



Computational study of *Curcuma zanthorrhiza* Roxb compounds as potential antidiabetic towards alpha-amylase, alpha-glucosidase, and Keap1 inhibition

[Estudio computacional de compuestos de *Curcuma zanthorrhiza* Roxb como posibles antidiabéticos frente a la inhibición de alfa-amilasa, alfa-glucosidasa e Keap1]

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Abstract

Context: *Curcuma zanthorrhiza* Roxb is traditionally used as a medicinal herb that is believed might cure some diseases. However, there is still a lack of information about the underlying mechanism of bioactive compounds from *C. zanthorrhiza*, which has antidiabetic properties.

Aims: To analyze the bioactive compounds of *C. zanthorrhiza* as inhibitors of alpha-amylase, alpha-glucosidase, and Keap1, which play a significant role in diabetes mellitus (DM) progression.

Methods: The bioactive compounds of *C. zanthorrhiza* were screened its antidiabetic activity by PASS server. To determine the interaction between selected active compounds of *C. zanthorrhiza*, molecular docking was performed by PyRx 0.8 software and visualized in Biovia Discovery Studio and PyMol, respectively. The pharmacological properties of selected active compounds of *C. zanthorrhiza* were then evaluated using the Lipinski rule and SwissADME.

Results: There were 20 from 60 bioactive compounds of *C. zanthorrhiza*, which have antidiabetic properties. The molecular docking analysis revealed that five from 20 bioactive compounds might be inhibiting alpha-amylase, alpha-glucosidase, and Keap1. Curcumin might be potential as an alpha-amylase, alpha-glucosidase, and Keap1 inhibitor. Curcumin and xanthorrhizol were the compounds that meet pharmacological properties criteria.

Conclusions: The data suggested that *C. zanthorrhiza* compounds may be a promising inhibitor candidate of three key target proteins that have been highly involved in DM. Further research is needed to validate the *in vitro* and *in vivo* activity of the *C. zanthorrhiza* compounds or be used as a primary compound for target DM progression.

Keywords: alpha-amylase; alpha-glucosidase; antidiabetic; *Curcuma zanthorrhiza*; Keap1.

Resumen

Contexto: *Curcuma zanthorrhiza* Roxb se usa tradicionalmente como una hierba medicinal que se cree que podría curar algunas enfermedades. Sin embargo, todavía falta información sobre el mecanismo subyacente de los compuestos bioactivos de *C. zanthorrhiza*, que tiene propiedades antidiabéticas.

Objetivos: Analizar los compuestos bioactivos de *C. zanthorrhiza* como inhibidores de la alfa-amilasa, alfa-glucosidasa y Keap1, que juegan un papel significativo en la progresión de la diabetes mellitus (DM).

Métodos: Los compuestos bioactivos de *C. zanthorrhiza* se cribaron su actividad antidiabética por el servidor PASS. Para determinar la interacción entre compuestos activos seleccionados de *C. zanthorrhiza*, se realizó el acoplamiento molecular mediante el software PyRx 0.8 y se visualizó en Biovia Discovery Studio y PyMol, respectivamente. A continuación, se evaluaron las propiedades farmacológicas de compuestos activos seleccionados de *C. zanthorrhiza* utilizando la regla de Lipinski y SwissADME.

Resultados: Se encontraron 20 de 60 compuestos bioactivos de *C. zanthorrhiza*, que tienen propiedades antidiabéticas. El análisis de acoplamiento molecular reveló que cinco de los 20 compuestos bioactivos podrían estar inhibiendo la alfa-amilasa, la alfa-glucosidasa y Keap1. La curcumina podría ser un inhibidor de la alfa-amilasa, alfa-glucosidasa e Keap1. La curcumina y el xanthorrhizol fueron los compuestos que cumplieron con los criterios de propiedades farmacológicas.

Conclusiones: Los datos sugirieron que los compuestos de *C. zanthorrhiza* pueden ser un candidato inhibidor prometedor de tres proteínas diana clave que han estado altamente involucradas en la DM. Se necesitan más investigaciones para validar la actividad *in vitro* e *in vivo* de los compuestos de *C. zanthorrhiza* o para usarse como compuesto principal para la progresión de la DM diana.

Palabras Clave: alfa-amilasa; alfa-glucosidasa; antidiabético; *Curcuma zanthorrhiza*; Keap1.

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INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disease characterized by a high glucose level due to either insufficient insulin or insulin resistance (Gofur et al., 2018; 2019). It has been predicted that DM will be continuing to grow worldwide around 693 million by 2045 (Cho et al., 2018). Type 2 DM (T2DM) covers most DM cases globally, affecting every individual and society nowadays and more susceptible to severe infection. DM patients get the second most requiring intensive treatment for coronavirus disease 2019 (COVID-19) and had higher mortality than those without DM (Wang et al., 2020; Zhu et al., 2020). Thus, the management of T2DM is urgently needed during the COVID-19 pandemic and before it will become a serious epidemic in the near future (Wild et al., 2004; Cho et al., 2018; Lim and Pranata, 2020).

