# Molecullar Docking of Epigallocatechin-3-gallate (EGCG) on Keap1-Nrf2 Complex Protein in Photoaging Prevention

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## Abstract

Photoaging is an extrinsic skin aging caused by ultraviolet radiation. It may affect patients' quality of life. Ultraviolet radiation causes increasing of Kelch-like ECH-associating protein1-nuclear factor erythoid 2-related factor 2 (Keap1-Nrf2) protein, that plays role in photoaging pathogenesis. Many substances, such as green tea catechins, have been developed in photoaging prevention. The most abundant catechin in green tea is epigallocatechin-3-gallate (EGCG). This study was an in silico study, aimed to obtain the effectiveness of EGCG through molecular docking on Keap1-Nrf2 protein. The bioinformatics tools used in this study, were Protein Data Bank (PDB), ChemDraw, Chem3D, and Molegro Virtual Docker (MVD) software. Mol Dock and ReRank score was evaluated in this study, reflected the interaction between Keap1-Nrf2 protein and compound molecules. The prediction of EGCG pharmacokinetics were performed using pkCSM On-Line Tool. The result of molecular docking between Keap1-Nrf2 protein with a candidate ligand (EGCG), a control ligand (arbutin), and a reference ligand (FB2_1615[A]) using MVD software, showed that the binding affinity of Keap1-Nrf2 protein with EGCG to be the lowest. The prediction of skin permeability of EGCG using pkCSM On-Line Tool was -2.735 cm/h and it was predicted that EGCG did not cause skin sensitization and AMES toxicity. EGCG has higher potential than arbutin and reference ligand to be an alternative agent in photoaging prevention. EGCG was predicted to have good skin absorption profile, without toxicity effect.

***Keywords:*** *photoaging, EGCG, Keap1-Nrf2, in silico, docking.*

## Introduction

Aging is a generalized impairment of function, resulting in an increasing vulnerability to environmental challenge and a growing risk of disease. Photoaging is an extrinsic skin aging, that mostly caused by ultraviolet radiation from the sun exposure. Photoaging manifests as wrinkle and dryness of the skin, and affect patient's quality of life, because skin is the outer organ seen by others.[1,2,3] Nowadays, the number of geriatric population increases. The geriatric population in Indonesia was 9.03% of all population in 2017. This fact plays role in the increasing of photoaging problems.[2,4]

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Ultraviolet radiation causes increasing of Keap1-Nrf2 protein, that plays role in photoaging pathogenesis. The number of free Nrf2 that translocates from cytoplasm to nucleus and the transcription of antioxidant response element (ARE) will decrease in photoaging.[5,6]

The effort in photoaging prevention have been developed, but the incidence of photoaging is still high. One of the biggest problems in photoaging prevention is the effectivity and the efficiency of the drugs. The new drugs that were needed, should have effective targets and easily to produce.[7,8]

Many plants, such as green tea, have been developed in photoaging prevention, but it still used multicompound approach. Green tea from tea plant *Camelia sinesis* has been planted since thousand years ago in Asia and it has been consumed by two-third of world population.

There were several studies showed the role of green tea in photoaging prevention.[7]

An in vitro study using human living skin equivalent about green tea polyphenol at 5 hours before and after ultraviolet B irradiation, showed that green tea polyphenol decreased the ultraviolet B irradiation induced apoptosis.[9] An animal study in SKH-1 hairless mice that were ultraviolet irradiated twice a week for 2 months and treated with green tea polyphenol in water orally, showed that green tea polyphenol inhibited the production of $H_2O_2$.[10] An in vivo study in SKH-1 hairless mice that were irradiated by ultraviolet B (5 times a week for 4 weeks) and were given green tea extract topically, showed that green tea extract decreased the wrinkle depth and decreased MMP-3 expression in irradiated group compared with control group.[11] A study about topical green tea extract that was topically administered on mice skin receiving ultraviolet B (UVB) irradiation, could prevent the increasing of matrix metalloproteinase-1 (MMP-1) and the decreasing of collagen number in dermal layer. The modern drug design uses single compound approach, and one of the most abundant compound in green tea is epigallocatechin-3-gallate (EGCG). EGCG is the main source of biologic activity of green tea. EGCG is the most abundant catechin in green tea.[7,12]

