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In silico analysis of phytochemical compound found in snake fruit (*Salacca zalacca*) peel as anti-aging agent

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ABSTRACT

Background: Skin aging is a complicated natural phenomenon characterized by progressive loss of structural integrity and physiological function of the skin. Snake fruit (*Salacca zalacca*) is an important fruit crop native from Indonesia. Its waste is a potential source bioactive phytochemical compound. **Objectives:** Present study investigates possible anti-aging potency of phytochemical compound found in salak fruit peel based on its binding towards enzyme related in skin aging process. **Materials and Methods:** Molecular docking was performed towards enzyme subjected to degradation of dermal matrix (MMP1, NEP) and hyper-pigmentation (PPO3). **Results:** Docked compound occupied the active site of each protein in which chlorogenic acid has the highest affinity among salak peel compound against MMP1, and Rutin against NEP and PPO3. Further intermolecular analysis revealed favourable interaction between chlorogenic acid, rutin towards respective protein. **Conclusions:** Taken together compound found in snake fruit, display possible inhibition towards protein related in skin aging and could be potentially used as anti-aging agent.

Keywords: Aging, molecular docking simulation, phytochemicals, *Salacca zalacca*, skin

INTRODUCTION

Skin aging is a complicated natural phenomenon characterized by progressive loss of structural integrity and physiological function of the skin. This change manifested as visible wrinkles and sagging on the skin.[1] Skin aging caused by various internal factors such as genetics, cellular metabolism, hormone, and metabolic processes and external factor such as chronic light exposure, pollution, ionizing radiation, chemicals, toxins.[2] UV radiation plays an important role in aging.[3] UV radiation, especially UVB, is a potent environmental stress that elevates the production of reactive oxygen species (ROS).[4] Even though ROS important in cellular signaling, unregulated production causing a condition known as oxidative stress that may lead to further cellular or tissue changes.[5]

There are two main components forming the skin, collagen, a fibrous protein responsible for the tensile strength of the dermal matrix and elastin responsible for its elasticity. Impairment of ROS production by chronic UV exposure is able to induce the production of collagen-degrading enzyme, matrix metalloproteinase-1 (MMP1), and results in detectable extracellular matrix (ECM) breakdown.[6] MMP1 functions in ECM breakdown by cleaving collagen type I, II, and III. Another study found UVB irradiation in animal model was found causing production of fibroblast elastase that effectively degrades skin elastin fibers.[7] Combined degradation of ECM by these type of proteins leads to visible structural changes of the skin tissue. Oxidative stress also induces melanin production through activation of tyrosinases (TYRs) that result in hyperpigmentation.[8,9] Since its importance, primary structural components of the skin, collagen and elastin, and

pigmentation process have been subjects of the majority of skin anti-aging research.[10] Therefore, inhibition of either oxidative stress or enzyme related to the degradation of skin ECM and hyperpigmentation offers a rational therapeutic strategy for treating skin aging.

Through the efforts of ongoing scientific researches, an increasing number of plant extracts and phytochemicals have been showed promising result as anti-aging agent. Snake fruit or Snake fruit (*Salacca zalacca*) is a plant species of the palm tree family (Arecaceae) that native to Indonesia. Snake fruit served as important crop in Indonesian and had been cultivated throughout the country. According to data from Ministry of Agriculture Republic Indonesia, in 2016, snake fruit production in Indonesia reached up to 700 ton.[11] Snake fruit consists of highly aromatic sweet pulp with a hard inedible peel. Snake fruit has high economic value in Indonesia while can be consumed directly, snake fruit also can be made other products such as syrup, chips, and coffee. Although its peel is inedible, it serves as a major waste of the snake fruit consumption. The previous study found that snake fruit peel contains important phenolic compound such as caffeic acid, protocatechuic acid, ferulic acid, chlorogenic acid, and rutin.[12] Natural compound belongs to phenolic and flavonoid group found in plants often exert beneficial biological activities, ranging to antioxidant to anti-inflammatory.[13,14] The previous studies have been found that all phytochemical compounds found in snake fruit peel were known to be active as an antioxidant.[15-18] Unfortunately, to this date, there is no information available regarding snake fruit waste potential as anti-aging agent, despite its abundance. Therefore, the present study investigates possible anti-aging potency of phytochemical compound found in salak fruit peel based on its binding toward enzyme related in skin aging process.

