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Original Research Article

Herbal Combination of *Tithonia diversifolia*, *Moringa oleifera*, and *Curcuma longa* as Antidiabetic Agent Against iNOS and COX-2 Proteins: An *in silico* Study

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ABSTRACT

Diabetes mellitus (DM) is a condition characterized by chronic hyperglycemia, which is caused by a combination of insulin resistance and pancreatic *β*-cell dysfunction. These pathological processes are further exacerbated by oxidative stress and chronic inflammation. The enzymes inducible Nitric Oxide Synthase (iNOS) and Cyclooxygenase-2 (COX-2) have been reported to be major contributors to the inflammatory processes associated with DM. Therefore, this study aims to investigate the antidiabetic potential of a herbal combination of *Tithonia diversifolia*, *Moringa oleifera*, and *Curcuma longa* (TMC) using an *in silico* approach. The study also evaluated the inhibition of iNOS and COX-2 proteins by bioactive compounds in the treatment sample through molecular docking and dynamics simulations. Aqueous extracts of the TMC combination were analyzed using LC-HRMS to identify 17 bioactive compounds. After the screening procedure based on drug similarity and molecular properties, selected compounds were subjected to further analysis. The results showed that xanthurenic acid, piperanine, ferulic acid, and 57-dihydroxy-4-methylcoumarin were major compounds with significant binding affinities towards iNOS (-8.2, -8.8, -7.2, and -8.6 kcal/mol, respectively) and COX-2 (-7.9, -8.1, -7.2, and -8.0 kcal/mol, respectively). Molecular dynamics simulations further showed stable protein-ligand interactions, supporting their potential as alternative treatments for diabetes. In addition, the compounds exhibited relatively low toxicity, good membrane permeability, as well as effective antioxidant, anti-inflammatory, and antidiabetic bioactivities. These results showed the potential of xanthurenic acid, piperanine, ferulic acid, and 5,7-dihydroxy-4-methylcoumarin as effective inhibitors of both iNOS and COX-2, contributing to the treatment of DM.

Keywords: *Tithonia diversifolia*, *Moringa oleifera*, *Curcuma longa*, inducible Nitric Oxide Synthase, Cyclooxygenase-2, Diabetes mellitus, Molecular docking.

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by persistent high blood sugar levels (hyperglycemia) due to impaired insulin action (insulin resistance) and pancreatic *β*-cell dysfunction. The development and complications of DM are based on chronic inflammation and oxidative stress, which contribute to the damage of pancreatic cells and other body tissues.[1] Insulin resistance and impaired glucose metabolism have been reported to be closely associated with inflammatory processes, which are driven partially by the activity of proinflammatory enzymes, such as iNOS (inducible Nitric Oxide Synthase) and COX-2 (Cyclooxygenase-2).[2,3] According to previous studies, activation of iNOS often leads to an increase in NO production, which reacts with superoxide free radicals in excess to form peroxynitrite. These highly reactive compounds can damage various cell components, including lipids, proteins, and DNA[4], leading to the programmed death (apoptosis) of *β*-cells.

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In addition, NO and peroxynitrite promote chronic inflammation through the activation of inflammatory cells and the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1*β* (IL-1*β*). The development of insulin resistance is significantly influenced by these cytokines.[5] Meanwhile, COX-2 is responsible for prostaglandin synthesis from arachidonic acid to prostaglandins. The inflammatory response is exacerbated by the overproduction of prostaglandins, particularly PGE_2, which activate immune cells and lead to the release of pro-inflammatory cytokines. This exacerbates insulin resistance and metabolic dysfunction.[6] By inhibiting the activity of these enzymes, it is expected to restore insulin sensitivity and enhance glucose control in diabetic patients.

Treatment of DM using synthetic drugs has been reported to have the potential to cause adverse side effects when used for a long period. The risk of severe side effects, such as organ damage, is also a consideration. In addition, limited access and high prices make synthetic drugs less favorable as the first choice for treatment.[7] To overcome these challenges, medicinal plants have been used to treat a wide range of diseases for millennia.[8] The World Health Organization (WHO) advocates for the use of medicinal plants as an alternative approach to DM treatment.[9,10] This is due to their safety, cost-effectiveness, and minimal side effects. Combination of several active ingredients in medicinal plants provides broader benefits and reduces the risk of toxicity.[11] Several medicinal plants have been recognized to exhibit bioactive compounds that can inhibit DM through inflammatory and oxidative pathways. Combination of *Tithonia diversifolia*, *Moringa oleifera*, and *Curcuma longa* (TMC) offers synergistic potential in addressing various inflammatory conditions. Each component of TMC contains several bioactive compounds with pharmacological activities.

Although existing literature has explored the antidiabetic effects of TMC in a single analysis, there are limited studies on their combination. Previous studies showed that sesquiterpenoids, diterpenoids, and flavonoids are the dominant compounds found in *Tithonia diversifolia* extract.[12] This extract exhibits inhibitory effects on the formation of proinflammatory cytokines, NO, and COX-2.[13] *Moringa oleifera* leaves represent a significant source of anti-inflammatory flavonoids and antioxidants, specifically quercetin, kaempferol, and myricetin.[14] The effect of the extract strengthens endogenous antioxidants, such as SOD, catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and glutathione-S-transferase (GST). This leads to a reduction in lipid peroxidation products and downregulates the expression of pro-inflammatory mediators, including COX-2, MCP-1, IL-6, 1L-*β*, TNF-α, and NO synthase.[15] *Curcuma longa* possesses both antioxidant and anti-inflammatory properties. By lowering NO and MDA levels, it efficiently reduces oxidative stress and also lowers inflammation by blocking the production of inflammatory mediators, such as COX-2, prostaglandins, NO, phospholipase, and lipoxygenase.[16] Therefore, this study aims to assess the antidiabetic potential of a herbal combination of TMC in inhibiting iNOS and COX-2 activities through the modulation of inflammatory and oxidative pathways *in silico*. Molecular docking analysis can be used to predict the interaction between bioactive compounds with key amino acid residues in the active sites of iNOS and COX-2 enzymes.