Lifestyle modification, such as alcohol consumption, westernization diet, and sedentary lifestyle, was considered a critical component responsible for elevated T2DM prevalence (Schmidhuber and Shetty, 2005; Mozaffarian et al., 2009). The high glucose level in T2DM stimulates the excessive production of reactive oxygen species (ROS), leading to an imbalance with the antioxidant defense system. Thus, it has been considered that molecular injury and T2DM pathogenesis were linked to oxidative stress state (Gofur et al., 2020). The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway plays a crucial role as a master antioxidant through controls multiple genes for self-defense caused by the presence of elevated free radicals (David et al., 2017; Atho'illah et al., 2019). Nrf2 is an inactive form in the cytoplasm in the normal state and binds with Kelch-like ECH-associated protein 1 (Keap1) as its negative regulator. Nrf2 will dissociate from Keap1 under cellular stress, translocate to the nucleus, and encode multiple antioxidants genes to upregulate antioxidant enzyme synthesis (Yang et al., 2014; David et al., 2017). Further, targeting the Nrf2/Keap1 signaling pathway might have a beneficial impact on the T2DM treatment.

The common diabetic treatment was currently used hypoglycemic agents, such as metformin, sulfonyleureas, and glucosidase inhibitor. The glucosidase inhibitor targeted the carbohydrate hydrolyzing enzyme, mainly alpha-amylase and alpha-glucosidase. Alpha-amylase breaks down the long starch chain, then alpha-glucosidase continues to degrade the oligosaccharides/disaccharides, thus increasing postprandial glucose. Blockade of both alpha-glucosidase and alpha-amylase will be the potential target to control postprandial hyperglycemia (Gong et al., 2020). However, the long-term gluco-

sidase inhibitor used will possibly raise serious adverse effects (Chiasson et al., 2002). T2DM was overgrowing worldwide at an alarming rate in the last few years. Therefore, there is an increasing demand for the development of antidiabetic therapies based on medicinal plants with low side-effect. It is estimated that from 30 000 species of plants existing globally, only 15% have known their pharmacological potential (De Luca et al., 2012). Interestingly, the previous study reported that approximately 800 plants possessed antidiabetic activity, with 33 plants that were possibly consistent with DM treatment, and several plant metabolites interfered either with the alpha-amylase and alpha-glucosidase activities or Nrf2/Keap1 signaling pathway (Patil et al., 2011; Nair et al., 2013; Tan and de Haan, 2014; Li et al., 2018; Alqahtani et al., 2019; Rahayu et al., 2021).

Curcuma zanthorrhiza Roxb (family *Zingiberaceae*), also known as 'Java turmeric', or 'temulawak' as its local Indonesian name, is an important traditional herb in the genus *Curcuma* that provides food and medicinal properties (Shahid et al., 2021). Secondary metabolites are abundant in the *Curcuma* genus, primarily in the form of curcuminoids and terpenoids. The major sesquiterpenoid in *C. zanthorrhiza* is xanthorhizol, which is abundant and not found in *C. domestica*. (Jantan et al., 2012). *C. zanthorrhiza* is traditionally used in Indonesia as a health supplement called "jamu" because it might cure several diseases (Ruslay et al., 2007). The previous study also demonstrated that *C. zanthorrhiza* or its active constituents possessed antioxidant properties (Jantan et al., 2012), anticancer (Tee et al., 2012), antihyperglycemic (Kim et al., 2014), and less toxic (Rahmayunita et al., 2018). However, although the beneficial effect of *C. zanthorrhiza* has been well studied, there is still a lack of information about how and which phytochemicals of *C. zanthorrhiza* might involve in Keap1/Nrf2 signaling on T2DM. Herein, the present study elucidated the molecular mechanism insight of *C. zanthorrhiza* as antidiabetic through computational studies.

MATERIAL AND METHODS

Biological activity prediction

The bioactive compounds from *C. zanthorrhiza* from the previous study by Jantan et al. (2012) were screened for their antidiabetic activity using PASS (Prediction of Activity Spectra for Substance) server (<http://www.way2drug.com/PASSOnline/predict.php>). Approximately 60 bioactive compounds predicted their Pa (probability of being active) related to the DM and Nrf2 pathway. Pa chosen for the current study was Pa > 0.5 to obtain a probability higher than

50% (Christina et al., 2021). The compounds with at least two mechanisms of action related to either DM or Nrf2 pathway were selected and used for molecular docking.

Ligands and proteins preparation

The 3D structure of ligands resulted from PASS server screening was collected in .sdf format from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Ligands were then converted into .pdb format using PyMol software (Schrödinger Inc., LLC) and minimized using Open Babel in Pyrx 0.8 (The Scripps Research Institute, California). The 3D structure of protein target, including alpha-glucosidase (2ZE0), alpha-amylase (3BAJ), and Keap1 (4IQK), were obtained from Protein Data Bank (PDB) (<https://www.rcsb.org/>). The protein target was removed for its water molecules and unnecessary ligands before performing the molecular docking. Acarbose (CID: 41774) was used as a natural inhibitor for alpha-amylase and alpha-glucosidase (Nazir et al., 2018), whereas N,N'-naphthalene-1,4-diylbis (4-methoxybenzenesulfonamide) (CID: 1073725) was used as a natural inhibitor of Keap1 (Marcotte et al., 2013).

Molecular docking evaluation

Molecular docking was performed using selected compounds of *C. zanthorrhiza* that related to the antidiabetic and Nrf2 pathway after screening by PASS Server (Table 1). Molecular docking was analyzed using Pyrx 0.8. Ligands docking to alpha-amylase was set to $x = 11.4281$, $y = 15.3272$, and $z = 40.6025$ with dimensions (Angstrom) $x = 18.2772$, $y = 18.7606$, and $z = 19.1048$. Ligands docking to alpha-glucosidase was set to $x = 7.9145$, $y = 4.3526$, and $z = 14.0891$ with dimensions (Angstrom) $x = 17.0178$, $y = 16.2121$, and $z = 18.8556$. Ligands docking to Nrf2 was set to $x = -45.9612$, $y = 4.8802$, and $z = -9.8586$ with dimensions (Angstrom) $x = 20.2192$, $y = 20.9256$, and $z = 20.9674$. The results were then visualized using PyMol and Biovia Discovery Studio v20 (Dassault System, Biovia corp.).

