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Insights into Molecular Interaction of Flavonoid Compounds in Citrus Peel Bound to Collagenase and Elastase Enzymes: A Computational Study

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ABSTRACT

Citrus peels contain various phytochemical active compounds such as flavonoids that are useful for antiaging cosmetic products. This study was conducted to identify the anti-collagenase and anti-elastase activities of flavonoid compounds in citrus peel and to determine the molecular interaction mechanism using the molecular docking method. The study was carried out through several stages, including preparation of enzyme macromolecules, preparation of flavonoid compound molecules, validation of molecular docking, identification of binding-free energy, visualization of interaction conformations, and predictions of molecular skin toxicity. The result showed that the flavonoid compounds in citrus peel (hesperidin, naringin, nobiletin, and tangeretin) could bind to collagenase and elastase enzymes. Naringin has the highest affinity for the collagenase enzyme with the binding-free energy of −9.52 kcal/mol, while nobiletin has the highest affinity for the elastase enzyme with the binding-free energy of −6.44 kcal/mol. Compared to EGCG (epigallocatechin gallate), the flavonoid compounds have a lower affinity for the collagenase enzyme but a higher affinity for elastase enzymes. Hydrogen bonds and the hydrophobic interactions dominate the interaction between citrus peel's flavonoids against the enzymes. When applied to the skin, flavonoid compounds are predicted to have no risk of skin toxicity. The flavonoid compounds of citrus peels are expected to have anti-collagenase and anti-elastase activities.

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INTRODUCTION

Skin aging is a complex biological process that can occur due to intrinsic (genetics, cellular metabolism, and hormone) and extrinsic (chronic sun exposure, pollution, and radiation) factors (Ganceviciene et al., 2012). Intrinsic skin aging is caused by changes in the elasticity of the skin over time. Extrinsic skin aging is predominately a result of chronic ultraviolet (UV) exposure. Excessive UV exposure significantly increases reactive oxygen species (ROS) formation (Singh et al., 2018). ROS can initiate complex molecular pathways, including degradation of extracellular matrix (ECM) biomolecules such as collagen and elastin (Eun Lee et al., 2019).

Collagen is the most abundant protein in the ECM and is responsible for the skin's elasticity, strength, and flexibility. Elastin is a vital protein for maintaining skin elasticity and resilience (Osorio et al., 2019). Reduction in collagen and elastin leads to wrinkling skin formation. Collagenase and elastase enzymes are responsible for breaking those biomolecules (Apraj & Pandita, 2016). Active compounds that can inhibit the collagenase and elastase enzyme's act can be used in antiaging cosmetic products (Azmi et al., 2014).

Many natural resources are known to have anti-collagenase and anti-elastase activities. Secondary metabolites such as polyphenols and flavonoids are known to support those activities. For example, the polyphenol compounds extracted from tea leaves, such as catechin, and epigallocatechin gallate (EGCG), are known to have potent anti-collagenase and anti-elastase activities (Thring et al., 2009). The flavonoid compounds in several plant extracts, including *Aloe barbadensis, Diospyros feet, Hylocereus sp., Lansium domesticum*, are proven to have similar activities (Vijayakumar et al., 2017). Another natural resource, known to contain many polyphenol and flavonoid compounds and the potential to have anti-collagenase and anti-elastase activities, is the citrus peel.

Citrus is one of the world's most popular fruit plants, containing many active compounds that are good for health (Lv et al., 2015). The production of citrus (orange) in Indonesia is very high. According to the data, it is known that in 2020, the production of citrus in Indonesia can reach 3 million tons a year (Fitri & Widyastuti, 2020). The high production and consumption of citrus fruit cause a high level of waste. Citrus peel has not been used optimally, even though several studies have shown many beneficial peel compounds (Gómez-Mejía et al., 2019).