The drug discovery and development is a complicated process. It needs high cost in a long duration of process. Nowadays, the modern drug design was started with virtual screening (in silico study) in order to decrease the cost. The in silico study in drug design or computer aided drug design (CADD) is one of bioinformatics branch. The in silico study consists of protein structure analysis; and docking interaction between protein and the new drug. The docking interaction can predict the drug action potential in a disease prevention or treatment.[13,14] This study was aimed to predict EGCG as potential agent for photoaging prevention through in silico study by evaluating the docking interaction between EGCG and Keap1-Nrf2 protein.

## Material and Method

The molecular structure of Keap1-Nrf2 protein was downloaded from protein data bank (PDB), and PDB ID: 5FZN was selected. The structure of ligands was drawn using ChemDraw software application, version 11 and copied into Chem 3D software application, version 11 to create the 3D structure and measure the minimum energy using Molegro Virtual Docker, version 5.5.

The docking study of EGCG on the Keap1-Nrf2 protein was conducted using Molegro Virtual Docker software, version 5.0 (processor: Intel (R) Pentium (R) CPU N4200 @1.10GHz; installed RAM: 4.00 GB; system type: 64-bit-operating system). The best docking results were detected visually by comparing the structure of the docked molecules with the structure of reference ligand (FB2_1615[A]) in the binding site. (15) The MolDock and ReRank scores were presented the energy needed in receptor-ligand bond. The lowest energy visualized the best binding pose between the ligand and amino acid residue of the protein.

The prediction of pharmacokinetics and toxicity of the ligands were performed using pkCSM On-Line Tool. The molecular structure of ligands were drawn as 2D molecular structures with ChemDraw software, copied into Chem3D software, stored as a .sdf file, and translated into SMILE format using SMILE Translator Online Help. The SMILE format was processed using the pkCSM Online Tool to predict the pharmacokinetics and toxicity of compounds.(16,17,18,19)

## Findings

Target selection was obtained using the PubChem compound database. Molecular docking was performed to evaluate the mode of binding between the compound and Keap1-Nrf2 protein. The result of molecular docking 3D structure between candidate ligand (EGCG), control ligand (arbutin), and reference ligand (FB2_1615[A]) in Keap1-Nrf2 cavity showed, that the ligands were able to interact with Keap1-Nrf2 protein as the target protein (PDB ID: 5FZN) on the same binding site (Figure 1).