Drug likeness and pharmacokinetic properties of *C. zanthorrhiza*

Drug likeness predictions were analyzed based on Lipinski's Rule of Five (Lipinski, 2004), including molecular weight (MW), LogP value, and the number of H-bond donor, H-bond acceptor, rotatable bond, and total polar surface area (TPSA) using Swiss-ADME physicochemical properties (<http://www.swissadme.ch/>). The term pharmacokinetics refers to the existence of therapeutic substances in the organism in various parameters, includ-

ing absorption, distribution, metabolism, excretion, and toxicity (ADMET). In the process of discovering new drugs candidate, an improved pharmacokinetic profile is essential. The drugs candidates may have not only high biological activity/low toxicity but also its mechanism as a therapeutic target in the organism. The early ADMET estimation for developing drug candidates was highly preferable due to it might prevent wasting time and resources (Daina et al., 2017; Isyaku et al., 2020).

RESULTS AND DISCUSSION

Antidiabetic-related activity screening

The biological activity prediction of *C. zanthorrhiza* was revealed by the PASS server. Surprisingly, from the 60 bioactive compounds which have tested, it was shown that only 20 bioactive compounds were related to both antidiabetic and Nrf2 (Fig. 1). The mechanism of action from *C. zanthorrhiza* bioactive compounds at least as sugar phosphatase inhibitor, HMOX1 expression enhancer, antioxidant, fructose 5-dehydrogenase inhibitor, NF-E2 related factor 2 stimulant, insulin promoter, and free radical scavenger. (E, Z)-Farnesol showed the highest probability as a sugar-phosphatase inhibitor ($P_a = 0.857$), among others. Camphor showed the highest probability of NF-E2 related factor 2 stimulants ($P_a = 0.706$). Curcumin showed the highest probability as an HMOX1 expression enhancer ($P_a = 0.826$) and free radical scavenger ($P_a = 0.766$). Alpha-phellandrene showed the highest probability as insulin promoter ($P_a = 0.649$), among others. (Z)- β -Ocimene showed the highest probability as an antioxidant ($P_a = 0.649$), among others. Xanthorhizol showed the highest probability as a fructose 5-dehydrogenase inhibitor ($P_a = 0.649$), among others.

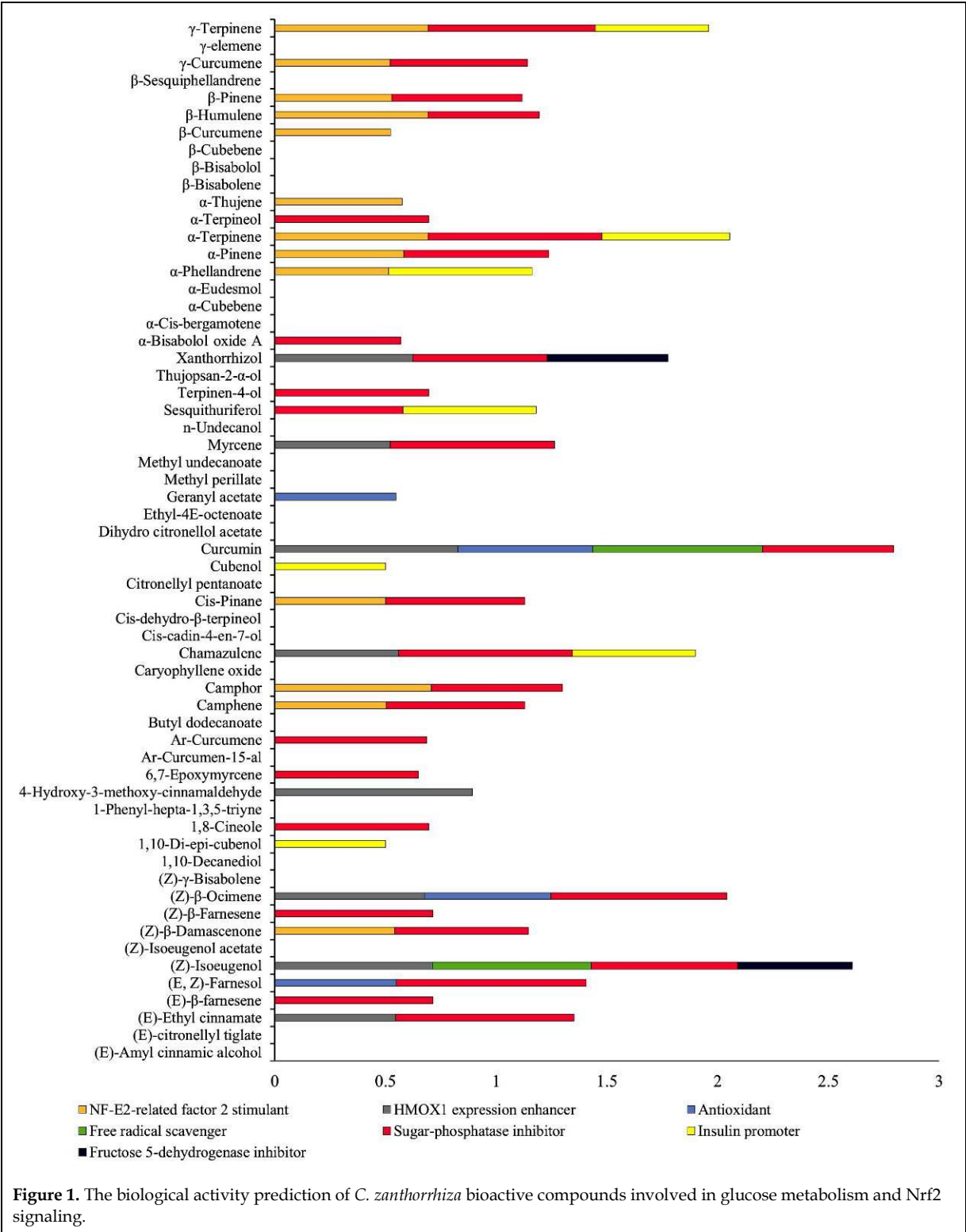
The development of drug candidates for curing several diseases, including T2DM, is still challenging throughout the world. Nowadays, the rapid development of computer science, resulting in a computational approach (*in silico*), gained many interests to evaluate many drug candidates in preclinical studies. Besides, it might reduce the cost and reduce the failure rates in the clinical phase of drug discovery (Wu et al., 2020). The present study was predicting the bioactive compounds from *C. zanthorrhiza*, which might be a promising candidate as an antidiabetic. According to the previous study, *C. zanthorrhiza* contained three curcuminoids, 19 monoterpenoids, 22 sesquiterpenoids, two phenols, one cinnamate, three fatty esters, one cinnamaldehyde, and the rest is fatty alcohol (Jantan et al., 2012). Thus, the one curcuminoid, 12 monoterpenoids, five sesquiterpenoids, one phenol, and one cinnamate, which might involve