Citrus peel is known to contain: essential oils (0.6-1%), fiber (6.30-42.13%), phenolic compounds (0.67-19.62%), and vitamin C (0.109-1.150%) (M'hiri et al., 2017). The main phenolic compounds contained in citrus peels are flavanones glucoside (hesperidin and naringin) and polymethoxylated flavones (PMFs) (nobiletin and tangeretin) (Rafiq et al., 2018). The phenolic compounds from citrus fruit peels have many pharmacological activities such as antioxidants, anti-inflammatory, anti-cancer, anti-proliferative, anti-viral, and anti-platelet aggregation (Chen et al., 2017).

This study was conducted to identify the anti-collagenase and anti-elastase activities of flavonoid compounds in citrus peel (hesperidin, naringin, nobiletin, and tangeretin) and to determine the mechanism of molecular interactions. In addition, this study aimed to predict the risk of skin toxicity from citrus peel flavonoids. EGCG was used as a reference compound in this study. EGCG is known as one of the active compounds that has potent anti-collagenase and anti-elastase activities. Some studies used EGCG as a reference in anti-collagenase and anti-elastase activity tests (Thring et al., 2009). Identification, evaluation, and exploration of the molecular interactions between these compounds against the collagenase and elastase enzymes were carried out using the molecular docking method. A computational study for predicting bioactive compound's pharmacological activity is widely applied because it is relatively more effective, easy, fast, inexpensive, and reliable.

METHODS

Matrix Metalloproteinase Macromolecules Preparation

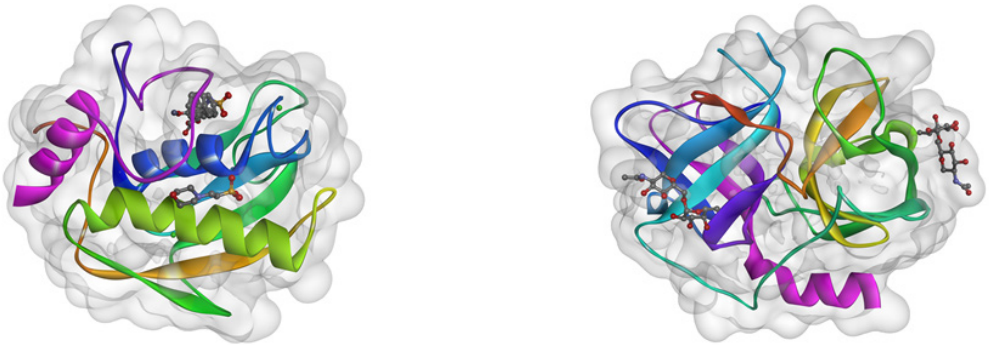
The matrix metalloproteinase macromolecules used were collagenase (MMP-8) and elastase (MMP-12) obtained from Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 5H8X (with a resolution of 1.30 Å) and PDB ID 5A8X (with a resolution of 2.23 Å) (Figure 1) (Tauro et al., 2016; Von Nussbaum et al., 2016). The two matrix metalloproteinase macromolecules were then prepared by removing water molecules, catechol (as the original ligand of collagenase) and dihydropyrimidone (as the original ligand of elastase), adding polar hydrogen atoms, and calculating Kollman's partial charge (Ugur et al., 2014).

Flavonoid Compound Molecules Preparation

The flavonoid compound molecules used were hesperidin, naringin, nobiletin, and tangeretin, which were contained in citrus peel. The EGCG structure molecule acts as a reference for the flavonoid compound's four molecules. EGCG is known as one of the active compounds that have potent anti-collagenase and anti-elastase activities. Some studies used EGCG as a reference in anti-collagenase and anti-elastase activity tests (Thring et al., 2009). The compound's entire molecular structure was modelled in the form of a three-dimensional conformation, and then the optimization used a semi-empirical method based on the AM1 basis set (Figure 2). The flavonoid and EGCG compound molecules that had been modelled and optimized were then modified on partial charge data to be used as input in the molecular docking simulation (Muttaqin et al., 2017).

