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In Silico Approach for Hepatoprotective Activity of *Piper crocatum* Leaf toward Cytochrome P450 2E1 Protein

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ABSTRACT

Liver plays important roles in metabolism of harmful xenobiotics. Prolonged exposure to chemicals, daily dietary supplements, or pharmaceutical drugs may cause liver damage or hepatotoxicity. Acetaminophen is a well-known pharmaceutical drug causing hepatotoxicity through generation of reactive metabolite called N-acetyl-p-benzoquinone imine through Cytochrome P450 2E1 (CYP2E1) metabolism. In the present study, we intended to predict the possible hepatoprotective properties of red betel (*Piper crocatum)* leaves. Quantitative structure activity relationship (QSAR) was used to predict the antioxidant and anti-inflammatory potential of major compounds of red betel, namely, eugenol, isoeugenol, chavibetol, hydroxychavicol, and allylpyrocatechol. Molecular docking was performed to analyze binding mode of the compounds toward CYP2E1 protein. Network analysis using Search Tool for the Retrieval of Interacting Genes was performed to determine pathways affected by CYP2E1. QSAR prediction shows that these compounds had moderate probability as antioxidant and anti-inflammatory agents. All of the docked compounds occupied the active site of the protein. Allylpyrocatechol and Hydroxychavicol had higher calculated binding affinity than indazole known as CYP2E1 inhibitor. CYP2E1 inhibition will probably reduce liver inflammation, as it is related to many inflammatory pathways. Based on QSAR, molecular docking, and network analysis, active compounds contain in red betel leaves had hepatoprotective property through inhibition of inflammatory pathway related to CYP2E1.

Keywords: Hepatoprotective, liver, molecular docking, piper, quantitative structure activity relationship

INTRODUCTION

Liver plays important roles in metabolism of harmful xenobiotics. Prolonged exposure to chemicals, daily dietary supplements, or pharmaceutical drugs may cause liver damage or hepatotoxicity.[1] The best known cytotoxicity mechanism is through the increase of reactive oxygen species (ROS) level which will deplete the antioxidant defense mechanism and leads to higher levels of oxidative stress and progressive inflammation.[2] This conditions lead to liver malfunction and liver disease.[3]

Mechanism of hepatotoxicity is diverse; it depends on the chemical agents inducing the hepatotoxicity. Acetaminophen (APAP) is a well-known pharmaceutical drug. It causes hepatotoxicity through the generation of reactive metabolite called N-acetyl-p- benzoquinone imine (NAPQI) through Cytochrome P450 2E1 (CYP2E1) metabolism. CYP2E1 is a P450 enzyme belonging to a superfamily of hemeproteins and mainly functioning in metabolizing hydrophobic low weight xenobiotics. CYP2E1 responsible for liver damage through glutathione decrease which cause mitochondrial oxidative stress.[4] Inhibition of CYP2E1 activity after APAP administration may act as natural protective mechanism of the cells against the NAPQI formation.[5] The amino metabolic enzymes, aspartate amino transferase, and alanine amino transferase levels generally increase in damaged liver, which mark liver dysfunction.[6] Liver damage also marked by the increase of inflammatory markers including Ccl2, Ccr2,

Ccl3, tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-12, and inducible nitric oxygen synthase.[7]

Ethnomedicine is traditionally used, especially in Indonesia, because of its easy accessibility, low cost, and perceived less side effects.[8] *Piper* is an important medicinal plants used in various traditional treatments.[9] It has been widely known due to its biological properties including detoxification, antimutagenic, antioxidative, anti-inflammatory, and anti-bacterial activities.[10] The previous study found that piper leaf extract contains phenolic compounds eugenol, isoeugenol, chevibetol, hydroxychavicol, and allypryrocatechol.[9] However, the mechanism of hepatoprotective effect of piper has not been completely reported. Rapid development of bioinformatics and chemioinformatics technologies has enabled preliminary drugs screening *in silico* by referring to their structures using quantitative structure activity relationship (QSAR) and receptor structure using molecular docking. This study aims to evaluate hepatoprotective potential of *Piper crocatum* leaf major compounds by assessing its antioxidant, anti-inflammatory and binding potential to CYP2E1 protein.