MATERIALS AND METHODS

Molecular Docking Simulations of Snake fruit Peel Compounds toward MMP1, Neutral Endopeptidase (NEP), and Polyphenol Oxidase 3 (PPO3)

Molecular docking analysis was performed to investigate possible binding mode of five major snake fruit peel compounds against protein subjected to skin aging process. The proteins used as receptor in the docking study were as follows: Matrix metalloproteinase-1 (MMP1), NEP 24.11/neprilysin, and PPO3. The three-dimensional protein structure MMP1 (PDB: 966C),[19] NEP (PDB: 5JMY),[20] and PPO3 (PDB: 2Y9X)[21] in complex with its known inhibitors were derived from X-ray crystallography and obtained from RCSB protein data bank (https://rcsb.org/). The compounds found in snake fruit peel: Caffeic acid, chlorogenic acid, ferulic acid, protocatechuic acid, and rutin were used as ligand in the docking study. The three-dimensional structure of each compound was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Receptor was prepared using AutoDockTools by removing crystallographic water and bound ligand, and cofactor and left as it is. The search space (grid box) was placed on the active site of each protein and position of the bound ligand in the crystal structure was used as reference. The grid box size was left in default size (30×30×30 Å). Molecular docking was performed using AutoDock Vina under default configuration. Molecular docking validated by redocking of the bound ligand toward the respective receptor. Root-mean-square deviation (RMSD) between modeled binding conformation and crystal conformation was then calculated. Binding affinity for each docked ligand toward receptor and its conformation was retrieved. Best docked conformation that ranked by Vina scoring was used in visual analysis using UCSF Chimera.[22] Intermolecular interaction of protein-ligand complex was inferred using PoseView accessible through Protein Plus web server (https://proteins.plus/).[23]

RESULTS

Proposed Binding of Snake Fruit Peel Compounds toward Enzyme Related in Skin Aging Process

Phytochemical compound found in salak fruit was subjected to molecular docking toward MMP1, NEP, and PPO3. To validate the methodology, redocking was performed. MMP1 bound ligand (N-hydroxy-2-[4-(4-phenoxy-benzenesulfonyl)-tetrahydro-pyran-4-yl]-acetamide), NEP bound ligand (LBQ657), and PPO3 bound ligand (tropolone) were extracted and docked back to the corresponding protein. All bound ligand docked conformation and the crystal conformation showed RMSD <2 Å (data not shown), which is a widely acceptable cutoff.[24] Thus, AutoDock Vina virtually was able to recover the actual experimental binding mode.

All compounds found in snake fruit peel were successfully docked to the MMP1, NEP, and PPO3 active site and the binding score presented in Table 1. Binding affinity was expressed as binding free energy. The more negative the value, the more likely the binding occurs. Chlorogenic acid showed the highest affinity toward MMP1 (−7.1 kcal/mol) among the compounds found in snake fruit peel. None of the snake fruit compounds exceeded the bound ligand affinity toward MMP1 and NEP, except toward PPO3. All the compounds had higher affinity toward PPO3 than its bound ligand. Rutin had considerably high affinity toward PPO3 (−7.8 kcal/mol).