Materials and Methods

TMC

Powdered extracts of *Tithonia diversifolia* (Batch No. 240429.PTN.F.MLG.357.121), *Moringa oleifera* (Batch No. 240130.KLR.F.KJY.001), and *Curcuma longa* (Batch No. 240614.KNT.L.MLG.596.227), which was referred to as TMC, were obtained from Materia Medica Batu (7°52'03"S 112°31'09"E), Malang, East Java, Indonesia. TMC were extracted using 500 mL of water boiled to 95ºC according to Adharini[17] with modification. These extracts were weighed at 50 g each and then extracted by brewing in boiling water and thoroughly stirred for 5 minutes. Following extraction, the crude extract was cooled and then filtered through a cloth. Subsequently, the filtrate was subjected to vacuum filtration to obtain a liquid extract. For 24 hours, the extract was stored at -80°C till dry, which was obtained by freeze-drying the frozen liquid extracts.

Bioactive compound analysis of extracts using LC-HRMS

The content of bioactive compounds within the TMC extract combination was investigated through LC-HRMS (Liquid Chromatography-High Resolution Mass Spectrometry) analysis conducted at the Laboratorium Riset Terpadu (LRT), Universitas Brawijaya. Furthermore, the HPLC analysis was performed using a Dionex Ultimate™ 3000 RSLCnano system equipped with a microflow meter (Thermo Fisher Scientific; USA). Using a mobile phase consisting of 0.1% formic acid in water and acetonitrile, supplied at a flow rate of 40 µL/min, a chromatographic separation was performed on a Hypersil GOLD™ PFP (Thermo Fisher Scientific; USA) at 30°C. Q Exactive™ (Thermo Fisher Scientific; USA) in positive ion mode was used as mass spectrometric detection. The full scan had a resolution of 70,000, while the data-dependent MS/MS scan exhibited a resolution of 17,500. Compound Discoverer software with the mzCloud MS/MS Library (Thermo Fisher Scientific; USA) was used for data acquisition and processing.

Data mining

A total of 17 bioactive compounds of the TMC combination were obtained from LC-HRMS results. The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was applied to retrieve the canonical SMILES and 3D structures of each compound. Furthermore, the RCSB Protein Data Bank (https://www.rcsb.org/) served as the source for the 3D structural data of iNOS (PDB ID: 3E7G) and COX-2 (PDB ID: 5F19) proteins.

Screening of bioactive compounds based on drug-likeness, physicochemical characteristics, and lipophilicity

Drug-likeness, physicochemical characteristics, and lipophilicity screening were analyzed using the SwissADME webserver (https://www.swissadme.ch/) to determine drug potency and understand the physicochemical as well as the metabolic properties of bioactive compounds. Drug-likeness assessment was conducted using a set of established rules, such as the Lipinski, Veber, Ghose, Egan, and Muegge criteria. Physicochemical characteristics of compounds include several H-bond donors, H-bond acceptors, rotatable bonds, and TPSA. Furthermore, lipophilicity properties were based on MLOGP, WLOGP, and XLOGP.[18] The results of compounds that passed the screening were then subjected to molecular docking analysis.

Molecular docking

A total of 7 compounds that passed drug-likeness screening and physicochemical characteristics were prepared using OpenBabel integrated with Pyryx software to minimize energy.[19] iNOS and COX-2 protein structures were preprocessed using Biovia Discovery Studio 2019 software, comprising the removal of water molecules and contaminant ligands. Furthermore, iNOS protein inhibitor and COX-2 native ligand were determined based on their binding to the target protein in the RCSB PDB database as a control. Molecular docking between compounds and proteins was performed with a specific docking approach at the active site using AutoDockVina[20] in PyRx 0.8 software.[21] iNOS and COX-2 were designated as receptors, while compounds were designated as ligands. The specific area for molecular docking was set for iNOS at grid center X: 53.981 Y:23.208 Z: 76.994 with dimensions X: 16.711 Y: 23.208 Z: 30.013 and COX-2 at grid center X: 28.130 Y: 30.046 Z: 62.798 with dimensions X: 27.348 Y: 27.794 Z: 30.374. Docking validation was performed by redocking with several replicates. PyMOL and Biovia Discovery Studio 2019 were applied to visualize the docking results.[22] The 4 compounds showing the weakest binding affinities were selected for subsequent investigation using molecular dynamics simulations.

