethanol and 15 mL of distilled water in an Erlenmeyer. Then the erlenmeyer was closed and left for four days. The mixture of simplicia and liquid filter was filtered until the extract was obtained. The extract obtained was then centrifuged at 5,000 rpm for 30 minutes. The supernatant obtained was then partitioned using a separating funnel extraction method. The n-hexane layer which is shown in clear color is slowly removed from the separating funnel, so that the n-hexane extract is produced. In the process of extracting Moringa leaf powder with acetone solvent, it is done by soaking 2 grams of MOLP with 20 mL of acetone for 10 hours. Through these two extractions, two kinds of extraction were produced, namely extraction with acetone solvent and extraction with n-hexane solvent.

Gas Chromatography-mass spectrometry (GC-MS) Test

GC-MS analysis of the extracts was carried out in the GCMS system with the BRAND: SHIMADZU; TYPE : GCMS QP2010 PLUS. The GC-MS test begins by taking one micro liter of the extracted sample, then inserting it into the tool for GC-MS analysis. The carrier gas used was Helium with a total rate of 50 ml/minute and a column rate of 1.88 ml/minute, the column temperature in the oven at 30 °C was held for 1 minute, then the temperature was slowly raised to 300 °C and held for 41 minutes. The ion source temperature is 250 °C, the interface temperature is 250 °C with the cut time starting from 3.00 minutes and ending at 50.99 minutes. In column chromatography, each compound is separated from the mixture. Each component will undergo ionization, then the ions will be separated according to their mass and obtained a mass spectrum. For each mass spectrum of the compound, it was matched with the Wiley-8 Library to be given a recommendation for the compound formula.

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Test

LC-HRMS analysis of MOLP and MOSEIL extracts was carried out at the Central Laboratory of Biological Sciences (LSIH) Universitas Brawijaya. The sample is diluted according to the solvent. The dilution was carried out by looking at the concentration of the sample with a final volume of 1500µ L, then vortexed for 2 minutes at a speed of 2000 rpm. Spindown was carried out at a speed of 6000 rpm for 2 minutes so that the superntant to be tested was produced. The composition of biochemical compounds was tested using a Thermo Scientific Dionex Ultimate 3000 RSLCnano with a microflow meter. Hypersil GOLD aQ analysis column 50 x 1 mm x 1.9µ particle size. The LC-HRMS test was initiated by inserting one microliter of extract into the column. The elution solvent used was 0.1% Formic acid in Water (A) and 0.1% Formic acid in Acetonitrile (B) for 30 minutes with an oven column temperature of 30˚C. The flow rate used was 40 L/min where the spectrum was recorded in negative and positive ionization modes. For each of the mass spectra of these compounds, matching is done with the mzCloud MS/MS Library.

Protein and Ligand Preparation

The main bioactive compounds in MOSEIL and MOLP that have been detected through GC-MS and LC-MS analysis were selected for further molecular docking studies to determine good molecular

interactions based on their affinity interactions with target proteins. The obtained bioactive compounds were validated for 3D structure by recording the CID number through the PubChem database (https://pubchem.ncbi.mlm.nih.gov). Post this step, all compound are then tested using the Lipinski rule of five parameters (https://www.scfbiolitd.res.in/software/drugdesign/lipinski.jsp). Then, for the next analysis, the results from previous analysis was then carried out to determine the ability of these compounds as oral drugs based on physical and chemical characteristics (27). The target protein used is TGFB1 downloaded from RCSB PDB (https://www.rscb.org/) in the form of PDB and the target protein purification process from native ligands and water molecules is carried out using the PyMol application (https://www.pymol.org). In this study, Metformin (CID: 4091) used as a control drug (28).

Molecular Docking and Visualization

The docking process is carried out with the help of the PyRx application program ((https://pyrx.sourceforge.io/) to demonstrate the potency of some compounds at the target. The coverage area of the TGF-β1 molecule in the middle is X: 0.0326, Y:-3.9430, Z: 4.7788 with dimensions (Angstrom) that is X: 89.0729, Y: 72.3868, Z: 136.2298. The results obtained from PyRx are the value of binding affinity or a measure of the strength of a compound in binding to the receptor. namely the value of binding affinity or a measure of the strength of a compound in binding to the receptor. Compounds and target proteins are converted to pdbqt format to facilitate the docking process. The results of the docking process continued with visualization using PyMOL Software (https://www.pymol.org) for 3D visualization and using the Discovery Studio 2021 application. After that, 5 bioactive compounds with the lowest binding affinity values were selected for the visualization process (29).