Molecular Docking Validation

The validation of the molecular docking method had to be done first using MGLTools 1.5.6 with AutoDock 4.2 to determine some of the parameters used in the molecular docking simulation between all the molecules of the flavonoid compound and EGCG towards the matrix metalloproteinase macromolecules. Validation of this molecular docking method was done by re-docking the original ligand. In this re-docking process, the parameters are declared to meet the criteria if the Root Mean Square Deviation (RMSD) value does not exceed the maximum distance of 2 Å (Zhu et al., 2014).



Collagenase (MMP-8) Elastase (MMP-12)

Figure 1. The matrix metalloproteinase macromolecules structure (Tauro et al., 2016; Von Nussbaum et al., 2016)

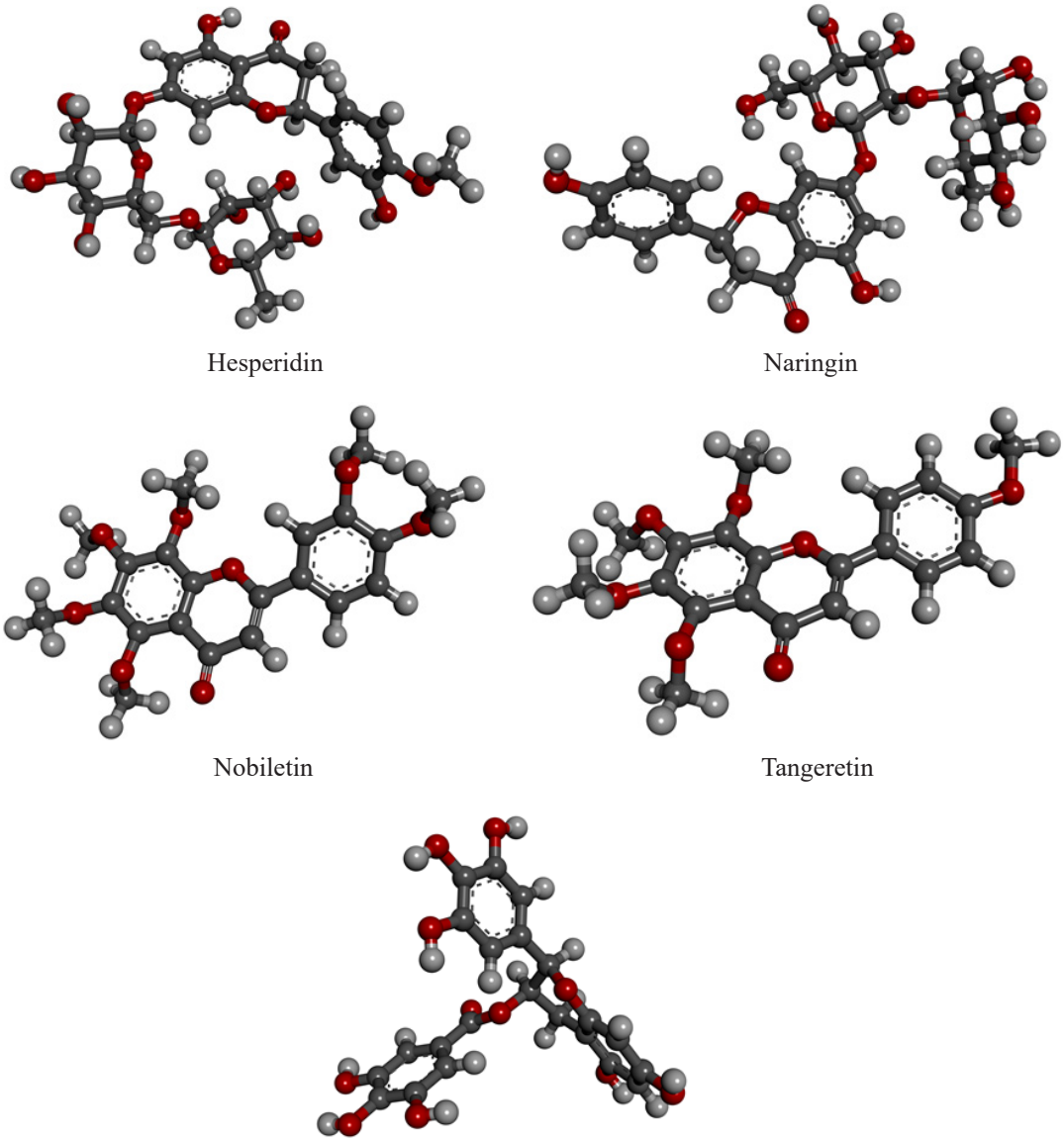


Figure 2. The three-dimensional compound molecules structure (Kim et al., 2021)

Molecular Docking Simulations

Molecular docking simulations were accomplished using MGLTools 1.5.6 with AutoDock 4.2. The molecular compounds of flavonoids and EGCG for molecular docking simulations were modelled and optimized, then added with hydrogen atoms and Gasteiger's partial charge. The distance between the surface area of the matrix metalloproteinase macromolecules and the compound molecules was limited to the maximum radius limit of 0.375 Å. The shape of the Connolly point surface of the molecule into different components, including convex, concave, and flat patches, was generated through the AutoDock 4.2 algorithm. All molecular docking simulations were demonstrated using a grid box measuring 64 x 60 x 60 and the Lamarckian Genetic Algorithm method with 100 conformations. The size limitation of the grid box is intended to prevent changes in the relative orientation of the compound molecules and the flexibility of the interacting surface's side chains is intended to allow the movement of small and rigid molecules (Forli et al., 2016).

Binding-Free Energy Result Identification

The results obtained from the molecular docking simulation were then observed, identified, and evaluated for the affinity of each molecule of the flavonoid and EGCG compounds based on the value of binding-free energy and inhibition constants through molecular interactions formed on matrix metalloproteinase macromolecules (Mobley & Klimovich, 2012).