Molecular dynamics

The Yet Another Scientific Artificial Reality Application (YASARA) software version 23.4.25 was used for molecular dynamics simulations, applying the AMBER14 force field. Furthermore, the system was set based on the physiological conditions of human cells, namely 37°C, pH 7.4, 1 atm, salt content 0.9%, water density 0.997 g/ml, and pressure 1 atm for 20 ns, which was automatically stored every 25 ps. Simulations were performed using the md_run program. The results were analyzed with the md_analyze program for RMSD and md_ analyze results for RMSF.[23]

Prediction of bioactivity, toxicity, and membrane permeability

The PerMM webserver (https://permm.phar.umich.edu/) was used to predict the cell membrane penetration ability of each compound. Environmental conditions were set based on human physiological conditions to 310 K and pH 7.4. Energy transfer values were analyzed and simulated through 3D bilayer membranes. The potential activity of bioactive compounds was analyzed using PASS Online Way2drug (http://www.way2drug.com/passonline/index.php).[24] Each compound was analyzed for toxicity type using the ProToxII webserver (https://tox-new.charite.de/protox_II/). Toxicity prediction included a range of adverse effects, such as hepatotoxicity, immunotoxicity, carcinogenicity, cytotoxicity, and mutagenicity.[25]

Results and Discussion

Oxidative stress and chronic inflammation were key contributors to the underlying mechanisms that drove DM. Pancreatic *β*-cells, the primary source of insulin, were susceptible to damage from high levels of NO generated by iNOS. Damage to *β* cells resulted in decreased insulin secretion.[26] Increased NO production through iNOS led to oxidative stress, which in turn impaired insulin signaling in tissues, thereby contributing to the development of insulin resistance. Consequently,

cells exhibited a reduced capacity to absorb glucose from the blood.[27] Increased COX-2 activity led to increased production of proinflammatory prostaglandins, such as PGE_2, which exacerbated inflammation and aggravated insulin resistance. The interaction of PGE_2, produced by COX-2, with cellular receptors resulted in the impairment of insulin signaling pathways, which were essential for the proper control of glucose metabolism.[28] Therefore, inhibition of these 2 proteins was an important strategy in DM management.

Identification of TMC bioactive compounds using LC-HRMS

LC-HRMS analysis showed the presence of 17 bioactive compounds within the TMC combination extract (Table 1). This chromatogram exhibited peaks corresponding to bioactive compounds within the sample, distinguished by retention times (RT) that varied between 0 and 30 minutes (Figure 1). The most prominent peak was indicative of the predominant compound.

Table 1: Bioactive compounds found in TMC combination extracts

Compound	Formula	CID	RT [min]
Maltol	$C_6H_6O_3$	8369	2.788
Betaine	$C_5H_{11}NO_2$	247	0.932
L-Valine	$C_5H_{11}NO_2$	6287	1.365
Uracil	$C_4H_4N_2O_2$	1174	1.428
D-(+)-Proline	$C_5H_9NO_2$	8988	1.184
4-Hydroxybenzaldehyde	$C_8H_8O_2$	126	5.312
Caffeine	$C_8H_{10}N4O_2$	2519	4.675
Xanthurenic acid	$C_{10}H_7NO_4$	5699	3.22
Isoleucine	$C_6H_{13}NO_2$	6306	0.14
3-Hydroxypyridine	C_5H_5NO	7971	1.371
Piperanine	$C_{17}H_{21}NO_3$	5320618	13.955
N-Acetyl-L-leucine	$C_8H_{15}NO_3$	70912	5.807
Pilocarpine	$C_{11}H_{16}N_2O_2$	5910	4.608
Ferulic acid	$C_{10}H_{10}O_4$	445858	7.009
Nicotinic acid	$C_6H_5NO_2$	938	1.396
Vanillin	$C_8H_8O_3$	1183	6.1
5,7-Dihydroxy-4 methylcoumarin	$C_{10}H_8O_4$	5354284	5.409

Screening drug-likeness, physicochemical characteristics, and lipophilicity

Drug-likeness screening of TMC combination compounds aimed to select compounds that had characteristics similar to drugs. The screening results showed that of the 17 compounds contained in the TMC combination, 7 bioactive compounds did not include amino acids or fatty acids and had violations of drug-likeness parameters of more than 2 (Figure 2A). Furthermore, the 7 bioactive compounds included caffeine, xanthurenic acid, piperanine, pilocarpine, ferulic acid, vanillin, and 5,7-dihydroxy-4-methylcoumarin. These compounds had molecular weights below 500 g/mol and TPSA below 140 Å2, showing their penetration into membrane lipids (Figure 2B). The partition coefficient values, which included MLOGP, XLOGP, and WLOGP, were below 10 ensuring that the semipermeable membrane could be penetrated (Figure 2C). Furthermore, the number of hydrogen bond donors and acceptors and rotatable bonds that were not too high also played a role in maintaining specificity and easily passing through cell membranes (Figure 2D).

Based on the screening results, 4 compounds (xanthurenic acid, piperanine, ferulic acid, and 5,7-dihydroxy-4-methylcoumarin) were predicted to penetrate the plasma membrane and reach the target protein. This was based on their characteristics, which were classified as small and less polar compounds (molecular weight < 500 g/mol and TPSA < 140 Å2) ensuring easy passage through the cell membrane.[29] Partition coefficients (MlogP, WlogP, and XlogP) that were not too high and low were used to predict the lipophilic characteristics of compounds.[30] The number of acceptor and donor hydrogen bonds a compound could form also affected its membrane permeability based on its polarity. Highly polar compounds had difficulty penetrating cell membranes.[31]

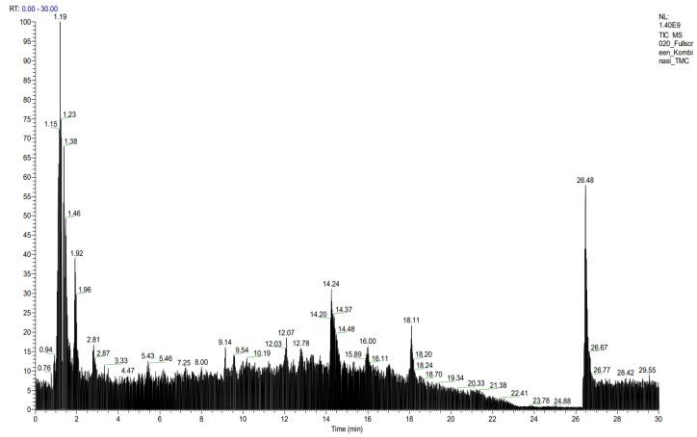

Figure 1: Total ion chromatogram (TIC) of LC-HRMS analysis results of TMC extract