METHODS

Biological Activity Spectra Prediction of *P. crocatum* Leaf

Biological Activity Spectra of compound found in *P. crocatum* leaf compounds (eugenol, isoeugenol, chevibetol, hydroxychavicol, and allypryrocatechol) were predicted using PASS online that is accessible through http://www.pharmaexpert.ru/PassOnline.[11] Simplified molecular-input line-entry system for every compound was used as input and was obtained from PubChem database. The calculated compounds Probability active (Pa) and Probability inactive (Pi) as antioxidant and anti-inflammatory saved. Pass online predicted the biological activity of query compound based on its structural similarity compared to the known active compound. Pa value was defined as probability for the query compound to be active for corresponding biological activity and vice versa for Pi. If Pa >0.9, the expected probability to find inactive compounds in the selected set was very low. If 0.5< Pa <0.7, the chance to find the activity in experiment was less, but the compound was not so similar to known pharmaceutical agents.[11]

Binding Mode and Binding Affinity Prediction of *P. crocatum* Leaf

The protein used as receptor in the docking study was protein subjected to hepatoxicity, namely CYP2E1. AutoDock Vina program was used for molecular docking of *P. crocatum* leaf compounds.[12] Crystal structure of CYP2E1 in complex with indazole was retrieved from RCSB protein data bank, with PDBID 3E6I.[13] Energy minimization using NAMD and VMD program was performed to find the best conformation of the protein structure. The receptor preparation was performed by extracting crystallography water and any other bound ligand. PubChem database was used to get three-dimensional structure of *Piper betel* major compounds: eugenol, isoeugenol, chavibetol, hydroxychavicol, and allylpyrocathecol.[9] *P. betel*'s compound was used as there was no publication yet about *P. crocatum* major compound during the analysis of this study. As *P. betel* and *P. crocatum* were closely related, they were expected to contain more and less similar active compounds. The search space used (grid box) was positioned on the active site of CYP2E1, using bound ligand as reference. The molecular docking was performed using default configuration then redocked to validate the results. Root mean square deviation (RMSD) of predicted binding mode and crystal binding mode was calculated using hungarian algorithm. Then, the calculated binding affinity was saved. Pymol molecular graphic program was used for the visualization.[14] Intramolecular interaction of protein-ligand complex calculated using PoseView accessible through proteinplus web server (https://proteins.plus/).

Interaction Network

CYP2E1 protein-protein network interactions were built on Search Tool for the Retrieval of Interacting Genes (STRING) (STRING/Proteins) which can be accessed through https://string-db.org/cgi. STRING is a web-based biological database to build prediction of protein-protein interactions using known experimental data, neighborhood, databases, gene fusion, co-occurrence, co-expression, and text mining. The analysis was performed to predict affected signaling pathway by CYP2E1 inhibition.

RESULTS AND DISCUSSION

QSAR Analysis

In this study, PASS online shows that five compounds found in *P. crocatum* leaf have antioxidant and anti-inflammatory properties. The biological activity prediction of these compounds showed various biological actions which can be seen from various Pa: Pi levels of antioxidant and anti-inflammatory activities. It can be observed that based on structural properties hydroxychavicol and allylpyrocatechol have the highest antioxidant probability around 0.533 compared to other compounds, while isoeugenol has the highest anti-inflammatory property about 0.5 [Table 1].

Docking of *P. crocatum* Leaf Compounds against CYP2E1

Molecular docking was performed with protein subjected to hepatotoxicity CYP2E1 as receptor. The docking results were validated using redocking. The extracted bound ligand (indazole) was docked back to CYP2E1. RMSD calculated from docked and crystal confirmation was <2 Å (data not shown), which was lower than the widely acceptable cut off. [12] Among the 5 ligands based on AutoDock vina score, allylpyrocatechol and hydroxychavicol possessed the highest binding affinity towards CYP2E1 around -7.3 kcal/mol than other compounds including bound ligand [Table 2].

The binding conformation visualization of the docked compounds against the CYP2E1 active site, which had close proximity to Heme as cofactor of CYP2E1 is shown in Figure 1.

