Urological cancer: molecular docking of the active compound *Scurrula atropurpurea* against nuclear factor erythroid2-related factor2 (Nrf2)

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**Abstract.** The nuclear factor erythroid2-related factor2 (Nrf2) is a transcription factor for redox homeostasis involved in antioxidant genes and detoxification enzymes. This transcription factor provides protection against organs and is involved in urological cancer progression. This study aims to investigate the interaction between the active compounds of *Scurrula atropurpurea* against the Nrf2 signal. This study was an in silico study. The research protocol consisted of searching for amino acids making up the Nrf2-Keap1 system, searching for the structure of the active component of *Scurrula atropurpurea*, modeling 3D protein structures, docking and visualization between protein-ligand, and analyzing bond interactions between proteins and ligands. The active compounds of *Scurrula atropurpurea* which are molecularly docking include aviculin, caffeine, catechin, epicatechin, kaempferol, quercetin, quercitrin, rutin, and theobromine. For interactions with Nrf2, rutin was easier to interact compared to other compounds. Energy interactions between caffeine, catechin, kaempferol, quercetin, quercitrin, quercitin, and rutin were lower than the energy of interaction between Nrf2 and Keap-1. It was concluded that some of the active compounds of *Scurrula atropurpurea* can modulate the Nrf2 signal. Thus, there is an active compound from *Scurrula atropurpurea* which can be an anticancer urological candidate via an Nrf2 signal.

1. Introduction
Cancer is a group of diseases in which uncontrolled cell growth occurs and has the potential to spread to other parts of the body. Tumor suppressing mechanisms present in normal cells always distinguish between abnormal growing cancer cells and normal cells, but the problem arises when the function of these tumors suppressing genes is limited by different environmental factors (such as pollution, radiation, certain infections, etc.) or human habits (such as tobacco, bad diet, alcohol, etc.) [1-5].

Malignancy of urology, including prostate cancer (PCa), bladder (BCa) and kidney cancer (renal cell carcinoma, RCC) is a major cause of morbidity and mortality worldwide. About 326,000 new
diagnoses of urological cancer and more than 63,000 deaths are estimated to occur in the United States in 2018, which contributes around 20% and 10% to total estimates of new diagnoses and deaths of each type of cancer [6]. Because of the high incidence, diversity in biology, and especially direct interaction with urine, urological cancer is an important resource for scientists and doctors for diagnostic discoveries and new therapies [7, 8].

Most aspects of cancer biology show a level of redox regulation. Carcinogenesis, cancer cell proliferation, migration, invasion, metastasis, and vascularization all appear to be under redox control [9]. Nuclear factor erythroid2-related factor2 (Nrf2) is the main regulator of redox homeostasis, is the main transcription factor that regulates various genes for antioxidant enzymes and detoxification. It protects the organs from various types of toxic insults. On the other hand, activation of Nrf2 also correlates with cancer projection and chemoresistance [10]. Downregulation of Nrf2 activity has attracted increasing attention because it may provide alternative cancer therapy.

Natural products isolated from medicinal plants have been used for the treatment of various diseases from ancient times. The first use of natural products as medicine dates to 2600 BC in Mesopotamia [11]. *Scurula atropurpurea* or known by the Javanese as parasite tea is a parasitic plant for tea (*Thea sinensis*). This downgraded plant has been used by the inhabitants of Java as a cancer drug [12, 13]. *Scurula atropurpurea* inhibits cervical cancer cell growth through an intrinsic pathway apoptosis mechanism [14]. *Scurula atropurpurea* also acts as an antioxidant. Some of the active components of this plant are antioxidants of quercetin, quercitrin, kaempferol [15-19]. Until now, the potential of *Scurula atropurpurea* for the treatment of urological cancer through Nrf2-Keap1 modulation has not been revealed. Therefore, this study aims to analyze the effects of modulation of Nrf2-Keap1 in the context of urological cancer therapy.

2. **Material and methods**

2.1. **Search for amino acids making up Nrf2 and Keap1**

The amino acid sequences of Nrf2 (GI: 693842) and Keap1 proteins (GI: 22027642) were obtained from the National Center for Biotechnology Information (NCBI) database, the United States National Library of Medicine (NLM), National Institute of Health (NIH) (http://www.ncbi.nlm.nih.gov). The 3D structure of Nrf2 and Keap1 in the form of .sdf file format, will be converted to .pdb file using the OpenBabel software [20].