Results and Discussion

Moringa plants have antioxidant, anticancer and antidiabetic activities (30). Another activity is acting as an antitumor (31), anti-inflammatory (32), antifungal, antibacterial and hepatoprotective (33). Moringa seed oil phytochemicals also have biological activities such as antioxidant and antifertility. Another benefit of Moringa seed oil is as a treatment for rheumatism and hypertension (34).

Phytochemical Composition of MOLP and MOSEIL by GC-MS Analysis

The GC-MS method is an analysis consisting of ion mobility spectrometry, capillary zone electrophoresis, ultraviolet spectroscopy, and infrared spectroscopy (35). GC-MS analysis in this study used two solvents, namely n-hexane and acetone. The bioactive compounds obtained through GC-MS analysis are shown in Figure 1.

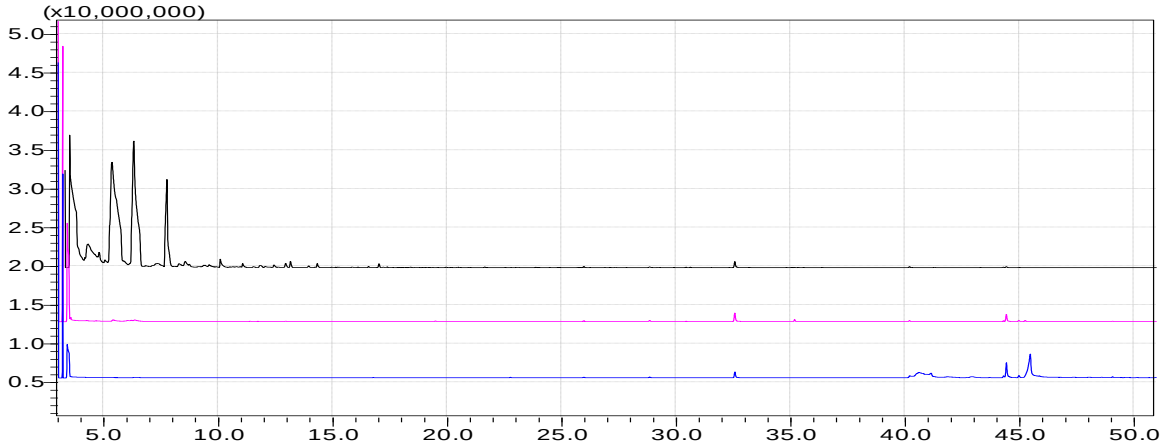

Figure 1. Graph of GC-MS analysis results on MOSEIL and MOLP (x-axis: Retention Time and y-axis: abundance). The blue graph shows the GC-MS analysis on MOSEIL with hexane solvent, the pink graph shows the results of GC-MS analysis on MOLP with n-hexane solvent, and the black graph shows the results of GC-MS analysis on MOLP with acetone solvent

The results of GC-MS analysis on MOSEIL with n-hexane solvent obtained 15 peaks of bioactive compounds shown in Table 1. n-hexane solvent is a polar solvent that can take polar compounds present in the sample, for example long chain fatty acids and esters. flavored esters (36). Kelompok senyawa pada minyak biji kelor yang diperoleh yaitu hidrokarbon, ester, asam lemak dan alkohol. The dominant

bioactive compounds found in MOSEIL include Hexadecanoic acid, methyl ester (0.534%); 9-Octadecanoic acid, methyl ester (E)- (16.453%); 9.12 Octadecanoic acid (Z,Z), methyl ester (1.093%); Methyl stearate (1.363%) and Oleic acid (37.546%).

Table 1. Components of bioactive compounds in MOSEIL by GC-MS analysis with n-hexane solvent

Compound Group	Name of Compound	% Area	% group
Hydrocarbons	*Nonanal*	32.445	32.79
	Cyclopentane, methyl-	0.130	
	2-Nonenal, (E)-	0.222	
Acid	*n-Hexadecanoic acid*	1.619	39.16
	Oleic acid	37.546	
Ester	*Dimethyl phthalate*	0.394	27.68
	Diethyl Phthalate	3.567	
	Hexadecanoic acid, methyl ester (Methyl palmitate)	0.534	
	6-Octadecenoic acid, methyl ester, (Z)-	3.497	
	9-Octadecanoic acid, methyl ester (E)-(Methyl elaidate)	16.453	
	9,12 Octadecanoic acid (Z,Z)-, methyl ester (methyl linoleat)	1.093	
	Methyl stearate	1.363	
	(E)-9-Octadecenoic acid ethyl ester	0.321	
	Eicosanoic acid, methyl ester	0.457	
Alkohol	Phenol, 2-methoxy-3-(2-propenyl)-	0.354	0.35