Molecular docking simulation of compounds in TMC combination

Molecular docking simulations were performed on 7 bioactive compounds with iNOS and COX-2 proteins to analyze the interaction of each compound with its target protein. All binding affinity values for all complexes were shown in Table 2. Based on their most negative binding affinity values, xanthurenic acid, piperanine, ferulic acid, and

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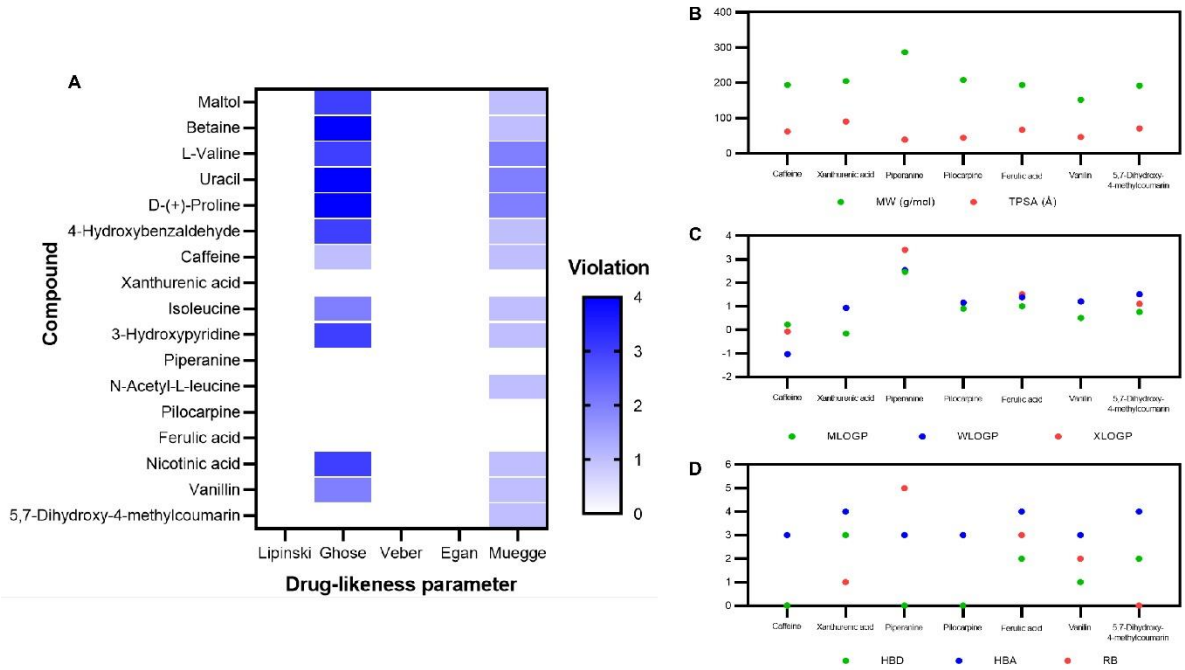

Figure 2: Analysis of drug-likeness, physicochemical characteristics, and lipophilicity of active compounds in TMC combination. A) drug-likeness of compounds resulting from LC-HRMS, B) molecular weight and TPSA, C) partition coefficient, and D) number of hydrogend bond acceptors, number of hydrogend bond donors, rotatable bond

Table 2: Interaction of TMC bioactive compounds on target proteins

Ligand	Protein	Binding affinity (kcal/mol)	Hydrogen bond	Hydrophobic interaction
Inhibitor/Native Ligand (Control)	iNOS	**-8.4**	Asn370	Ile201, Ala439, Met434, Met374, Phe369, Trp194, Tyr489
	COX-2	**-12.0**	Asn382, Thr212, Tyr148, His214, Gln203	Leu391, His386, His388, His207, Val447, Ile408, Phe395, Val295, Phe200, Ala199, Phe210, Ala202, Tyr385, Trp387
Caffeine	iNOS	-6.9	Asn370, Cys200	Phe369, Trp194, Cys200, Ala197
	COX-2	-6.2	Thr206	Ala202, Tyr385, His386, His207, His388
Xanthurenic acid	iNOS	-8.2*	-	Trp194, Phe369, Cys200, Leu209
	COX-2	-7.9*	Gln203, Asn382, Tyr385, Trp387	Ala202
Piperanine	iNOS	-8.8*	Tyr489	Phe369, Trp194, Met355, Arg199, Ala197
	COX-2	-8.1*	Thr206	Ala202, His386
Pilocarpine	iNOS	-6.3	Asn370	Trp194, Trp372, Cys200

	COX-2	-6.5	Asn382, His207, Tyr385	Leu391, His207, His388
Ferulic acid	iNOS	-7.2*	-	Phe369, Trp194
	COX-2	-7.2*	Asn382	Ala202
Vanillin	iNOS	-6.6	Asn370	Phe369, Trp194
	COX-2	-6.2	Thr206, His388, Trp387	Ala202
5,7-Dihydroxy-4-methylcoumarin	iNOS	-8.6*	-	Phe369, Trp194, Leu209, Cys200
	COX-2	-8.0*	Tyr385, Thr206	Ala199, Leu391, Ala202, Leu390

Note: An asterisk (*) denotes the four compounds with the lowest binding affinities, which were selected for further analysis due to their affinities being comparable to or better than the control. The underlined amino acid residues mark the potential inhibition sites of protein similar to the control.