Binding residues of CYP2E1 with ligands were also analyzed. Residue Thr303 interacted with the docked ligands conformation except isoeugenol which interacted with Phe478, whereas Ala299 only interacted with hydroxychavicol

Table 1: Biological activity prediction of the major compounds of *Piper crocatum* leaf

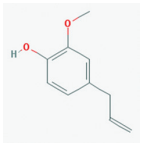
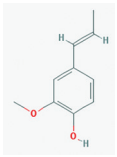
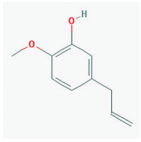
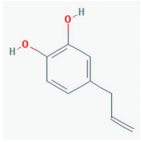
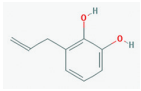
Compound	Structure	Antioxidant		Anti-inflammatory	
		Pa	Pi	Pa	Pi
Eugenol		0.474	0.008	0.503	0.056
Isoeugenol		0.47	0.008	0.56	0.041
Chavibetol		0.474	0.008	0.503	0.056
Hydroxychavicol		0.533	0.005	0.533	0.048
Allylpyrocatechol		0.533	0.005	0.518	0.052

Table 2: Details of calculated binding affinity of five major compounds found in *Piper crocatum* leaf toward CYP2E1

Compound	Binding Affinity (kcal/mol)
Allylpyrocatechol	-7.3
Hydroxychavicol	-7.3
Eugenol	-5.2
Isoeugenol	-4.4
Chavibetol	-4.2
Bound Ligand	-6.3

[Table 3]. Both allylpyrocatechol and hydroxychavicol had directed hydrophobic interaction toward Phe298 [Figure 2].

In this study, QSAR and molecular docking were used on major compounds to assess hepatoprotective potential. QSAR analysis showed that the compounds found in *P. crocatum* have moderate probability activity as antioxidant and anti-inflammatory agents. These properties are related to class of compound found in *P. crocatum* which are phenolic compounds. Phenolic compound is known for its free-radical scavenging ability. Thereby, Hepatoprotective mechanism of *P. crocatum* was probably due to the active compounds' antioxidant and anti-inflammatory activities.

All of the docked compound occupies the active site of the protein in which allylpyrocatechol and hydroxychavicol have higher calculated binding affinity than CYP2E1 inhibitor

Table 3: Hydrogen bonding residues between CYP2E1 and major compounds of *Piper crocartum* leaf

Compound	Hydrogen Bond
Allylpyrocatechol	Thr303
Hydroxychavicol	Thr303, Ala299
Eugenol	Thr303
Isoeugenol	Phe478
Chavibetol	Thr303
Bound Ligand	Thr303

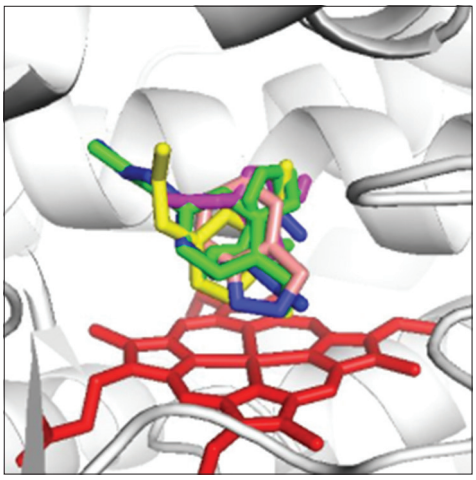


Figure 1: Superimposed binding mode docked compounds towards Cytochrome P450 2E1. The protein is shown as helical ribons with Heme cofactor (Red). The ligand (Yellow) eugenol, (Green) isoeugenol, (Blue) chavibetol, (Purple) hydroxychavicol, (Pink) indazole, and (Cyan) allypryrocatechol are shown as stick representation with only polar hydrogen shown