Table 1. The total energy and the GAP energy values of the geometry optimization results

Compound molecule	Total energy (a.u)	GAP energy (a.u)
Hesperidin	−0.97	0.30
Naringin	−0.91	0.32
Nobiletin	−0.32	0.30
Tangeretin	−0.27	0.30
Epigallocatechin gallate (EGCG)	−0.61	0.30

Interaction Conformation Visualization

The amino acid residues, that played a role in the molecular interactions, formed were then observed using BIOVIA Discovery Studio 2020. Some of these molecular interactions include hydrogen bonds, hydrophobic interactions, and electrostatic interactions. Molecular interactions between flavonoid and EGCG compound molecules against collagenase and elastase enzymes can illustrate small molecular ligand's ability to stabilize the active binding sites of target macromolecules (Dar & Mir, 2017).

Molecular Skin Toxicity Prediction

Toxicity prediction was performed to observe the effects of flavonoid and EGCG molecules on the skin surface using Toxtree v.3.1.0. Two parameters were used to predict toxicity at this stage, namely Skin Irritation/ Corrosion and Skin Sensitization Reactivity Domain. Skin Irritation/Corrosion is a parameter that can be used to assess the potential for irritation or corrosion or a combination of both of the molecular compound structures. Then Skin Sensitization Reactivity Domains are used to determine the sensitivity of compound molecules in the surface area of the skin contact (Han et al., 2019).

RESULTS AND DISCUSSION

Total Energy Calculation of Compound Molecules Optimization

Modelling and geometry optimization were demonstrated on hesperidin, naringin, nobiletin, tangeretin, and EGCG compound molecules in the form of three-dimensional structures using the semi-empirical method based on the AM1 basis set. The five compound molecule's geometry optimization results with the best conformation were selected based on the total energy value and the difference between Highest Occupied Molecular Orbital-Lowest Unoccupied Molecular Orbital (HOMO-LUMO) (GAP energy).