The dominant compound in MOSEIL is oleic acid (37.546%) which is a fatty acid group. Oleic acid is the main component of Moringa seed oil with a percentage of approximately 72.27% (37). The benefits of oleic acid are to prevent and treat various types of diseases such as cardiovascular or autoimmune, metabolic disorders and as an anticancer and are used for obesity diets (38). Oleic acid can also reduce lipid accumulation and cell death in hepatocellular carcinoma through autophagy mechanism (39). Moreover, oleic acid reduced cyclin D1 expression and prevent HCC proliferation while it has no effect on healthy hepatocytes. Furthermore, oleic acid also induced inhibition effect on antiapoptotic protein such as Bcl-2 and c-Flip in HCC cell (40).

There are 16 peaks of bioactive compounds identified in the results of GC-MS MOLP analysis with n-hexane as solvent which are shown in Table 2. The dominant compounds in MOLP with n-hexane solvent include Cyclopentane, methyl- (53.965%); Hexadecanoic acid, methyl ester (0.375%); 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (0.247%); 9-Octadecenoic acid, methyl ester, (E)- (2.915%); and Methyl stearate (0.413%).

Groups of compounds found in MOLP, including hydrocarbons (55.221%), esters (8.802%), and alcohols (35.976%). The type of compound that has the most benefits for the body in MOLP with n-hexane solvent is ester with a percentage of 8.802%. In the Moringa plant, ester compounds are formed naturally. The chemical reaction between alcohol and acid will form an ester as the final product. Esters are widely used in medicine, including relieving pain, affecting solubility and bioavailability in patients (41).

The bioactive compounds found in MOLP with acetone as solvent are shown in Table 3. Almost 40 bioactive compounds were identified, which is the dominant compounds that are safe for the body in MOLP with acetone solvent include 3-Penten-2-one, 4-methyl- (22.592%); Hexadecanoic acid, methyl ester (0.037%); 6-Octadecenoic acid, methyl ester, (Z)- (0.044%) and Cyclohexanone(0.124%). Acetone is a semipolar solvent (42). Acetone solvent can extract small molecular mass polar compounds in Moringa seed oil and leaf powder samples.

Table 2. Components of bioactive compounds in n-hexane solvent MOLP with GC-MS analysis

Compound Group	Name of Compound	% Area	% group
Hydrocarbons	Cyclopentane, methyl-	53.965	55.22
	Toluene	1.147	
	Butylated Hydroxytoluene	0.109	
Ester	*Dimethyl phthalate*	0.391	8.80
	Diethyl Phthalate	3.792	
	Oxalic acid, cyclohexylmethyl tridecyl ester	0.669	
	Hexadecanoic acid, methyl ester	0.375	
	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	0.247	
	9-Octadecenoic acid, methyl ester, (E)-	2.915	
	Methyl stearate	0.413	
Alcohol	*1-Pentanol, 2,3-dimethyl-*	34.009	35.97
	3-Hexanol	0.317	
	2-Hexanol	0.836	
	Cyclohexanol, 2-(1-methylethyl)-	0.209	
	Phenol, 2-methoxy-3-(2-propenyl)-	0.215	
	Oleyl alcohol, chlorodifluoroacetate	0.388	

Table 3. Components of bioactive compounds in acetone solvent MOLP by GC-MS analysis

Compound Group	Name of Compound	% Area	% group
Hydrocarbons	*Propane, 2,2-dimethoxy-*	23.831	63.98
	Cyclohexane, methyl-	6.941	
	Toluene	30.1889	
	Propane, 2,2'-[methylenebis(oxy)]bis-	0.047	
	3-Ethyl-3-methylheptane	0.141	
	Cyclohexane, ethyl-	0.285	
	Ethylbenzene	0.385	
	p-xylene	0.630	
	1-Ethyl-3-methylcyclohexane (c,t)	0.062	
	o-Xylene	0.144	
	Nonane	0.284	
	Benzene, (1-methylethyl)-	0.029	
	Cyclohexane, propyl-	0.048	
	2,4-Difluorobenzene, 1-benzyloxy-	0.050	
	Benzene, 1-ethyl-3-methyl-	0.202	
	Mesitylene	0.384	
	2,2,3,3,4,4-Hexamethyltetrahydrofuran	0.186	
	Benzene, 2-ethyl-1,4-dimethyl-	0.019	
	o-Cymene	0.038	
	Undecane	0.040	
	Benzene, 1,2,3,5-tetramethyl-	0.046	