glucose or fructose metabolism, and Keap1 inhibitor candidates (Fig. 2).

The energy activity of selected *C. zanthorrhiza* with alpha-amylase

The molecular docking result discovered that five from 20 bioactive compounds of *C. zanthorrhiza* have binding affinity values closed with acarbose as the

natural inhibitor of alpha-amylase. The summarized of the four bioactive compounds are listed in Table 1. The four bioactive compounds from *C. zanthorrhiza* are sesquiterpenoids, including β -humulene, chamazulene, γ -curcumene, and xanthorrhizol and the rest is curcumin as curcuminoid. Curcumin was found to serve the strongest ability to bind with alpha-amylase complex compared with other bioactive compounds.



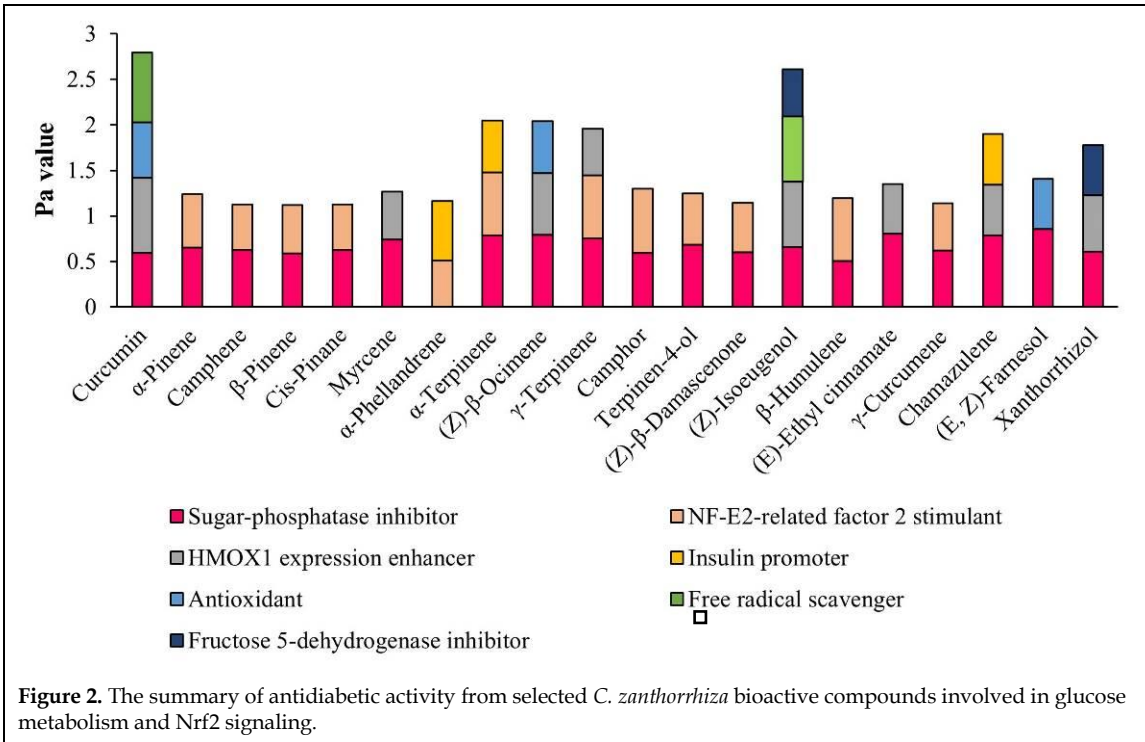


Table 1. The binding affinity and the amino acid interaction between selected *C. zanthorrhiza* bioactive compounds with alpha-amylase complex.