5,7-dihydroxy-4-methylcoumarin were selected for subsequent molecular dynamics simulations. Piperanine and 5,7-dihydroxy-4-methylcoumarin had more negative binding affinity values compared to the inhibitor when interacting with iNOS. This suggested that the compound had the potential to be a more effective inhibitor of the target protein than the control because it had a stronger receptor-ligand interaction.[32] Meanwhile, all 4 compounds when bound to COX-2 had higher binding affinity values than the native ligand.

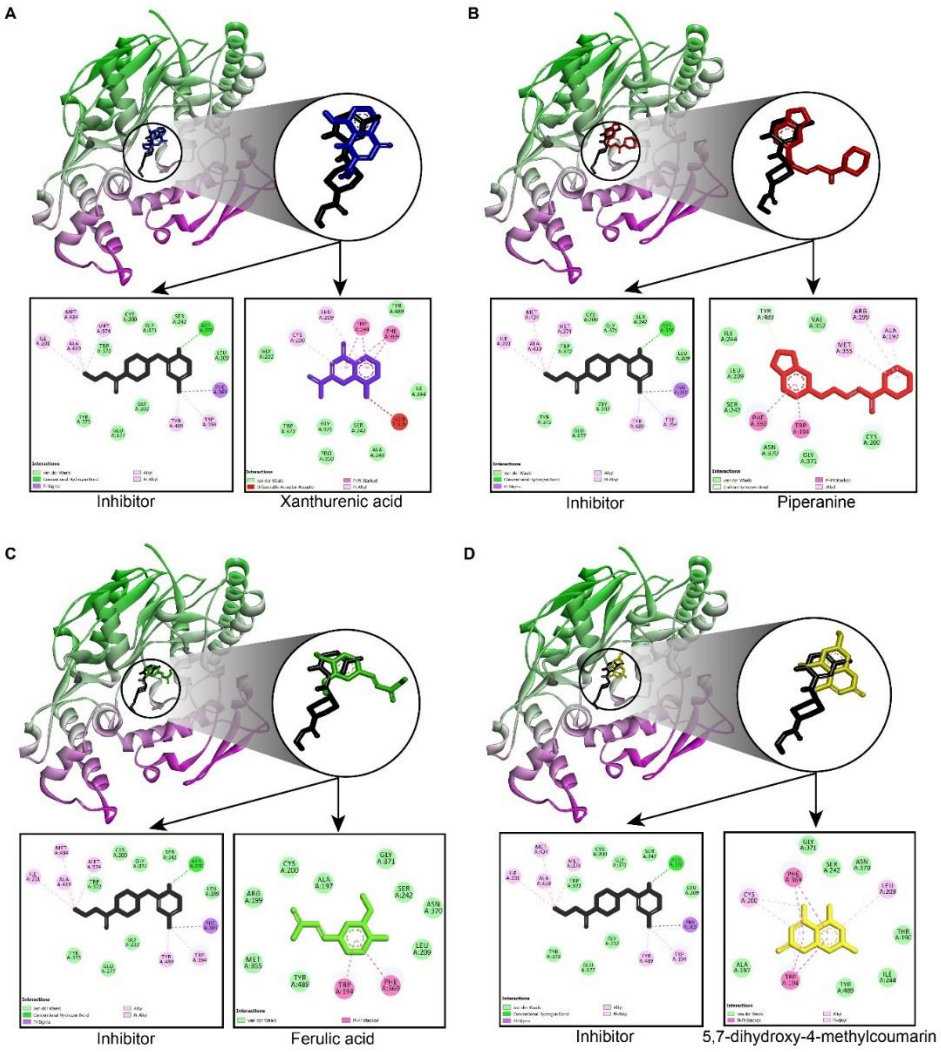

Figure 3: Interaction of bioactive compounds on iNOS. A) xanthurenic acid-iNOS, B) piperanine- iNOS, C) ferulic acid- iNOS, and D) 5,7-dihydroxy-4-methylcoumarin- iNOS.

Visualization of protein and ligand interactions was shown in Figures 3 and 4. The compounds within the TMC combination showed a binding affinity for iNOS and COX-2 within the same active site as the control. Xanthurenic acid, piperanine, ferulic acid, and 5,7-dihydroxy-4-methylcoumarin bind to iNOS by forming hydrophobic bonds at the same residues as the inhibitors, namely Trp194 and Phe369. In comparison, the inhibitor bonded to iNOS at residues Ile201, Ala439, Met434, Met374, Phe369, Trp194, and Tyr489. Furthermore, xanthurenic acid is bound to COX-2 by forming a hydrophobic bond similar to the native ligand, Ala202, and 5 hydrogen bonds at residues Gln203 and Asn382. Piperanine bound to COX-2 active site by creating 3 hydrophobic bonds at the same residues as the native ligand, Ala202, and His386. Ferulic acid formed 1 hydrogen bond as well as 1 hydrophobic bond and was bound to residues similar to the native ligands, namely Asn382 and Ala202. 5,7-dihydroxy-4-methylcoumarin bound to COX-2, forming 5 hydrophobic bonds at the same residues as the native ligand, namely Ala199, Leu391, and Ala202.