Compound Group	Name of Compound	% Area	% group
Acid	*1-Methoxy-2-propyl acetate*	0.151	0.15
Ester	*Acetic acid, butyl ester*	0.160	0.73
	4,7-Dioxooctanoic acid, methyl ester	0.158	
	2-Propenoic acid, 6-methylheptyl ester	0.020	
	Dimethyl phthalate	0.029	
	Diethyl Phthalate	0.283	
	Hexadecanoic acid, methyl ester	0.037	
	6-Octadecenoic acid, methyl ester, (Z)-	0.044	
Ketones	*Methyl Isobutyl Ketone*	1.951	34.84
	4-Penten-2-one, 4-methyl-	0.968	
	3-Penten-2-one, 4-methyl-	22.592	
	2-Pentanone, 4-methoxy-4-methyl-	0.611	
	Cyclohexanone	0.124	
	5-Hexen-2-one, 5-methyl-	0.032	
	2-Pentanone, 4-hydroxy-4-methyl-	8.394	
	Phorone	0.169	
Alcohol	*Ethanol, 2-butoxy-*	0.042	0.29
	1,3-Dioxolane-4-methanol, 2,2-dimethyl-, (S)-	0.215	
	Phenol, 2-methoxy-3-(2-propenyl)-	0.033	

The most common type of compound found in acetone solvent MOLP was hydrocarbon compound with a percentage of 63.985%. The types of ketones, acids, esters and alcohols respectively were 34.841%, 0.151%, 0.732% and 0.291%, respectively. However, from the n-hexane extract, these compounds may also come out. Ketone compounds have anticancer activity, because they are attached to aromatic amides that exhibit micromolar inhibitory activity present in fibrosarcoma (43). This inhibition is carried out by regulating lipid metabolism and suppressing blood sugar and insulin levels which will then have an impact on reducing the proliferation and differentiation activity of cancer cells (44). Toluene group, which dominates the percentage of hydrocarbon compound (30.1889%), can act as antioxidant and prevents the occurence of HCC by inducing catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxide (GPx) (45). GCMS data only shows volatile compounds present in the extract. Column heating treatment is intended to detect compounds that are rather heavy but heating is also limited to a temperature of 280 ˚C, furthermore during the GCMS process it is possible that some compounds are damaged. Heavier compounds will not be eluted so further tests are needed with LCMS.

LC-HRMS Analysis of Phytochemical Constituents of MOLP and MOSEIL

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) is a combined method of LC and MS. LC is used as a separator and MS is used as a detection system. The combination of the two provides more accurate qualitative and quantitative analysis results (46). LC-HRMS uses a high-throughput system to analyze various biomolecules. Liquid chromatography is also more powerful to elucidate bigger components in biomaterials. Some of the big compounds cannot be eluted in gas mobile phases in GC methods, but they appear in LC as far as the mobile phases are suitable. Biomedical research has used LC-HRMS as an important analytical tool to support research data (47). The total ion chromatograms of LC-HRMS MOSEIL and MOLP analysis are shown in Figure 2.