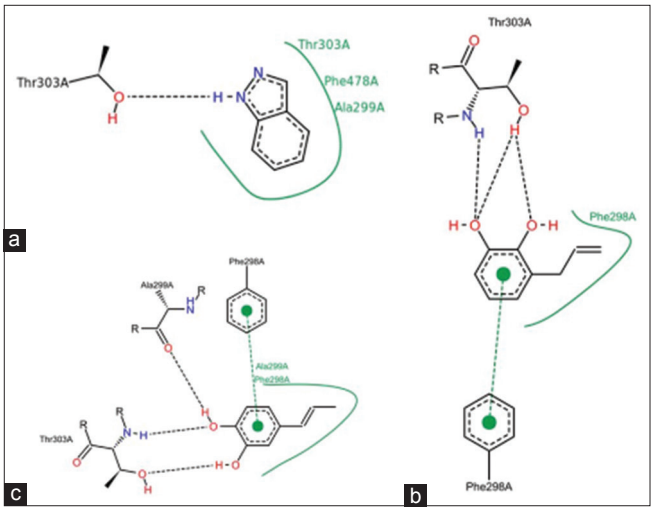


Figure 2: Intramolecular interaction of (a) bound ligand indazole compared to (b) Allylpyrocatechol and (c) Hydroxychavicol against Cytochrome P450 2E1. Most striking difference between indazole and allylpyrocatechol and hydroxychavicol was the existence of directed hydrophobic interaction

known as indazole. This interaction is important in drug design, since this interaction increases the ligands affinity. Based

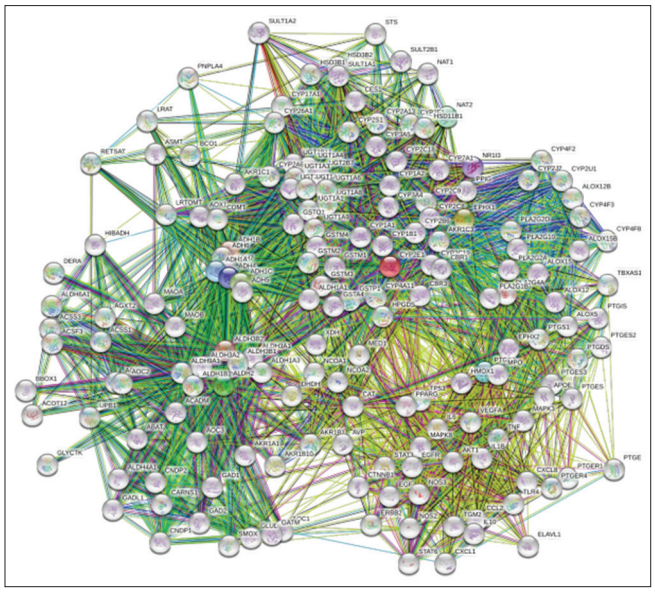


Figure 3: Mechanism of Cytochrome P450 2E1 protein towards Hepatoprotective Activity used Search Tool for the Retrieval of Interacting Genes. The network had 171 nodes and 2712 edges. The lines represent the interaction of protein between the genes, the circles represent the genes, and the results within the circles show the protein structure. The network has far more interaction as the proteins interact more with each other

on this, compound founds in *P. crocatum* serve as potential binder/inhibitor of CYP2E1. Since CYP2E1 is pivotal protein in production of harmful metabolite like NAPQI leading to hepatotoxicity. Compounds found in *P. crocatum* leaf also exert its possible hepatoprotective activity through the inhibition of hepatotoxic metabolite by CYP2E1.

To emphasize the anti-inflammatory and anti-oxidant effect of CYP2E1, protein networking analysis was performed on STRING. A network of 171 nodes with 2712 edges was build. As can be seen in Figure 3, CYP2E1 is closely related to other metabolic proteins, the CYPs proteins and antioxidant and detoxifying enzymes such as Alanine Dehydrogenase, Glutathione S-transferase, and UDP-glucoronosyl transferase. In a more distance network, it can be seen that it is related to many inflammatory cytokines such as NOS2, IL-10, CxCL1, CCl2, TLR4, CxClB, IL1b, TNF, CxCl8, PPARg, NOS3, ERB2, STAT, ERB2, and many more as well as to many inflammatory related proteins such as Arachidonate-5lipoxygenase. It is also related to many apoptosis related pathway such as TP53.

CONCLUSION

Five major compounds found in *P. crocatum* leaf have moderate antioxidant and anti-inflammatory potential. They also have potential binding activity towards CYP2E1 protein. Protein network analysis shows that CYP2E1 protein is related to many antioxidant and detoxifying enzymes, as well as inflammatory and apoptotic pathway. Inhibition of CYP2E1 may result in reduction of inflammation and ROS which resulted in hepatoprotective activity. *In vitro* and *in vivo* tests are necessary to determine the effect of *P. crocatum* Leaf.

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