Protein	Compound	Binding affinity (kcal/mol)	Hydrogen bond interaction	Van der Waals interaction
Alpha-amylase (3BAJ)	Acarbose	-7.6	Asp197, Asp300, Gln63, Thr163, Tyr151	Ala106, Ala198, Glu233, Gly104, Gly164, His101, His305, Ile235, Ile51, Leu162, Leu165, Lys200, Trp58, Tyr62, Val107, Val234
	Curcumin	-8.5	Asp300, Gln63, Glu233, Thr163	Arg195, Asn105, Asp197, Gly164, His299, Ile51, Leu165, Trp58, Trp59, Val107
	β-Humulene	-7.0	-	Ala198, Asp197, Asp300
	Chamazulene	-7.3	-	Asp300, Gln63
	γ-Curcumene	-7.0	-	Arg195, Asp197, Asp300, Gln63, Thr163, Trp58
	Xanthorrhizol	-7.5	Asp197	Ala198, Arg195, Asp300, Gln63, His101, His305, Trp58

The binding affinity values are based on the Gibbs free energy, hence larger negative values result in a more stable and intense ligand-protein interaction (Pertami et al., 2021; Atho’illah et al., 2021). The molecular docking analysis revealed that all ligands have bound in the same site in alpha-amylase complexes, such as Asp197 and Asp300. The hydrogen bond in-

teraction with the alpha-amylase complex showed only in curcumin and xanthorrhizol (Fig. 3).

One of the common treatments for T2DM was controlling postprandial hyperglycemia through inhibiting the breakdown of the carbohydrates. Acarbose is a common drug to inhibit carbohydrate digestion though it suppresses alpha-glucosidase and alpha-amylase enzymes work. Pancreatic alpha-amylase is

an important enzyme that breakdown the carbohydrates into monosaccharide. The monosaccharide is then degraded by alpha-glucosidase to produce glucose then enter the bloodstream. Moreover, inhibiting these two key enzymes might delay the cells' glucose uptake and reduce blood sugar levels (Kajaria et al., 2013). The present study demonstrated that four sesquiterpenoids were interacting with the alpha-amylase complex. Curcumin and xanthorizol interact with Asp197 and Asp300 amino acid residues in the alpha-amylase complex. The previous study reported that Asp197 works as the nucleophile in the hydrolysis of polymeric substances. Further, the Asp300 is considered the main residue during hydrolysis by optimizing substrate orientation (Nazir et al., 2018; Alqahtani et al., 2019). The docking result confirmed that the selected *C. zanthorrhiza*, especially curcumin and xanthorizol, interacts with several key amino acid residues of alpha-amylase, which beneficial impact glucose metabolism in T2DM patients.

The energy activity of selected *C. zanthorrhiza* with alpha-glucosidase

The molecular docking result also found that five from 20 bioactive compounds from *C. zanthorrhiza* had close binding affinity values with acarbose as the

natural alpha-glucosidase inhibitor. Then the summarized of the five bioactive compounds are listed in Table 2. Curcumin was found to serve the strongest ability to bind with alpha-glucosidase complex compared with other bioactive compounds. The molecular docking analysis revealed that all ligands have bound in the same site in alpha-glucosidase complexes, such as Asp60, Asp199, Asp326, and Gln167. Surprisingly, only curcumin demonstrated the hydrogen bond interaction between selected *C. zanthorrhiza* bioactive compounds with alpha-glucosidase complex (Fig. 4).

The following result on alpha-glucosidase also found that the selected compounds of *C. zanthorrhiza* were occupied the same binding site with acarbose, including Asp60, Asp199, Asp326, and Gln167. Previous studies reported that Asp199 and Asp326 are highly conserved catalytic residues in alpha-glucosidase and the critical amino acid residue required for enzyme activation (Hung et al., 2005; Jhong et al., 2015; Puranik et al., 2016). The docking result confirmed that the selected *C. zanthorrhiza*, especially curcumin, interacts with several key amino acid residues of alpha-glucosidase, which is further expected to inhibit monosaccharide breakdown and delayed the postprandial hyperglycemic in T2DM patients.