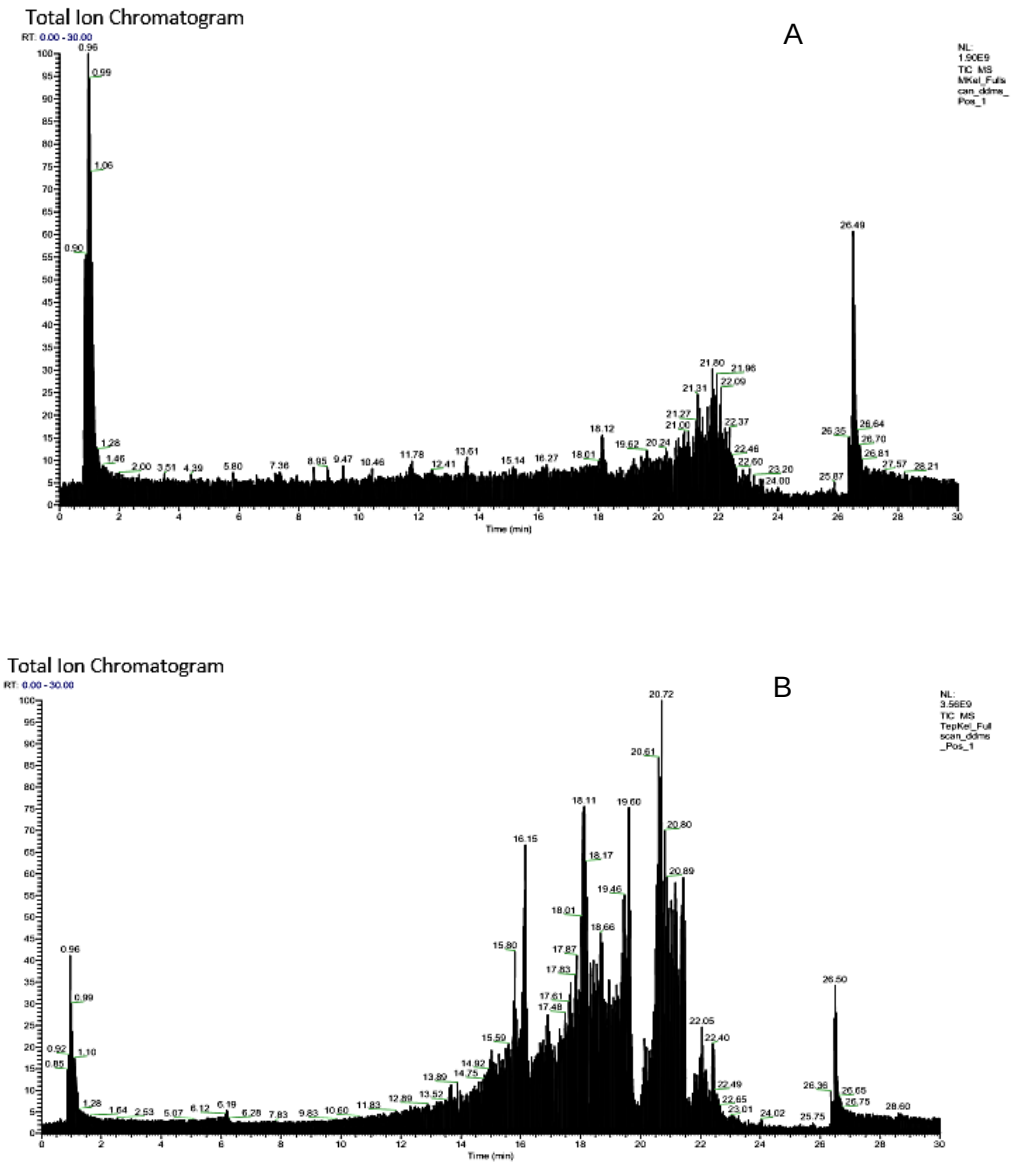

Figure 2. Chromatogram of LC-MS analysis results on MOSEIL (A) and Chromatogram of LC-HRMS analysis results on MOLP (B)

Table 4. Components of bioactive compounds analysis of LC-HRMS on MOSEIL

Compound Group	Name of Compound	% Area
Aldehyde	4-Methoxybenzaldehyde	1.04
	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	
Acid	Palmitoleic Acid	7.85
	12-Oxo phytodienoic acid	
	9-Oxo-10(E),12(E)-octadecadienoic	
	α-Eleostearic acid	
	α-Linolenic acid	
	octadec-9-ynoic acid	
	Pinolenic acid	
	Eicosapentaenoic acid	

Compound Group	Name of Compound	% Area
Ester	Diisobutylphthalate	8.83
	Reserpine	
	γ-Linolenic acid ethyl ester	
Alcohol	Cafestol	3.23
	Diacetoxyscirpenol	
Ketones	5α-Dihydrotestosterone	3.83
	Sedanolide	
	Nictoflorin	
	Andrographolide	
Amida	Trigonelline	72.55
	Oleoyl ethanolamide	
	Palmitoyl ethanolamide	
	DL-Leucineamide	
Amina	Choline	1.88
	L-Tyrosine	
	Adenosine	
Acid	Isoleucine	
	D-(+)-Tryptophan	
	Isoleucine	
	L-Histidine	
	L-Histidine	
	L-(+)-Arginine	
Ester	Acetylcholine	
Sulfur	2-(Methylthio)benzothiazole	

The results of the LC-HRMS MOSEIL analysis in Table 4 there are 7 groups of compounds, namely aldehydes, acids, esters, alcohols, ketones, and nitro compounds. The percentage of compounds that dominate is nitro compounds (72.55%). The therapeutically significant organic compounds mostly contain a nitro group. Nitro compounds have the potential to treat various diseases, especially the acid group (48). One of the constituents of alkaloids that play a role in preventing the development of cancer is trigonelline which has a potential role as an antinociceptive (49). Meanwhile, the content of other nitro compounds such as oleyl ethanolamide can reduce hepatic oxidative stress and endoplasmic reticulum stress which is the initial initiation of HCC development (50). The compounds detected through the LC-MS test were compounds with higher molecular weights such as proteins and carbohydrate derivatives. The choice of eluent can also be more varied. These compounds could be the mainstay active compounds of this plant and further studies are needed to find out more about the potential compounds in moringa seed oil.