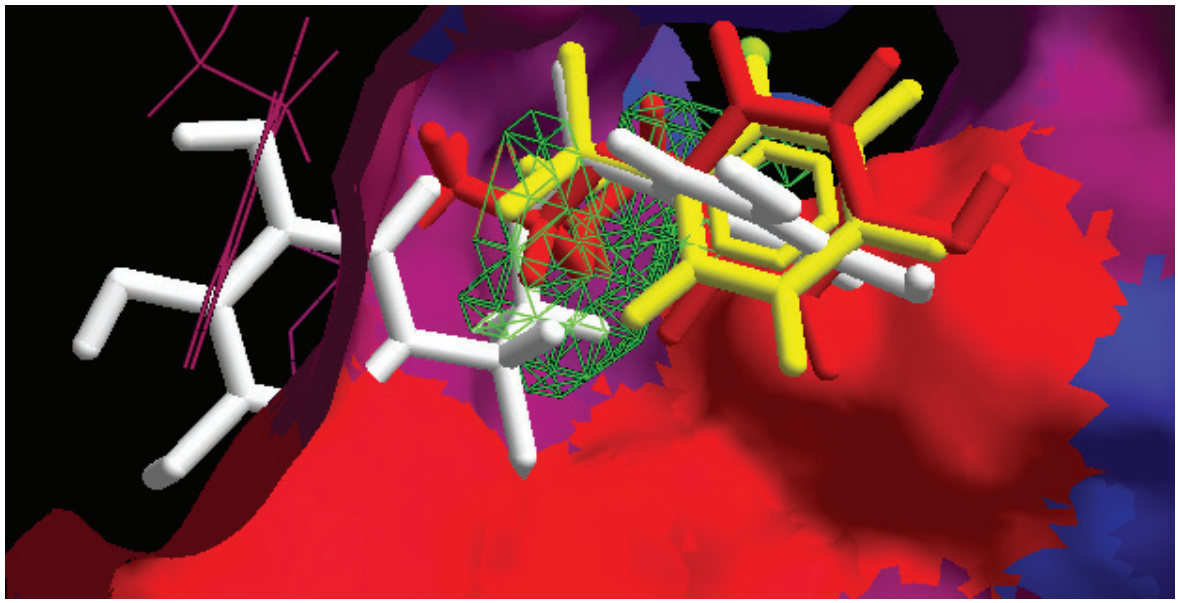

**Figure 1. The result of molecular docking 3D structure between candidate ligand (EGCG), control ligand (arbutin), and reference ligand (FB2_1615[A]) in Keap1-Nrf2 cavity. Description: green (Keap1-Nrf2 cavity), white (EGCG), red (arbutin), yellow (FB2_1615[A]).**

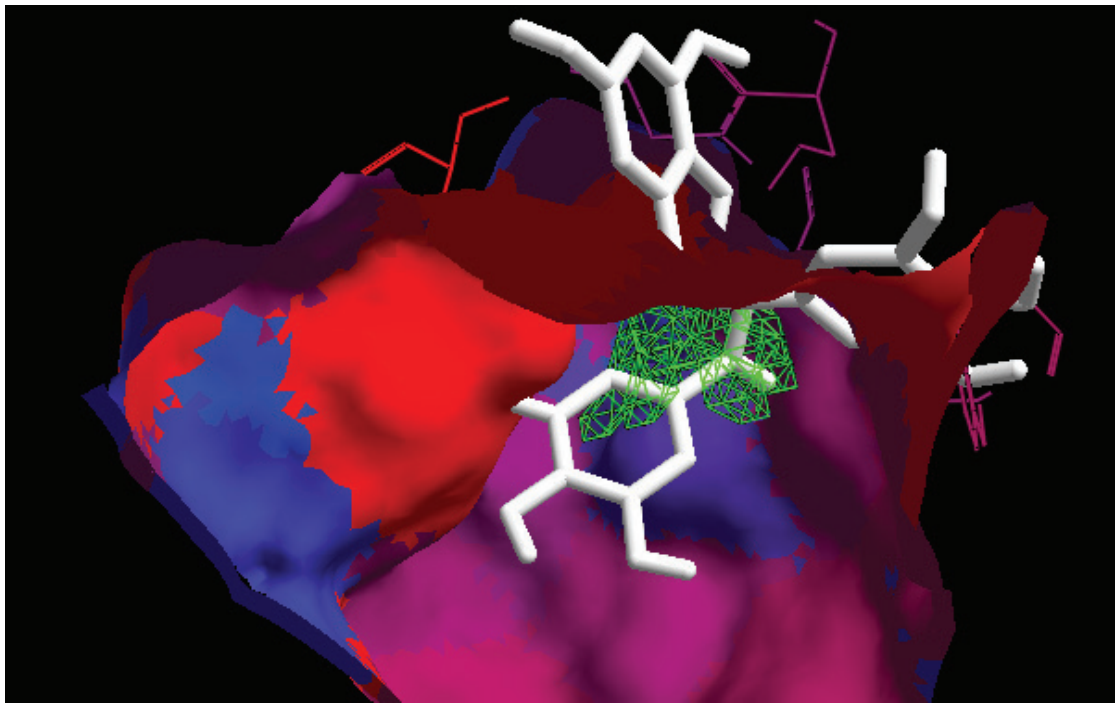

**Figure 2. Hydrophobicity view of EGCG in Keap1-Nrf2 cavity using Molegro Virtual Docker software. Description: white (EGCG); green (Keap1-Nrf2 cavity).**