Visualization analysis showed the snake fruit peels compounds conformation occupied the active site of MMP1 [Figure 1]. MMP1 is a metal-containing enzyme, whereas a catalytic zinc atom coordinated to his401, his405, and his411 residues of the HExxHxxGxxH motif located on its active site.[25] The docked chlorogenic acid conformation showed

Table 1: Binding affinity of docked snake fruit peel compounds

Compound	Binding affinity (kcal/mol)		
	MMP1	NEP	PPO3
Caffeic acid	−7.1	−7.0	−6.2
Chlorogenic acid	−9.1	−7.3	−7.2
Ferulic acid	−7.0	−6.8	−6.4
Protocatechuic acid	−6.4	−6.2	−6.1
Rutin	−8.7	−7.6	−7.8
Bound ligand	−9.9	−9.0	−5.8

in close proximity toward Zn. Based on intermolecular analysis result, none of the compounds except N-hydroxy-2-[4-(4-phenoxy-benzenesulfonyl)-tetrahydro-pyran-4-yl]-acetamide was able to chelate the cofactor [Figure 2 and Table 2]. Chlorogenic acid and rutin formed hydrogen bond similar to MMP1 bound ligand at Ala182 and glu219, except Leu181 residue. MMP1 bound ligand also interacted hydrophobically with S1 cavity.

All the docked compounds also reside the active site of NEP [Figure 1]. Similar to MMPs, NEP is a zinc-dependent enzyme consisted of two major domains forming a large cavity on its center.[20] Based on analysis, LBQ657 coordinated with zinc atom, while rutin formed a π cation interaction with the cofactor. Even though other ligands also occupied the S1 pocket, only LBQ657 formed strong hydrophobic interaction with the cavity [Figure 2 and Table 2].