2.2. **Search for the structure of the active compound Scurula atropurpurea**

The 3D structure of the active compound of *Scurula atropurpurea* was obtained from the PubChem Open Chemistry Database. Nine active compounds were obtained, namely aviculins (CID 10391477), caffeine (CID 2519), catechin (CID: 9064), epicatechin (CID: 72276), kaempferol (CID 5280863), quercetin (CID 5280343), quercitrin (CID 5280459), routine (CID 5280805), and theobromine (CID 5429). The 3D structure of various compounds in the form of .sdf file format, will be converted to .pdb files using the OpenBabel software [20].

2.3. **3D protein structure modeling**

The 3D structure of target proteins was predicted using the SWISS-MODEL webserver with the modeling homology method. The structure then validated by using Ramachandran plot analysis [21, 22].

2.4. **Docking and visualization between protein-ligand**

Docking simulations between active compounds *Scurula atropurpurea* with target proteins were carried out using HEX 8.0 software [23]. The docking protocol consists of three stages of visualization, namely minimization of rigid-body energy, semi-flexible repairs, and finishing refinement in explicit solvents. The docking results are then visualized with Chimera 1.6.2 software and Discovery Studio 4.1.
2.5. Analysis of bond interactions between proteins and ligands

The results of the next docking analysis will be visualized using Discovery Studio 4.1, LigPlot + and LigandScout 3.1 [24, 25] software. Analysis of interactions between proteins and ligands was done to find the type of bond.

3. Results

Table 1 shows the interactions between various active compounds from *Scurulla atropurpurea* to Nrf2. The smallest bond energy for interaction with Nrf2 is found in rutin (-277.62 kJ/mol). Sequentially the energy of the quercitrin bond (-268.08 kJ/mol), aviculin (-261.81 kJ/mol), catechin (-194.92 kJ/mol), quercetin (-192.86 kJ/mol), epicatechin (-191.44 kJ/mol), kaempferol (-189.96 kJ/mol), caffeine (-161.98 kJ/mol), and theobromine (-142.86 kJ/mol).

| Interaction   | Point Interaction | Category            | Binding energy |
|--------------|-------------------|---------------------|---------------|
| Nrf2 – aviculin | Aviculin – Cys514 | Hydrophobic Bond    | -261.81 kJ/mol |
|              | Aviculin – Ala511 | Hydrophobic Bond    |               |
|              | Aviculin – Arg449 | Hydrophobic Bond    |               |
|              | Aviculin – Pro469 | Hydrophobic Bond    |               |
|              | Aviculin – Lys472 | Hydrophobic Bond    |               |
|              | Aviculin – Arg517 | Hydrophobic Bond    |               |
|              | Aviculin – Gln512 | Hydrophobic Bond    |               |
|              | Aviculin – Asn513 | Hydrophobic Bond    |               |
|              | Aviculin – Lys516 | Hydrophobic Bond    |               |
| Nrf2 - caffeine | Caffeine – Leu497 | Hydrophobic Bond    | -161.98 kJ/mol |
|              | Caffeine – Ile498 | Hydrophobic Bond    |               |
|              | Caffeine – Phe468 | Hydrophobic Bond    |               |
|              | Caffeine – Ile466 | Hydrophobic Bond    |               |
|              | Caffeine – Pro469 | Hydrophobic Bond    |               |
|              | Caffeine – Ile473 | Hydrophobic Bond    |               |
|              | Caffeine – Ile501 | Hydrophobic Bond    |               |
|              | Caffeine – Ala461 | Hydrophobic Bond    |               |
|              | Caffeine – Val470 | Hydrophobic Bond    |               |
| Nrf2 - catechin | Catechin – Val478 | Hydrogen Bond       | -194.82 kJ/mol |
|              | Catechin – Pro477 | Hydrophobic Bond    |               |
|              | Catechin – Lys506 | Hydrophobic Bond    |               |
|              | Catechin – Arg503 | Hydrophobic Bond    |               |
|              | Catechin – Arg502 | Hydrophobic Bond    |               |
| Nrf2 - epicatechin | Epicatechin – Cys514 | Hydrophobic Bond | -191.44 kJ/mol |
|              | Epicatechin – Lys516 | Hydrophobic Bond |               |
|              | Epicatechin – Asn513 | Hydrophobic Bond |               |
|              | Epicatechin – Gln512 | Hydrophobic Bond |               |
|              | Epicatechin – Arg517 | Hydrophobic Bond |               |
| Nrf2 - kaempferol | Kaempferol – Asn521 | Hydrophobic Bond | -189.96 kJ/mol |
|              | Kaempferol – Ile522 | Hydrophobic Bond    |               |
|              | Kaempferol – Lys462 | Hydrophobic Bond    |               |
|              | Kaempferol – Glu520 | Hydrophobic Bond    |               |
| Nrf2 - quercetin | Quercetin – Leu519 | Hydrophobic Bond    | -192.86 kJ/mol |
|              | Quercetin – Lys516 | Hydrophobic Bond    |               |
|              | Quercetin – Asn513 | Hydrophobic Bond    |               |
Table 2 shows the comparison of the interaction energy between the Nrf2-Keap1 complex with the Nrf2-Keap1 complex when there is an active compound from *Scurulla atropurpurea*. The bond energy of the formation of the Nrf2-Keap1 complex is -678.33 kJ/mol. The presence of an active compound *Scurulla atropurpurea* changes the energy of the Nrf2-Keap1 bond. Compounds that cause more negative bond energy are caffeine (-703.03 kJ/mol), catechins (-710.72 kJ/mol), kaempferol (-708.53 kJ/mol), quercetin (-722.01 kJ/mol), quercitrine (-749.01 kJ/mol), and rutin (-753.54 kJ/mol).