Table 5. Components of bioactive compounds analysis of LC-HRMS on MOLP

Compound Group	Compound	Percentage
Acid	*Pinolenic acid*	33.10
	Palmitoleic Acid	
	α-Linolenic acid	
	4-Methoxycinnamic acid	
	Tetranor-12(S)-HETE	
	(R)-3-Hydroxy myristic acid	

Compound Group	Compound	Percentage
Ester	*Ethyl palmitoleate*	12.28
	Arachidonic acid ethyl ester	
	Docosahexaenoic acid methyl ester	
Alcohol	*2-(14,15-Epoxyeicosatrienoyl) glycerol*	45.87
	1-Linoleoyl glycerol	
	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	
	Levalbuterol	
	Andrographolide	
Amida	*Oleamide*	6.56
	Lidocaine	
	Palmitoyl ethanolamide	
	Piperine	
	Anandamide (AEA)	
	Oleoyl ethanolamide	
Amina	*Cetrimonium*	0.51
	Choline	
	2-(Methylthio)benzothiazole	
	Tridemorph	
	Diphenhydramine	

Analysis of LC-HRMS on MOLP in Table 5. identified as many as 5 groups of compounds. These compounds are acids, esters, alcohols, hydrocarbons, amides, amines, ketones, aldehydes and phosphate compounds. The highest percentage value was the alcohol compound group (45.87%). The results show that MOLP is a source of antioxidants and anti-inflammatory because it is rich in organic compounds (51).

Molecular Docking Analysis and VIsualization of MOLP and MOSEIL

The main bioactive compounds in MOLP and MOSEIL that have been detected through GC-MS and LC-MS analysis were selected for further molecular docking studies to determine good molecular interactions based on their affinity interactions with target proteins. The bioactive compounds in MOLP are docked with the target protein TGF-β1 and compared with control drugs, the results of which can be seen in Table 6. In Autodock Vina, the higher the negative number in the binding affinity value, the stronger and more stable the binding molecule (52). The six components are also visualized in Figure 3.

Table 6. Docking values of major compounds on MOLP and Metformin against TGF-β1

CID	Compound	Binding Affinity (kcal/mol)	Amino Acid Residue and Interaction
5318517	Andrographolide	-7.4	Hydrogen bond (Asp235, Val234, Ser127, Ser130) van der waals bond (Thr240, Ile131, Phe239, Phe124, Tyr132, Lys125, Gln126, Thr128, Leu232, Ile236)
638024	Piperine	-6.8	Hydrogen bond (Ser127, Ser130, Phe124) van der waals bond (Phe239, Thr116, Lys125, Tyr121, Tyr132, Thr128)
12788231	Cannabicyclohexa	-6.7	Hydrogen bond (Ser127) van der waals bond (Gln126, Ser130, Tyr132, Phe124, Leu232, Thr128, Ile131, Thr240, Phe239, Thr116, Thr241)

CID	Compound	Binding Affinity (kcal/mol)	Amino Acid Residue and Interaction
			Pi bond (Lys125) Alkyl bond (Tyr121)
123600	Levalbuterol	-6.5	Hydrogen bond (Phe124, Ser127, Thr116) van der waals bond (Gln126, Thr241, Tyr121, Tyr132, Ile131, Thr128, Ser130, Leu232) Pi bond (Lys125) Unfavorable acceptor (Phe239)
75536014	(12Z)-9,10,11-trihydroxy	-6.4	Hydrogen bond (Ser130, Ser127) van der waals bond (Ile131, Leu232, Thr128, His129, Val234, Phe124, Thr241, Thr116, Tyr121) Alkyl bond (Phe239) Unfavorable acceptor (Lys125)
965	9-octadecanoic acid, methyl ester	-5.4	Hydrogen bond (Seu127) van der waals bond (Asp235, Thr128, Thr240, Ile131, Tyr132, Ser130, Leu232, Thr116, Phe124) Alkyl bond (Phe239, tyr121) Pi bond (Lys125)
11634	6-octadecanoic acid, methyl ester	-5.2	Hydrogen bond (Asp235) van der waals bond (Ile236, Val234, Ser127, Thr128, Tyr132, Thr116, Thr241, Phe124, Ser130, Thr240) Pi bond (Lys125, Tyr121) Alkyl bond (Phe239, Ile131, Leu232)
4091	Metformin	-5.0	-