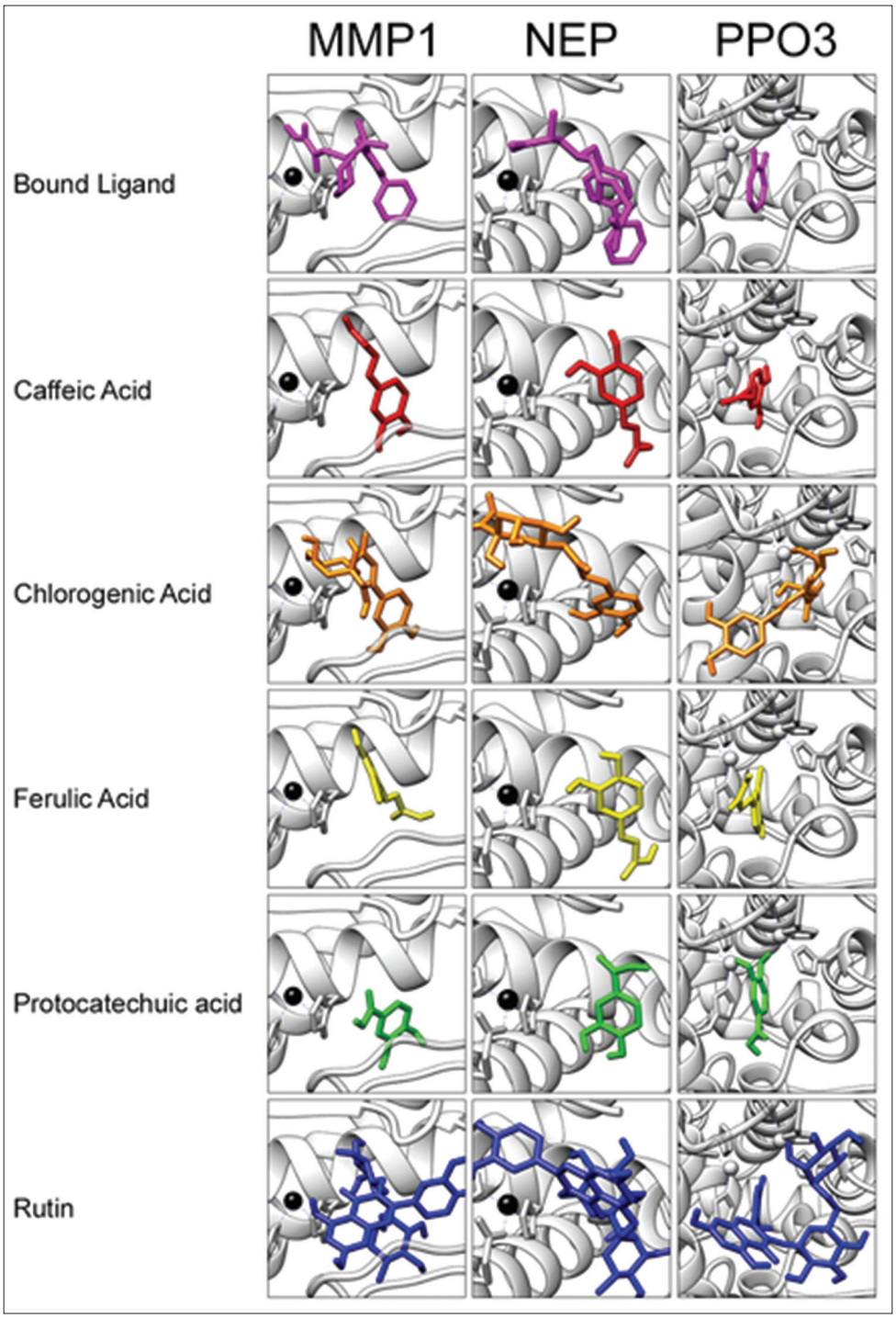

Figure 1: Superimposed binding mode of (Red) caffeic acid, (Orange) chlorogenic acid, (Yellow) ferulic acid, (Green) protocatechuic acid toward, and (Blue) rutin toward matrix metalloproteinase-1, neutral endopeptidase, and PPO3. The protein showed as surface representation. The ligand showed as stick representation with only polar hydrogen showed. Cofactor showed as sphere representation

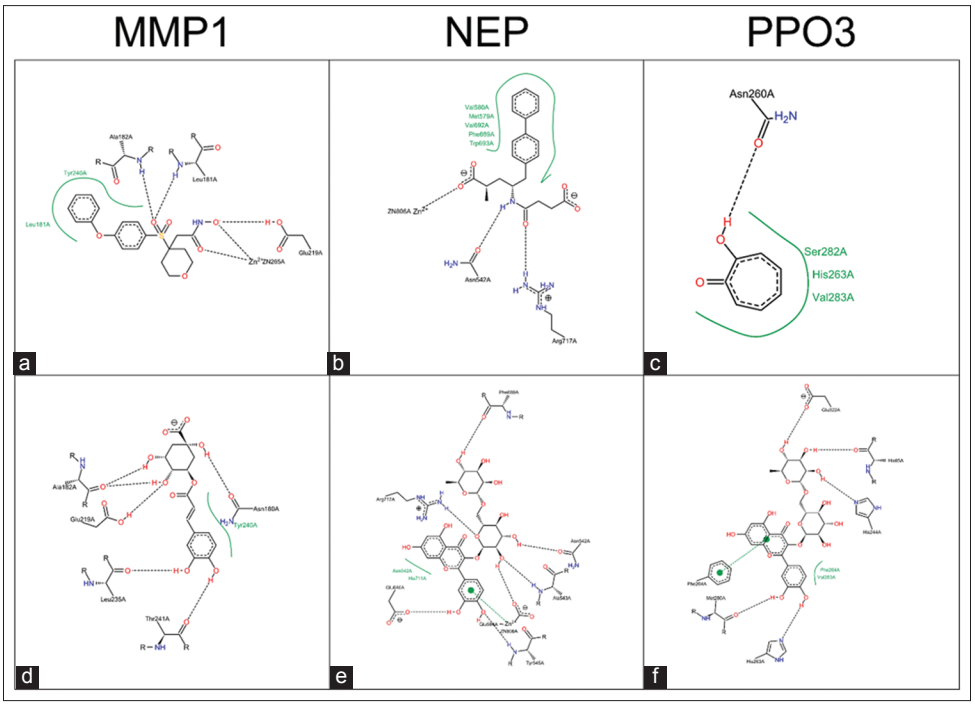

Figure 2: Intermolecular interaction of bound ligand of (a) matrix metalloproteinase-1, (b) neutral endopeptidase, and (c) polyphenol oxidase 3 compared to (d) chlorogenic acid and rutin (e and f). Visible difference between the bound ligand compared to snake fruit peel compound is the existence of cofactor interaction, hydrophobic interaction (directed and indirected), and hydrogen bond number. Hydrogen bond presented as dashed lines, hydrophobic interaction as green line, directed hydrophobic as dashed green line