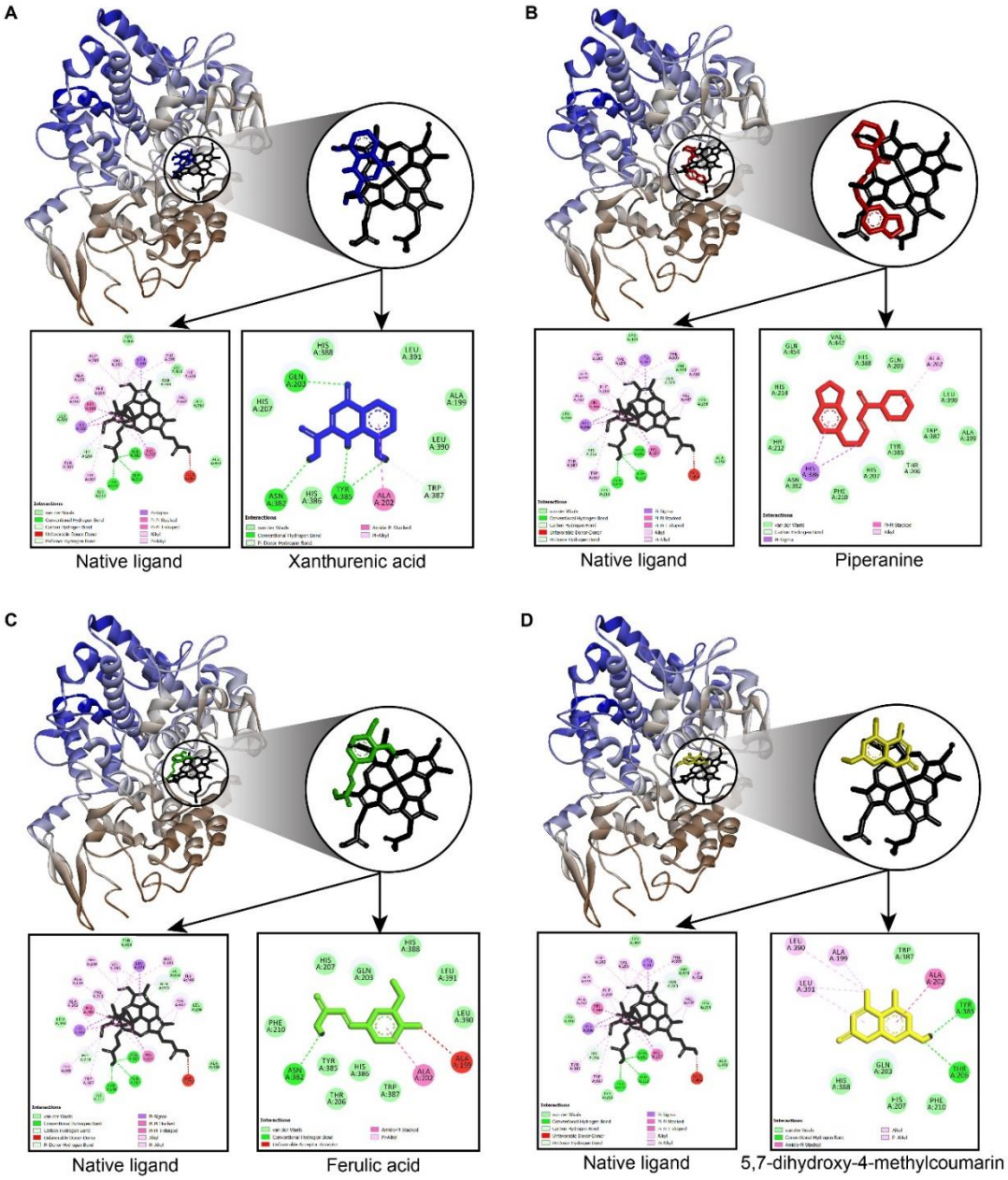

Figure 4: Interaction of bioactive compounds on COX-2. A) Xanthurenic acid-COX-2, B) piperanine-COX-2, C) ferulic acid-COX-2, and D) 5,7-dihydroxy-4-methylcoumarin-COX-2.

Molecular dynamics

The stability of protein-ligand complexes and the structural integrity of protein after ligand binding were examined by applying molecular dynamics simulations, with particular emphasis on the interaction between iNOS and COX-2 and the bioactive substances of the TMC combination. This simulation incorporated the following parameters, namely the number of hydrogen bonds, the root-mean-square deviation (RMSD) of the ligand conformation, the RMSD of backbone atoms, and the RMSF of amino acid residues. Molecular dynamics results showed that the entire complex was stable. This was distinguished by RMSD values below 3 Å and negligible fluctuations (Figures 5A-B, 6A-B). The backbone and conformation RMSD values in all complexes had values below 3Å, suggesting that the ligand when interacting with protein during the simulation was stable.[33]

In this study, the 4 compounds exhibited strong interactions with iNOS, as evidenced by the high number of hydrogen bonds formed, potentially rivaling the interactions observed with the control compound. However, fewer hydrogen bonds were observed with COX-2. The formation of more hydrogen bonds contributed to stronger protein-ligand binding.[34] RMSF analysis showed that the values of all compounds were similar to the control. This showed that the 4 compounds had potential as iNOS and COX-2 inhibitors (Figures 5D, 6D). Fluctuations in the RMSF values of some residues were also observed, and residues with below 3Å also showed residue stability.[35] Despite these potentials, some protein-ligand interactions were found to be unstable at certain residues similar to the control.