The best docking position in the 3D structure molecules of EGCG to Keap1-Nrf2 protein as the target protein (PDB ID: 5FZN) can be seen in Figure 2. The docking was carried out at cavity 2, vol. 23.552. The bond location of the ligand binding site and target protein showed, that EGCG interacted with Keap1-Nrf protein through 54 number of bonds. Hydrogen and steric bond from 12 amino acids (Arg 415, Phe 335, Arg 336, Gln 337, Ser 338, Tyr 334, Asn 382, Ser 602, Gly 379, Asp 389, Arg 380, Asn 414) were showed at Figure 3.

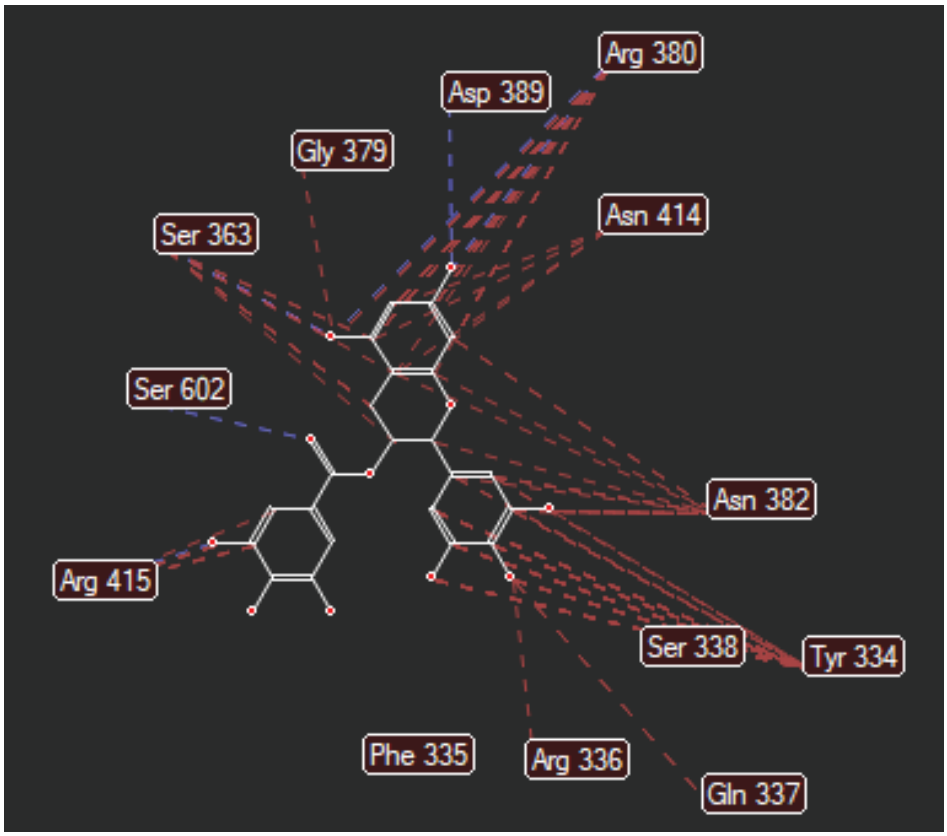


**Figure 3. Hydrogen and steric bound between EGCG and target protein (Keap1-Nrf2 protein) using Molegro Virtual Docker software. Description: blue line (hydrogen bound); red line (steric bound).**

The best docking position in the 3D structure molecules of arbutin (as the standard therapy of photoaging) to Keap1-Nrf2 protein as the target protein (PDB ID: 5FZN) can be seen in Figure 4. The docking was carried out at cavity 2, vol. 23.552. The bond location of the ligand binding site and target protein showed, that arbutin interacted with Keap1-Nrf protein through 36 number of bonds. Hydrogen and steric bond from 6 amino acids (Arg 415, Ser 363, Tyr 334, Ser 602, Gly 603, Gly 364) were showed at Figure 4.