Based on the results of the optimization of the molecular geometry of compounds listed in Table 1, flavonoids and EGCG had good total energy and GAP energy values and could be predicted to be able to interact well at the active site of the target macromolecule binding site (Bakalova & Kaneti, 2000). The total energy and GAP energy were generated to describe the conformation of the molecular structure of flavonoids and EGCG compounds that had approached their original state and were expected to form stable molecular interactions with target macromolecules. All flavonoid molecular structures that had been energy minimized were then verified for their partial charge as input in molecular docking simulations.

Binding Affinity of Molecular Compounds Structure

The molecular compounds of flavonoids and EGCG that had been modelled and optimized were then identified, evaluated, and compared to their affinity and molecular interaction capabilities on matrix metalloproteinase macromolecules prepared through molecular docking simulations using MGLTools 1.5.6 with AutoDock 4.2. This simulation was accomplished using several methods that had been validated in the previous stage. Based on the validation of the molecular docking method, the RMSD values were 0.50 Å (MMP-8) and 0.83 Å (MMP-12). Validation of the docking method is declared valid and can be used if the RMSD is less than 2 Å. This RMSD value can show the closeness of the original ligand conformation before and after the re-docking process (Pitaloka et al., 2021). Some of the parameters used were the size of the grid box, the number of conformations, and the maximum value of the conformation search evaluation.

The molecular docking simulation results in Table 2 show that all flavonoid compounds from citrus peels have an affinity for binding with collagenase and elastase enzymes. Naringin has the best affinity with the active binding site of collagenase (MMP-8) compared to other flavonoid compound molecules, with the binding-free energy value of −9.52 kcal/mol. Nevertheless, compared to the original ligand and EGCG, the flavonoid compounds of citrus peel have a lower affinity for the collagenase enzyme (Fakih & Dewi, 2020).

A different phenomenon is shown when all the molecular compounds interact with elastase (MMP-12). The

Table 2. The affinity of the molecular docking simulations

Macromolecule receptor	Compound molecule	Binding-free energy (kcal/mol)	Inhibition constant
Collagenase (MMP-8)	Original ligand	−11.50	3.70 nM
	Hesperidin	−8.83	334.47 nM
	Naringin	−9.52	105.51 nM
	Nobiletin	−8.73	400.02 nM
	Tangeretin	−8.79	359.11 nM
	Epigallocatechin gallate (EGCG)	−9.93	52.57 nM
Elastase (MMP-12)	Original ligand	−9.67	81.93 nM
	Hesperidin	−6.16	30.12 uM
	Naringin	−6.17	19.06 uM
	Nobiletin	−6.44	42.93 uM
	Tangeretin	−5.96	49.48 uM
	Epigallocatechin gallate (EGCG)	−5.87	32.79 uM

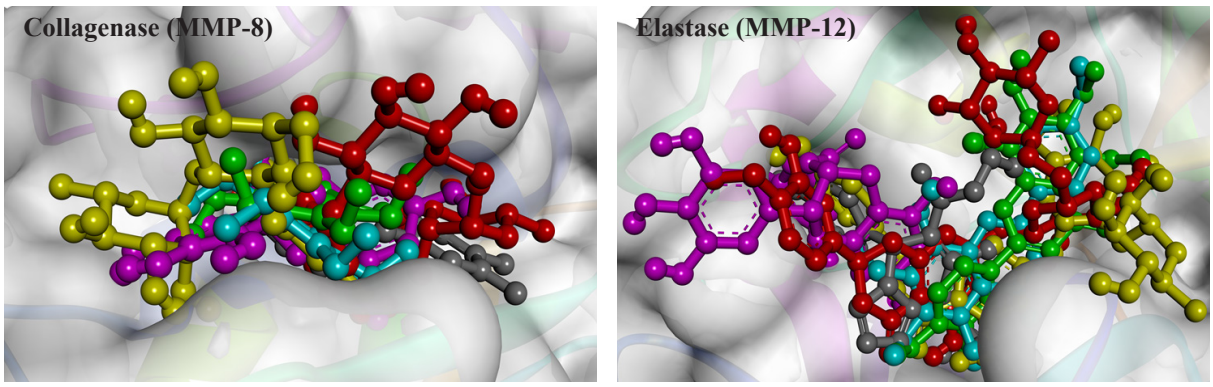


Figure 3. The overlay conformations of compound molecules in the binding-site area of macromolecules