Table 2: Hydrogen bond of docked snake fruit peel compounds toward MMP1, NEP, and PPO3 residues

Compound	Hydrogen bond		
	MMP1	NEP	PPO3
Caffeic acid	Tyr237, thr241	Asn542, Glu584	Met280
Chlorogenic acid	Asn180, Ala182, glu219, leu235, thr241	Tyr545, Glu646	Asn260, Thr261
Ferulic acid	Ala182, thr241	Asn542	Met280
Protocatechuic acid	Leu235, Thr241	Asn542, Asp650, Trp693	His85, Asn260
Rutin	Ala182, Gly179, Tyr210, arg214, glu219, thr241	Asp650, Trp693	His85, His244, His263, Met280, glue322
Bound ligand	Leu181, Ala182, glu219	Asn542, Arg717	Asn260

MMP1: Matrix metalloproteinase-1, NEP: Neutral endopeptidase, PPO3: Polyphenol oxidase 3

Docked conformation showed all the compounds located on hydrophobic cavity of the PPO3 active site [Figure 1]. TYRs including PPO3 are copper-containing enzyme, whereas each binuclear copper coordinated with histidine residue which located in the bottom of the pocket.[21] None of the compounds including tropolone interacted directly with copper ion, except protocatechuic acid. Rutin formed the numerous hydrogen bonds with the residues around the active site and π-π stacking with phe264 residue [Figure 2 and Table 2].

DISCUSSION

The present study characterized the binding of phytochemical compound found in snake fruit peel toward enzyme subjected to degradation of dermal matrix (MMP1 and NEP) and hyperpigmentation (PPO3). Results showed that chlorogenic acid was potential binder of MMP1 since it had the highest binding affinity value among compounds found in snake fruit peel. The synthetic inhibitor of MMPs often tries to copy binding of MMPs with its natural inhibitor TIMPs. This approach may be through binding to the catalytic site of the enzyme, chelating the active site zinc, binding to the recognition site, or interacting hydrophobically to S1 site.[25] Even though none of the compounds was able to chelate zinc, chlorogenic acid formed numerous hydrogen bond with the active site of MMP1.

The previous study was found that *Zingiber officinale* (L.) rose extract able to inhibits human skin fibroblast elastase (HSFE).[26] It is possible that other natural compounds may also inhibit HSFE activity. Recent studies found that HSFE was identical with NEP; thus, in this study, NEP used in molecular docking.[27] LBQ657 is the active form of pro-drug sacubitril and a known potent inhibitor of NEP. This inhibitory property was achieved through interaction to the catalytic zinc and hydrophobic S1 pocket.[20] Due to this, interaction of ligand and zinc and S1 residues played an important role in NEP inhibition. Binding prediction showed among compound found in snake fruit peel, only rutin interacted with catalytic zinc, through the π cation interaction.

Until now, the three-dimensional structure of human TYR has not been solved yet. PPO3 is a TYR originated

from *Agaricus bisporus* that is often used in aging assay and molecular docking study of TYR inhibitor.[21] Previous crystallographic study suggested that the slow-acting inhibitor of PPO3, tropolone, was located in van der Waals distance toward his263 and formed directed hydrophobic interaction to phe264,[21] which was similar to binding prediction of rutin found in snake fruit peel.

Taken together, compound found in snake fruit, specifically chlorogenic acid and rutin display possible competitive inhibition toward enzyme related in skin aging process based on *in silico* binding conformation and binding affinity. The previous study found that direct inhibition of MMP1 and HSFE was beneficial and prevented skin changes.[28,29] Thus, conclude phytochemical compound found in snake fruit peel could be potentially used as anti-aging agent.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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