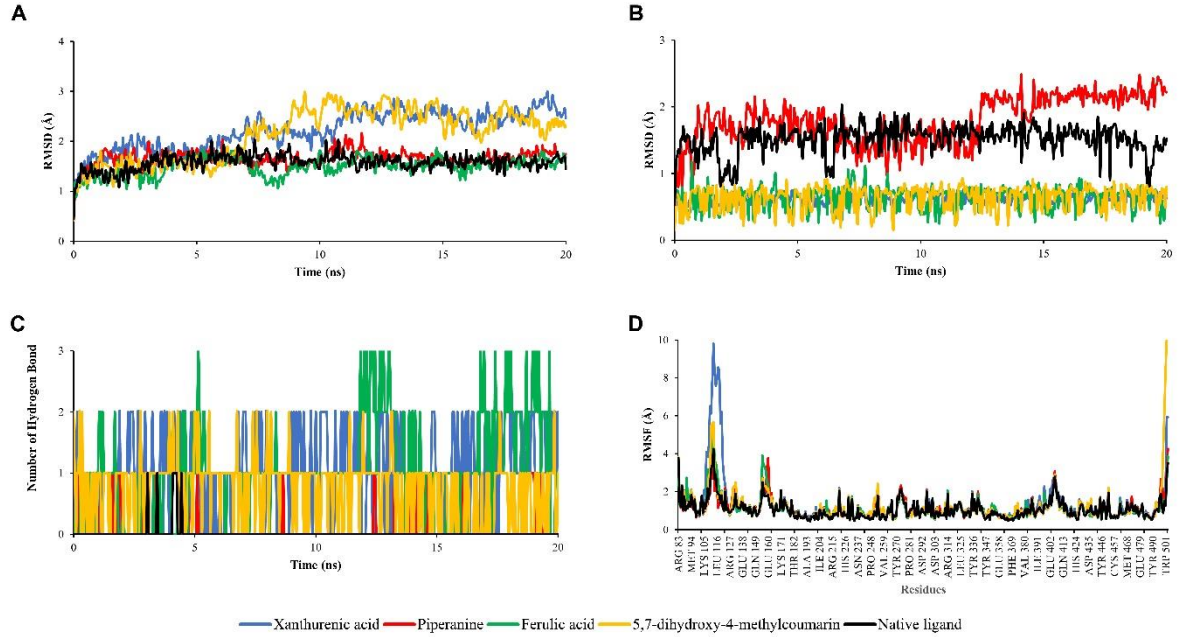

Figure 5: Structural dynamics of selected bioactive compounds from TMC complexed with iNOS. A) RMSD of backbone atoms, B) RMSD of ligand conformation, C) number of hydrogen bonds, D) RMSF per residue of each complex. Blue: xanthurenic acid, red: piperanine, green: ferulic acid, yellow: 5,7-dihydroxy-4-methylcoumarin, black: native ligand/inhibitor.

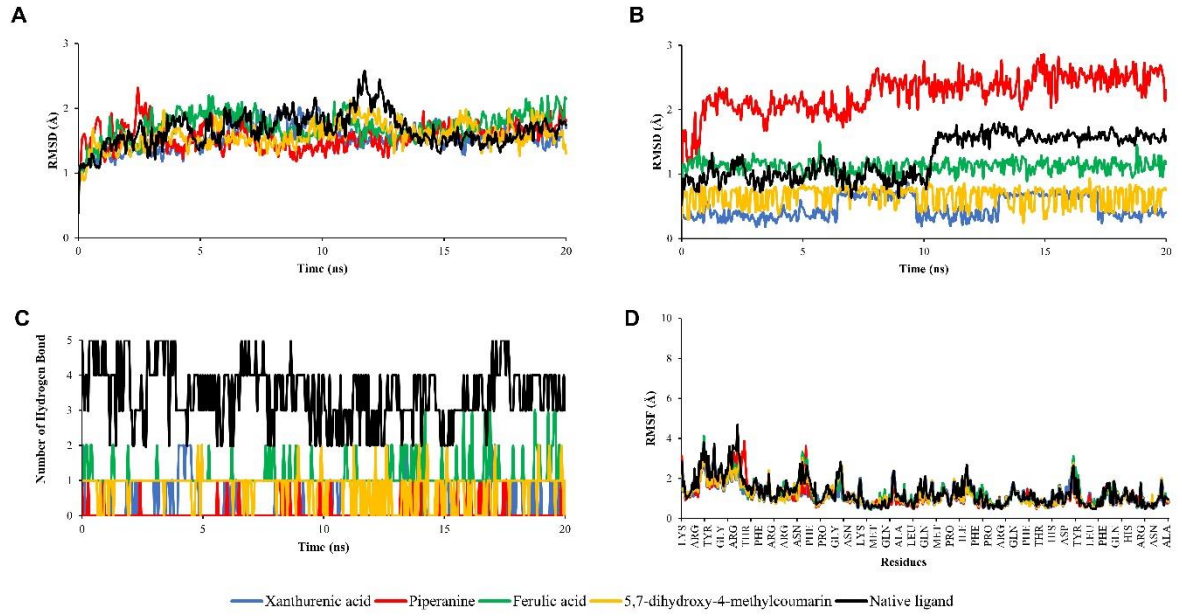

Figure 6: Structural dynamics of selected bioactive compounds from TMC complexed with COX-2. A) RMSD of backbone atoms, B) RMSD of ligand conformation, C) number of hydrogen bonds, D) RMSF per residue of each complex. Blue: xanthurenic acid, red: piperanine, green: ferulic acid, yellow: 5,7-dihydroxy-4-methylcoumarin, black: native ligand/inhibitor.