flavonoid compound molecules have a better affinity to elastase enzyme than EGCG. Thus, hesperidin, naringin, nobiletin, and tangeretin are predicted to have the potential as elastase inhibitors (MMP-12). The result of molecular docking simulations shows that nobiletin has the best binding-free energy value of −6.44 kcal/mol with the elastase enzyme. However, similarly when interacting with collagenase (MMP-8), all compounds have binding-free energy that is not better than original ligands.

Conformational Modes in the Binding-Site Area

Based on the visualization of the active binding sites for each target macromolecule, in general, hesperidin, naringin, nobiletin, and tangeretin showed conformational similarities to the EGCG compound molecule as a reference (Figure 3). Most of the compound molecules are able to interact with amino acid residues Leu160, Ala161, Ala163, Leu193, Val194, His197, and His201 on collagenase (MMP-8). They are able to interact also with amino acid residues in the active site area of elastase (MMP-12), including His57, Leu99, Val190, Cys191, Phe192, Asp194, Ser195, Ala213, Ser214, Phe215, and Val216. This phenomenon can occur because the AutoDock 4.2 algorithm supports small molecules of compounds to move freely without rigid bonds with the molecular docking methods used.

The interaction between flavonoid and EGCG compound molecules against the two matrix metalloproteinase macromolecules is dominated by hydrogen bonds and the formed hydrophobic interactions (Table 3). Significantly, the EGCG compound molecule has electrostatic interactions with Glu198 (in collagenase (MMP-8)) and Asp102 (in elastase (MMP-12)). Importantly, the original ligands found in elastase (MMP-12) can bind firmly to the active site due to the contribution of halogen interactions with several amino acid residues, including Cys191, Asp194, Ser195, Ala213, Ser214, and Phe215. It is predicted that this makes the interactions of each original ligand and EGCG against the enzymes more stable.

Table 3. The molecular interactions of molecular docking simulation

Macromolecule receptor	Compound molecule	Type of interactions	Amino acid residues
Collagenase (MMP-8)	Original ligand	Hydrogen bond	Ile159, Leu160, Ala161, Ala163
		Hydrophobic interaction	Leu160, Leu193, Val194, His197
		Electrostatic interaction	Glu198
	Hesperidin	Hydrogen bond	Leu160, Ala161, Glu198, Asn218, His201, His207
		Hydrophobic interaction	Ile159, Leu160, Val194, His197
	Naringin	Hydrogen bond	Gly158, Leu160, Ala161, Tyr189, Asn218, Tyr219
		Hydrophobic interaction	Leu160, Tyr189, Val194, His197, Tyr219
	Nobiletin	Hydrogen bond	Gly158, Ala213, Leu214, Tyr216, Pro217, Asn218, Tyr219, Ala220
		Hydrophobic interaction	Leu160, Val194, His197, Tyr219
	Tangeretin	Hydrogen bond	Ile159, Gly158, Tyr219, Ala220
		Hydrophobic interaction	Leu160, Val194, His197, Tyr219
	Epigallocatechin gallate (EGCG)	Hydrogen bond	Gly158, Leu160, Ala161, Tyr189, Tyr219, Ala220
		Hydrophobic interaction	Leu160, His197
		Electrostatic interaction	Glu198
Elastase (MMP-12)	Original ligand	Hydrogen bond	Ser195, Phe215, Val216
		Hydrophobic interaction	His57, Leu99, Val190, Phe192, Ala213, Val216
		Halogen interaction	Cys191, Asp194, Ser195, Ala213, Ser214, Phe215
	Hesperidin	Hydrogen bond	Phe41, His57, Ala60, Pro96, Ser195, Val216
		Hydrophobic interaction	Leu99, Phe192
	Naringin	Hydrogen bond	Phe41, His57, Pro96, Phe192, Gly193, Ser214, Val216
		Hydrophobic interaction	His57, Leu99, Phe192, Leu143
	Nobiletin	Hydrogen bond	His57, Ala60, Gly193, Ser195, Val216
		Hydrophobic interaction	His57
	Tangeretin	Hydrogen bond	His57, Phe192, Ser195, Ser214, Val216
		Hydrophobic interaction	Phe192
	Epigallocatechin gallate (EGCG)	Hydrogen bond	His57, Tyr94, Val97, Leu100, Ser214
		Hydrophobic interaction	His57, Leu99
		Electrostatic interaction	Asp102