The binding affinity for each compound in the MOLP ranged from -7.4 kcal/mol to -5.2 kcal/mol, so it was lower than the control (Metmorphine). Metformin is one of the widely used anti-diabetic drugs and has been shown to have a protective effect against various specific diseases other than diabetes such as cardiovascular disorders, polycystic ovary syndrome and cancer. TGF- β1 as one of the cytokines involved in cancer pathogenesis is a new target for metformin. Metformin can suppress TGF- β1 receptor dimerization thereby interfering with downstream signal transduction (53). Compounds that have potential, including Andrographolide, Piperine, Cannabicyclohexa, Levalbuterol, (12Z)-9,10,11-trihydroxy, 9-octadecanoic acid, methyl ester and 6-octadecanoic acid, methyl ester can be a new therapeutic potential as an inhibitor. TGF-1 activity. 3D visualization of MOLP compound docking results with TGF-β1 is presented in Figure 3.

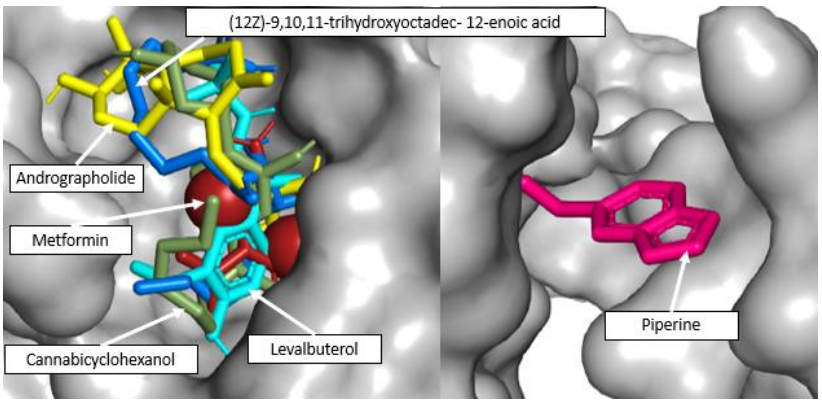

Figure 3. Visualization of MOLP compounds with lower binding affinity compared to the control on TGF-β1

Andrographolide compounds themselves have activity as anti-inflammatory, anti-platelet aggregation, potential as anti-neoplastic, as well as cell signaling, immunomodulation, and stroke (54). This compound has also been used successfully in the treatment of melanoma, prostate cancer, colorectal cancer and oral squamous carcinoma (55). The dominant compound in MOLP is piperine, where piperine has analgesic, anticonvulsant, antitumor, and anti-inflammatory roles. The anti-inflammatory activity of piperine in this chronic disease is achieved through downregulation of inflammatory pathways such as NF-κB, MAPK, AP-1, COX-2, NOS-2, IL-1β, TNF-α, PGE2, STAT3 (56). 9-octadecanoic acid, methyl

ester and 6-octadecanoic acid, methyl ester is an ester group compound that plays a role in inhibiting the proliferation and apoptosis of cancer cells, especially breast cancer cells (54). Based on the amino acid residue parameters in Table 6, it is known that there is a strong bond between all MOLP bioactive compounds and the target protein through hydrogen bonds. Hydrogen bonds play a role in the interaction of the substrate with the target protein by forming new hydrogen bonds in the protein-ligand complex (57,58). Parameters of amino acid residues in molecular docking studies are used to estimate the presence of these bonds (59). Meanwhile, the bioactive compound in MOSEIL was docked with the target protein TGF-β1 and compared with the control drug, the results of which can be seen in Table 7.

Table 7. The docking value of bioactive compounds in MOSEIL and Metformin against TGF-β1

CID	Compound	Binding Affinity (kcal/mol)	Amino Acid Residue and Interaction
108052	Cafestol	-12.2	Hydrogen bond (Ser130, Ser127, Phe124) van der waals bond (Thr128, Ile131, Tyr132, Tyr121, Thr116, Thr241) Pi bond (Lys125, Phe239)
5318767	Nicotiflorin	-9.0	Hydrogen bond (Trp330, Arg277, Leu332, Asp333, Cys389, Thr282,Glu290) van der waals bond (Cys294, Asn292,Cys285, Glu98, Pro97, Gln359, Lys388, Ser331) Pi bond (Pro99)
5280343	Quercetin	-8.1	Hydrogen bond (Tyr132, Val234) van der waals bond (Thr116, Phe124, Tyr121, Phe239, Ile131, Leu232, Gln233, Asp235, Ile236, Thr240, Thr241) Pi bond (Lys125)
422111	Diacetoxyscirpenol	-7.8	Hydrogen bond (Phe124, Ser130, Ser127) van der waals bond (Phe239, Thr116, Lys125, Tyr121, Tyr132, Thr128)
5770	Reserpine	-7.7	Hydrogen bond (Glu100, Gln959, Ser986) van der waals bond (Asp933, Ala960, Pro99, Asn283, Glu96, Glu98, Thr282, His276, Leu271, Ser274) Pi bond (Ala279) Alkyl bond (Arg989, Arg277)
965	9-octadecanoic acid, methyl ester	-5.4	van der waals bond (Asp235, Thr128, Thr240, Ile131, Tyr132, Ser130, Leu232, Thr116, Phe124) Hydrogen bond (Seu127) Alkyl bond (Phe239, tyr121) Pi bond (Lys125)
4091	Metformin	-4.9	-