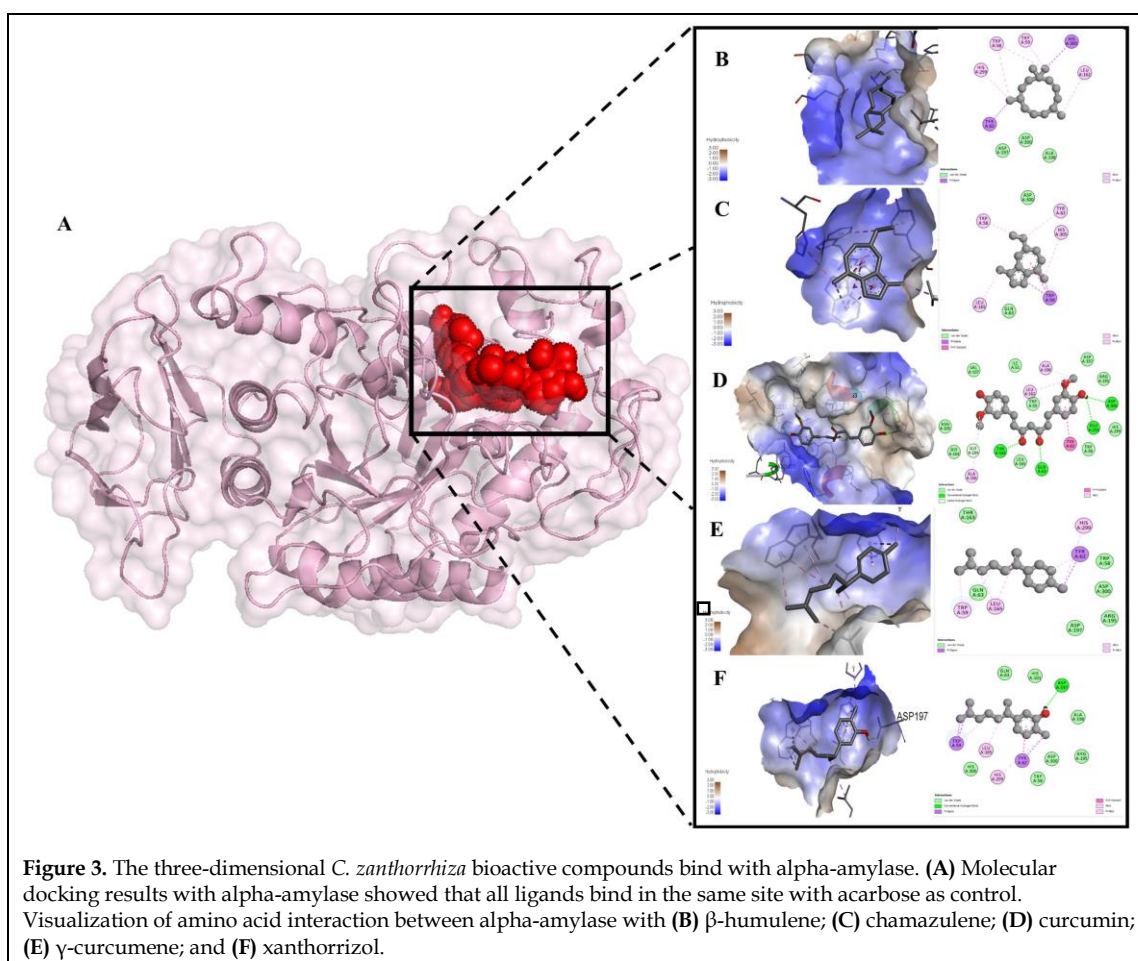


Table 2. The binding affinity and the amino acid interaction between selected *C. zanthorrhiza* bioactive compounds with alpha-glucosidase complex.

Protein	Compound	Binding affinity (kcal/mol)	Hydrogen bond interaction	Van der Waals interaction
Alpha-glucosidase (2ZE0)	Acarbose	-9.1	Arg407, Arg411, Asn58, Asp326, Glu256	Ala59, Arg17, Arg197, Arg381, Asp199, Asp382, Asp60, Gln167, His325, Phe144, Phe163, Ser384, Tyr15, Val383
	Curcumin	-8.6	Asp326, Gln167	Ala200, Arg197, Arg407, Arg411, Asn324, Asn61, Asp199, Asp60, His103, His325, Phe144, Phe163, Phe282, Val100,
	β-Humulene	-7.1	-	Asn61, Asp199, Asp326, Asp60, Gln167, His325, Phe163, Val100
	Chamazulene	-8.2	-	Asn61, Asp60, Asp199, Asp326, Gln167
	γ-Curcumene	-6.9	-	Asn61, Asp199, Asp326, Asp60, Gln167, Glu256, Phe163
	Xanthorrhizol	-7.7	-	Arg197, Asn61, Asp199, Asp326, Asp60, Gln167, Glu256