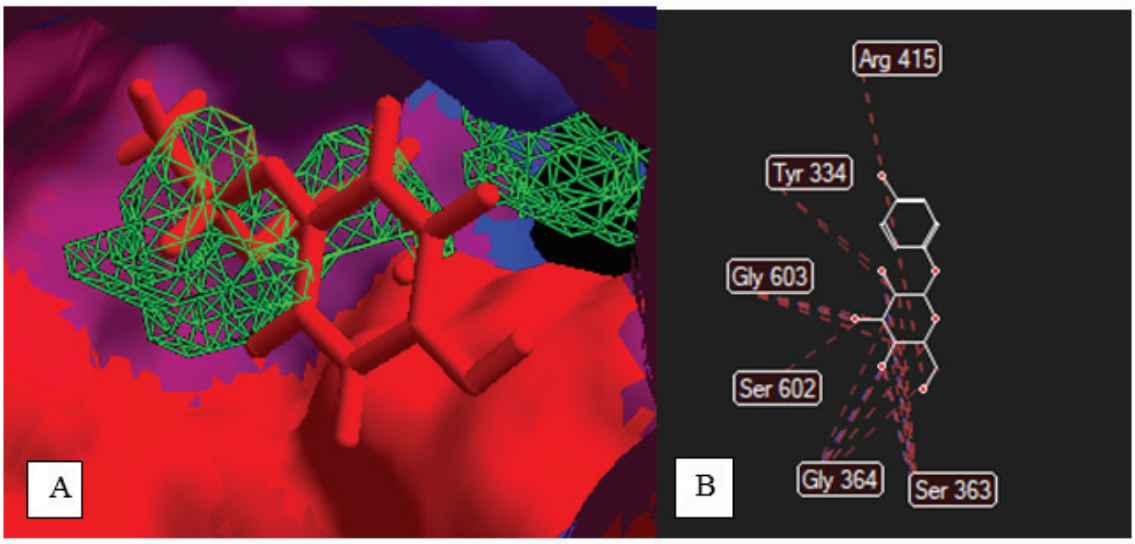


**Figure 4. A. Hydrophobicity view of arbutin in Keap1-Nrf2 cavity using Molegro Virtual Docker software. Description: red (arbutin); green (Keap1-Nrf2 cavity). B. Hydrogen and steric bound between arbutin and target protein (Keap1-Nrf2 protein) using Molegro Virtual Docker software. Description: blue line (hydrogen bound); red line (steric bound).**

The Mol Dock score and ReRank score of interaction between EGCG and 5FZN in Nrf2 cavity were shown in Table 1. The result of molecular docking between Keap1-Nrf2 protein with a candidate ligand (EGCG), a control ligand (arbutin), and a reference ligand (FB2_1615[A]) using Molegro Virtual Docker software, showed that the binding affinity of Keap1-Nrf2 protein with EGCG to be lower than that of arbutin and the reference ligand. The average Mol Dock and ReRank score of interaction between Keap1-Nrf2 protein and EGCG were -142.53±0.37 kcal/mol and -122.39±0.89 kcal/mol; between Keap1-Nrf2 protein and arbutin were -88.46±0.79 kcal/mol and -86.52±0.67 kcal/mol; and between Keap1-Nrf2 protein and FB2_1615[A] were -66.16±0.03 kcal/mol and -58.70±4.08 kcal/mol.