Similar to MOLP, the bioactive compound MOSEIL has a lower binding affinity value than Metformin. The five bioactive compounds in MOSEIL namely Cafestol, Nicotiflorin, Quercetin, Diacetoxyscirpenol, 9-octadecanoic acid, methyl ester and Reserpine can be new therapeutic potentials as inhibitors of TGF-β1 activity. Visualization of the docking results of MOSEIL compound with TGF- β1 is presented in Figure 4.

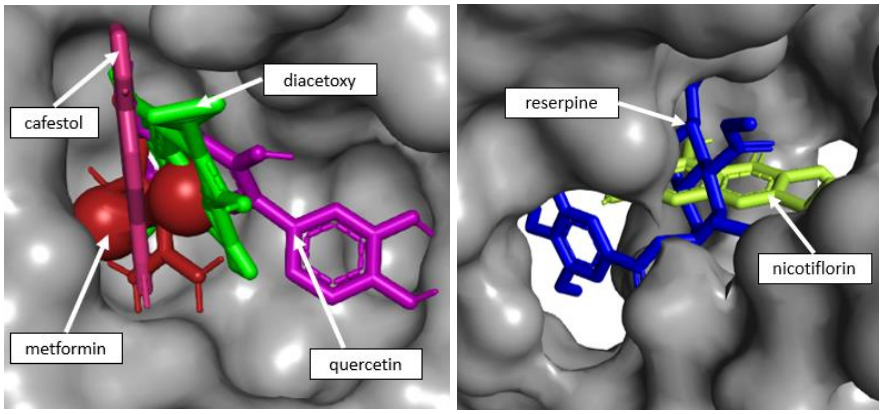

Figure 4. Visualization of MOSEIL compounds with lower binding affinity compared to the control on TGF-β1

The Cafestol compound present in MOSEIL is a type of diterpene compound that has a role in the process of inhibiting the regulation of inflammatory activity, increasing glutathione (GSH), induction of apoptosis in tumor cells and anti-angiogenesis (60). The role of Cafestol is to prevent the development of cancer by blocking the activation of carcinogens and increasing the detoxification function of the liver by reducing the effects of exposure to free radicals that cause oxidative stress (61). Other compounds such as Nicotiflorin has a protective effect and are often used in the pre-treatment of liver cancer cases to reduce serum levels of pro-inflammatory cytokines such as interleukin-1β. (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF- α) and interferon-γ (IFN- γ) (62). In the epithelial-mesenchymal transition, Quercetin compounds can signal inhibition of TGF-β1 which is the initial initiation of this transition activity through the Smad pen pathway and becomes a potential therapeutic agent, especially in the prevention of cancer cell proliferation (63). Diacetoxyscirpenol is also a potential compound in the treatment of malignant tumors, especially in patients with hypoxia. This compound will inhibit the hypoxia-inducible factor 1 (HIF-1) signaling pathway which is the pathway for angiogenesis stimulation (64). Reserpine also demonstrated the anti-fibrotic and anti-inflammatory activity of the compounds present in MOSEIL, this compound will prevent the formation of fibroblast cells by inhibiting the differentiation of mesenchymal stromal cells (MSCs) (65). After the active compounds MOSEIL and MOLP are molecularly docked with the same target protein, namely TGF-β1, both can be known to cause cancer cells. *Moringa oleifera* is also possible in the development of existing cancer treatment therapies (66). The various potentials of each compound in MOSEIL and MOLP can be used as the first step as a better cancer treatment therapy.

Conclusions

In conclusion, the analysis through GC and LC-MS tests as well as computational experiments, the active phytochemical compounds in MOLP, and MOSEIL indicates a lower binding affinity than the control drug metformin. In addition, the therapeutic potential is also supported by the presence of antioxidant properties, hepatoprotective and anti-inflammatory effects. Therefore, both materials can be proposed as candidate green materials for the prevention of the development of HCC-linked fibrosis through inhibition of TGF-β1 activity.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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