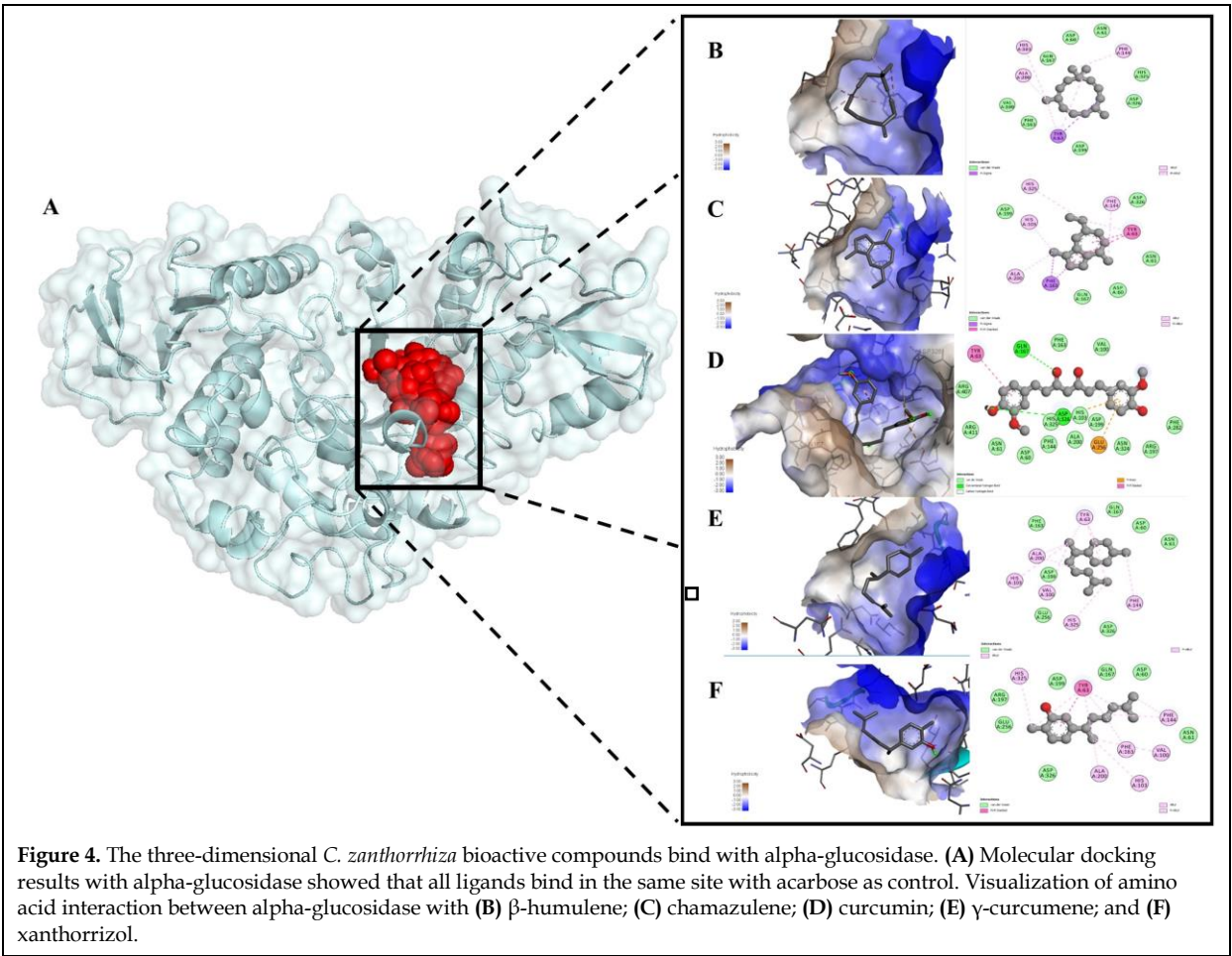


Table 3. The binding affinity and the amino acid interaction between selected *C. zanthorrhiza* bioactive compounds with Keap1.

Protein	Compound	Binding affinity (kcal/mol)	Hydrogen bond interaction	Van der Waals interaction
Keap1 (4IQK)	N,N'-naphthalene-1,4-diylbis(4-methoxybenzenesulfonamide)	-10.4	Ala556, Asn382, Gly364, Ser508, Ser602	Arg483, Gln530, Gly333, Gly462, Phe557, Tyr525
	Curcumin	-8.3	Ser363	Arg380, Arg483, Asn382, Asn414, Gln530, Gly364, Gly603, Phe577, Ser508, Tyr525, Tyr572
	β -Humulene	-6.7	-	Arg380, Arg415, Asn414, Gly364, Gly603, Phe577, Ser363, Ser555, Ser602, Tyr572
	Chamazulene	-6.9	-	Ala556, Arg380, Asn382, Gly364, Gly603, Ser363, Ser602
	γ -Curcumene	-6.3	-	Arg380, Asn382, Asn414, Gly364, Gly603, Ser363, Ser602
	Xanthorrhizol	-7.0	Gly364	Arg380, Asn414, Gly462, Gly509, Gly603, Ile416, Leu365, Leu557, Ser363

The energy activity of selected *C. zanthorrhiza* with Keap1

The molecular docking with Keap1 inhibitor summarized that only five from 20 bioactive compounds from *C. zanthorrhiza* had closed binding affinity values with Keap1 inhibitor (Table 3). Curcumin was found to serve the strongest ability to bind with Keap1 if compared with other bioactive compounds. The molecular docking analysis revealed that all ligands have bound in the same site in Keap1, such as Gly364. Surprisingly, curcumin and xanthorrhizol showed the hydrogen bond interaction among other selected *C. zanthorrhiza* bioactive compounds with Keap1 (Fig. 5).

In addition, T2DM has been linked to oxidative stress caused by excessive free radicals' production. The blockade of Keap1 as a negative regulator for Nrf2 may improve the outcome of T2DM. The molecular docking result showed that five compounds might be a candidate to inhibit Keap1. Xanthorrhizol interacts with Gly364 through hydrogen bond interaction, whereas curcumin through van der Waals interaction in the Keap1 complex. A previous study reported that Gly364 is one of the amino acid residues responsible for inducing the Keap1-dependent mechanism through H-bond and H-benzene, leading to Nrf2 activation (Staurengo-Ferrari et al., 2019). A previous study reported that curcumin binds to Keap1,

causing Nrf2 to be released and translocated into the nucleus, where it binds to an antioxidant response element in DNA to trigger gene activation (Suprihatin et al., 2017). In the current study, curcumin interacts with Ser363 through hydrogen bond interaction. Ser363 has a significant role in electrostatic interaction and maintains the Keap1-Nrf2 complexes' stability (Londhe et al., 2019). Other residues, such as Arg380, Arg415, and Arg483 were also reported to have a crucial role in Nrf2 binding (Lo et al., 2006). The Keap1 inhibition from interacting with Nrf2 will have an advantageous impact on T2DM, such as improving diabetic nephropathy and angiogenesis impairment (David et al., 2017). In addition, from the molecular docking results, five bioactive compounds from *C. zanthorrhiza* were identified as promising candidates to inhibit alpha-amylase, alpha-glucosidase, and Keap1 for improving the treatment or possibly enhancing the quality of life the T2DM patients.

Drug-likeness analysis

From the molecular docking result, five potential candidates were obtained, which predict might possess antidiabetic activity through its interaction with alpha-amylase, alpha-glucosidase, and Keap1. The analysis of drug-likeness will meet the optimum criteria if following several parameters, including having a molecular weight (MW) between 150-500 g/mol,

LogP value less than 5, number of hydrogen bond donor and acceptor less than 5 and 10 respectively, and the polar surface area (TPSA) range 20-130Å² (Christina et al., 2021). Based on these criteria, the curcumin and xanthorrhizol met the optimum criteria

range. The other compounds at least have one violation according to the Lipinski rule of five (Table 4). Due to the rule of five, a molecule can only be orally active/absorbed if it does not break any two or more of the initial conditions (Isyaku et al., 2020).