Potential bioactivity, toxicity, and membrane permeability of TMC bioactive compounds

A total of 4 compounds were analyzed to predict their possible bioactivity in inhibiting DM. Based on the bioactivity results, these compounds had relatively high Pa values according to parameters related to antioxidant, anti-inflammatory, and antidiabetic (Figure 7A). Prediction of bioactivity of the compounds was shown by the principle of quantitative structure-activity relationship (QSAR) which was

determined from the values of Pa (probable activity) and Pi (probable inactivity). When Pa > 0.7 the most likely similar drug, a value of 0.5 < Pa < 0.7 suggested less similarity to known drugs but a lower probability of experimental activity. Meanwhile, when Pi < Pa < 0.5, then the likelihood of activity was low, this condition could signify the emergence of a new chemical class.[36] The compounds ferulic acid and 5,7-dihydroxy-4-methylcoumarin had more active bioactivity compared to the other 2 compounds. Toxicity prediction results showed that piperanine and ferulic acid exhibited toxic potential for carcinogenicity and mutagenicity, respectively (Figure 7B). Meanwhile, other compounds, xanthurenic acid, and 5,7-dihydroxy-4-methylcoumarin, were relatively non-toxic. The other compounds could cause a type of toxicity and their dosage just be monitored. Conformational changes of the compounds when penetrating the cell membrane were showed in Figure 7C. Compounds with high energy transfer values exhibited decreased membrane permeability. Based on the energy transfer value, piperanine penetrated the cell membrane more easily compared to the other compounds (Figure 7D).

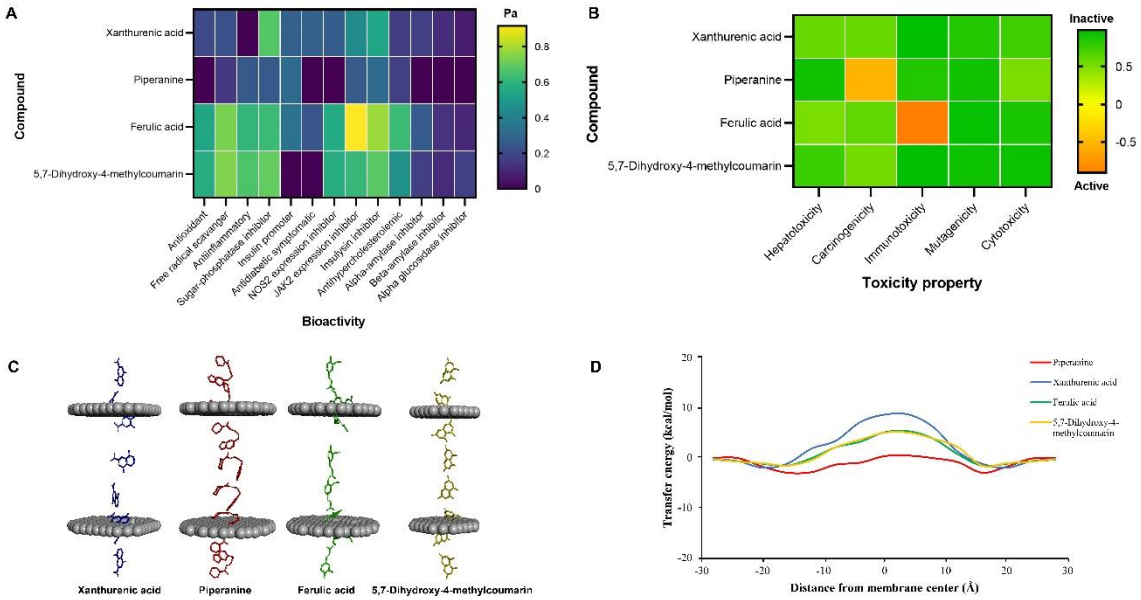

Figure 7: Potential bioactive compounds of TMC. A) Bioactivity, B) toxicity, C) membrane permeability, and D) energy transfer value

A molecule's penetration ability was inversely proportional to its energy. Lower energy molecules could readily translocate across the plasma membrane.[37] The easiest compounds to penetrate the plasma membrane started from piperanine and the hardest from xanthurenic acid. Molecules exhibited dynamic behavior within the cell membrane, constantly reorienting to align their polar and nonpolar regions with the membrane's hydrophilic and hydrophobic domains. The nonpolar portion integrated into the lipid bilayer, while the polar portion interacted with the aqueous environment at the membrane surface.[38]

The 4 compounds showed potential to inhibit iNOS and COX-2 in DM. Previous studies suggested that xanthurenic acid had antioxidant properties and could increase the production of reduced GSH.[39] Ferulic acid reduced inflammation by preventing the generation of inflammatory cytokines and the release of harmful molecules (ROS and RNS). This procedure was carried out by suppressing the activity of iNOS and COX-2 through the NF-κB pathway process.[40] Ferulic acid, known for its ability to lower blood sugar, protect against cell damage, reduce inflammation, and promote cell survival, potentially prevent kidney damage caused by oxidative stress.[41] Piperanine was an alkaloid compound that had been shown to have several health benefits, particularly in combating chronic conditions such as reducing insulin resistance, anti-inflammatory effects, and improving fatty liver disease.[42] 5,7-Dihydroxy-4-methylcoumarin was known for its ability to neutralize harmful free radicals, making it a valuable substance in antioxidant applications.[43] This compound also reduced the production of NO and the activity of genes in inflammation, specifically iNOS and COX-2.[44] However, this study was a predictive in silico approach, and further analysis was needed to validate the potential of this compound in DM.

Conclusion

In conclusion, combination of TMC extracts contained 4 potential bioactive compounds, namely xanthurenic acid, piperanine, ferulic acid, and 5,7-dihydroxy-4-methylcoumarin, which were predicted to have antidiabetic activity by inhibiting iNOS and COX-2 activity, respectively. This combination had potential as a safe alternative DM treatment compared to conventional drugs. Further validation through *in vitro* and *in vivo* tests was needed to evaluate the effectiveness and safety of using this combination of extracts in living organisms.

Conflict of Interest

The authors declared no conflict of interest with the data contained in the manuscript.

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