**Table 1. MolDock Score and ReRank Score of interaction between 5FZN protein and the compounds**

| The compounds | MolDock Score (kcal/mol) | ReRank Score (kcal/mol) |
|---|---|---|
| EGCG | -142.53±0.37 | -122.39±0.89 |
| Arbutin | -88.46±0.79 | -86.52±0.67 |
| FB2_1615[A] as reference ligand | -66.16±0.03 | -58.70±4.08 |

**Table 2. Pharmacokinetics properties of EGCG and arbutin**

| Pharmacokinetics properties | Model name | Predicted value (EGCG) | Predicted value (Arbutin) | Unit |
|---|---|---|---|---|
| Absorption | Skin Permeability | -2.735 | -2.743 | Log Kp (Numeric) |
| Toxicity | Skin Sensitization | No | No | Yes/No (Categorical) |
| | AMES toxicity | No | No | Yes/No (Categorical) |

The prediction of pharmacokinetics and toxicity were performed using pkCSM On-Line Tool. The prediction result using pkCSM On-Line Tool showed that the molecular weight value of EGCG was 458.375 (<500), and the value of the log of octanol/water partition coefficient (log P) was 2.2332. The result of pharmacokinetic prediction of EGCG can be seen in Table 2. The skin permeability of EGCG that were performed using pkCSM On-Line Tool was -2.735 cm/h. It was predicted using pkCSM On-Line Tool that EGCG did not cause skin sensitization and AMES toxicity.

## Discussion

The result of molecular docking between Keap1-Nrf2 protein with EGCG, arbutin, and a reference ligand (FB2_1615[A]) using Molegro Virtual Docker software, showed that the binding affinity of Keap1-Nrf2 protein with EGCG to be lower than that of arbutin and the reference ligand. These results showed the prediction of EGCG having higher potential in photoaging prevention than arbutin and the reference ligand.

The administration of topical formulation depends on the skin permeability. The skin permeability using pkCSM On-Line Tool was expressed as constant log Kp (cm/h). The skin permeability of EGCG was -2.735 cm/h. It was predicted, that EGCG has good skin permeability, because low skin permeability was expressed as log Kp more than -2.5 cm/h.[17,18]

Toxicity of compound can be predicted from AMES toxicity and skin sensitization. The mutagenic potential of the compounds can be predicted from the AMES

test. Mutagenic indication of compound is indicated from positive AMES test, and also indication that the compound has potential as a carcinogenic agent. The most important adverse effect from topical agent application is skin sensitization. The safety consideration of topical drug is the evaluation of whether a compound can induce allergic contact dermatitis.[17,18] The result of pkCSM On Line tool showed, that EGCG has no mutagenic and skin sensitization potential.

This study showed Keap1-Nrf2 protein as the potentially interactive target protein with EGCG. The Keap1-Nrf2 plays role in photoaging pathogenesis. Ultraviolet radiation causes increasing of Keap1-Nrf2 complex, that plays role in photoaging pathogenesis.[5,6] It was predicted in this in silico study, that binding of EGCG to Keap1-Nrf2 protein would be able to prevent photoaging. The prediction of pharmacokinetics and toxicity using pkCSM On Line tool showed that EGCG has good absorption profile without toxicity effect.

## Conclusion

This in silico study showed, that EGCG has potential in photoaging prevention, by interacting with Keap1-Nrf2 protein (PDB ID: 5FZN). The binding affinity of EGCG to Keap1-Nrf2 protein was lower than that of arbutin and reference ligand (FB2_1615[A]), it showed that EGCG has higher potential to be an alternative agent in photoaging prevention, with good phamacokinetics profile.

**Conflict of Interest:** No conflict of interest regarding the publication.

**Source of Funding:** This research was financially supported by Directorate of Research and Community Service - Directorate General of Research and Development - Ministry of Research, Technology and Higher Education (Direktorat Riset dan Pengabdian Masyarakat - Direktorat Jenderal Riset dan Pengembangan - Kementerian Riset, Teknologi dan Pendidikan Tinggi/Kemenristekdikti).

**Ethical Clearance**: taken from Ethical Committee in Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia.

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