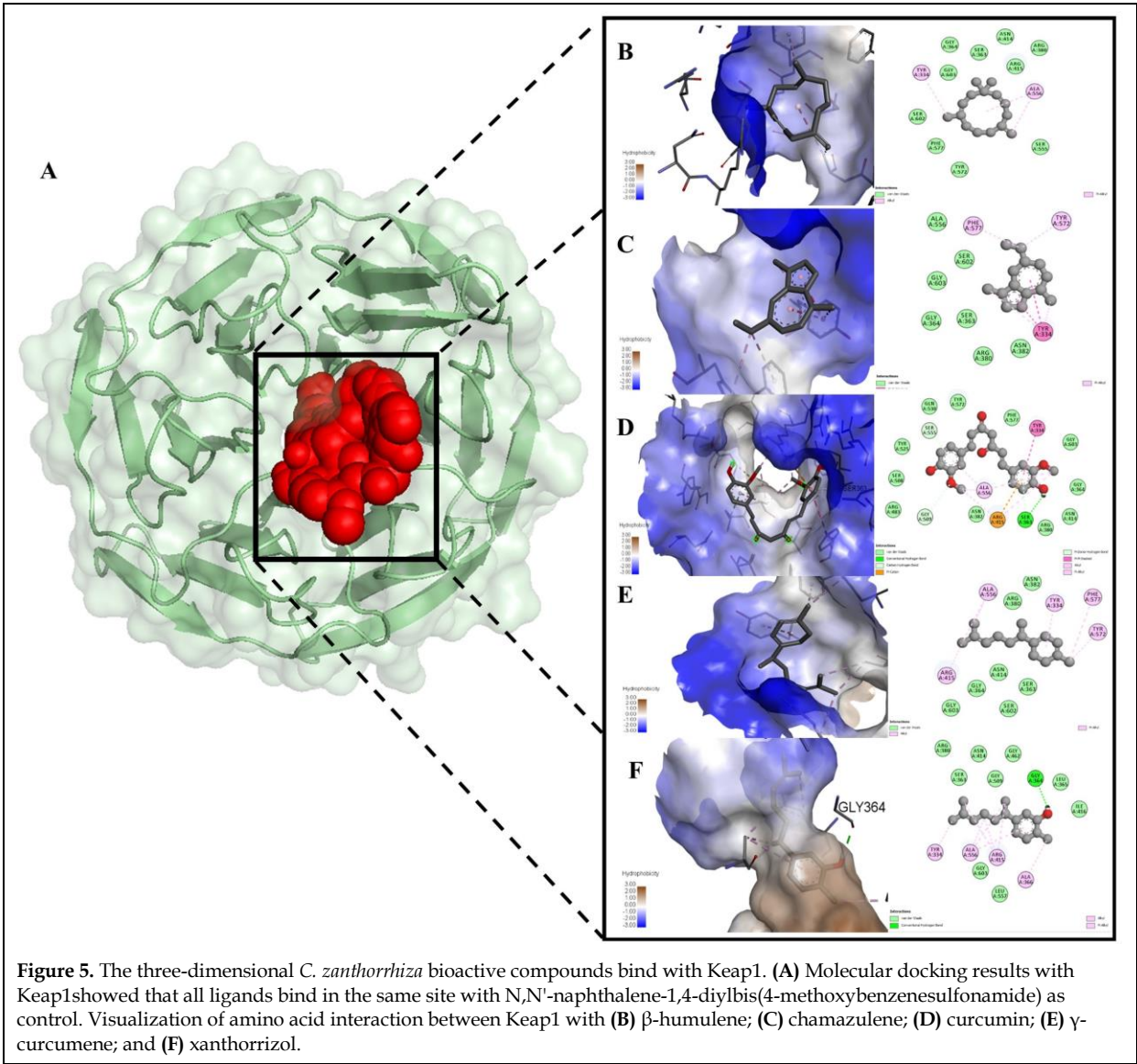


Table 4. Pharmacological properties of selected *C. zanthorrhiza* bioactive compounds.

Compound	Molecular weight (g/mol)	LogP value	H-bond donor	H-bond acceptor	Rotatable bonds	TPSA
β -Humulene	204.35	5.04	0	0	0	0.00 Å ²
Chamazulene	184.28	3.97	0	0	1	0.00 Å ²
Curcumin	368.38	3.27	2	6	8	93.06 Å ²
γ -Curcumene	204.35	5.04	0	0	4	0.00 Å ²
Xanthorrhizol	218.33	4.55	1	1	4	20.23 Å ²

CONCLUSION

The present study suggested five from 20 bioactive compounds, including β -humulene, chamazulene, curcumin, γ -curcumene, and xanthorrhizol from *C. zanthorrhiza* might have the potential to inhibit α -amylase, α -glucosidase, and Keap1. Curcumin was predicted as α -amylase, α -glucosidase, and Keap1 inhibitor. Curcumin and xanthorrhizol were the bioactive compounds in the current study that meets the pharmacological properties criteria based on the Lipinski rule of five. Further research *in vitro* and *in vivo* should be conducted to validate the activity of the *C. zanthorrhiza* bioactive compounds or be used as a primary compound for target T2DM progression.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Prasetyawan S	Safitri A	Rahayu S
Concepts or ideas	x		x
Design	x		x
Definition of intellectual content		x	
Literature search		x	
Experimental studies	x	x	x
Data acquisition	x	x	x
Data analysis		x	x
Manuscript preparation		x	x
Manuscript editing	x	x	
Manuscript review	x	x	x

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