Table 4. The skin toxicity prediction of compound molecules

Compound molecule	Skin Irritation / Skin Corrosion	Skin Sensitization Reactivity Domains
Hesperidin	Not corrosive to skin	No skin sensitization reactivity
Naringin	Not corrosive to skin	No skin sensitization reactivity
Nobiletin	Not corrosive to skin	No skin sensitization reactivity
Tangeretin	Not corrosive to skin	No skin sensitization reactivity
Epigallocatechin gallate (EGCG)	Not corrosive to skin	No skin sensitization reactivity

In molecular docking simulations, the most responsible complex molecular interactions are hydrogen bonds, especially the flavonoid and EGCG compounds, which act as hydrogen bond donors, and amino acid residues on matrix metalloproteinase macromolecules, which act as hydrogen bond acceptors. Most hydrogen bonds are relatively strong, with average bond lengths less than 3 Å (Boyken et al., 2016). Apart from hydrogen bonding, there are also contributions from hydrophobic and electrostatic interactions that play a role in stabilizing small molecular ligand's flexibility on the active sites of target macromolecules.

Skin Toxicity of Compound Molecules Prediction

The prediction of molecular toxicity in the surface area of the skin contact from hesperidin, naringin, nobiletin, tangeretin, and EGCG, was accomplished using two parameters, namely Skin Irritation/Corrosion and Skin Sensitization Reactivity Domain. Skin Irritation/Corrosion is a parameter that can assess whether a compound's molecular structure has the potential to cause irritation or corrosion or a combination of both. Furthermore, Skin Sensitization Reactivity Domains are used to determine the sensitivity of compound molecules to areas that have direct contact with the skin surface (Deshmukh et al., 2012).

In this case, it is important to carry out toxicity testing to estimate the degree of damage caused by a compound to biological or non-biological materials. Toxicological screening is essential in developing new drugs and determining the therapeutic potential of a drug molecule. Toxicity testing is generally intended to determine the undesirable effects of a drug, especially on the incidence of cancer, heart disorders, and skin or eye irritation. Some herbal bioactive compounds are known to have a risk of skin irritation (Seitz et al., 2013; Xu et al., 2020).

Based on the results of the skin toxicity prediction in Table 4, it was found that the flavonoid and EGCG compound molecules had less potential to cause irritation or corrosion on the skin surface. This category of irritation or corrosion indicates that the compound's molecular structure has a low risk of causing severe burns that can irritate the skin surface. Then, similar to the results of the Skin Irritation/Corrosion parameters in the previous stage, the molecules of flavonoids and EGCG were included in the low category in causing severe sensitivity to the skin surface based on the skin Sensitization Reactivity Domains parameter. Thus, the compound molecules are predicted to be safe for use as active compounds in topical preparation formulations.

CONCLUSION

Based on the study results, it can be concluded that the flavonoid compounds in citrus peel (hesperidin, naringin, nobiletin, and tangeretin) could bind to collagenase and elastase enzymes. Naringin has the highest affinity for the collagenase enzyme with the binding-free energy of −9.52 kcal/mol, while nobiletin has the highest affinity for the elastase enzyme with the binding-free energy of −6.44 kcal/mol. Compared with EGCG, flavonoid compounds have a lower affinity for the collagenase enzyme, but a higher affinity for elastase enzymes. When applied to the skin, flavonoid compounds are predicted to have no risk of skin toxicity.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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