### Table 2. Interaction between Keap1 and Nrf2 with or without the active compound *Scurulla atropurpurea*.

| Molecule                  | Binding energy  |
|---------------------------|-----------------|
| Nrf2 – Keap1              | -678.33 kJ/mol  |
| Nrf2, aviculin – Keap1    | -632.96 kJ/mol  |
| Nrf2, caffeine - Keap1    | -703.73 kJ/mol  |
| Nrf2, catechin - Keap1    | -710.72 kJ/mol  |
| Nrf2, epicatechin - Keap1 | -655.82 kJ/mol  |
| Nrf2, kaempferol - Keap1  | -708.53 kJ/mol  |
| Nrf2, quercetin - Keap1   | -722.01 kJ/mol  |
| Nrf2, quercitrine - Keap1 | -749.84 kJ/mol  |
| Nrf2, rutin - Keap1       | -753.54 kJ/mol  |
| Nrf2, theobromine – Keap1 | -652.39 kJ/mol  |
4. Discussion
The docking method considers the interaction between small molecules and activity on the target, and predicts the degree of attachment of their binding interactions from the docking orientation and the strength of the interaction between them [26]. Nrf2 is protective of normal cells against stress conditions. While in tumor cells, Nrf2 can also be protective due to its activation which is able to win stress or redox regulation based therapy. Thus, there is still controversy over whether activation or inhibition of Nrf2 in tumor cells is a useful therapeutic strategy [27]. In this study, we first analyzed the interaction of nine active compounds from *Scurrula atropurpurea* against Nrf2. The interaction between rutin and Nrf2 has the most negative bond energy. This indicates that rutin interactions with Nrf2 are easier to occur among other active compounds. This study extends previous findings that interactions between routine and Keap can inhibit Keap-Nrf2 interactions [28].

In the second model we simulate the change in the interaction energy of Nrf2 with Keap1 without the presence of active compounds compared to the active compound is available. Active compounds that facilitate interaction between Nrf2 and Keap1 include caffeine, catechin, kaempferol, quercetin, quercitrin, and rutin. If Nrf2 with Keap1 forms stronger complexes, the more difficult Nrf2 translocation is for modulation of endogenous antioxidants. In cancer cells this will be destructive. In contrast, compounds that inhibit the interaction of Nrf2 with Keap1 include aviculins, epicatechin, and theobromine. This inhibition will make it easier for Nrf2 translocation into the nucleus for antioxidant modulation. In cancer cells this will be protective against redox regulation based therapy.

It was concluded that some of the active compounds of *Scurrula atropurpurea* can modulate the Nrf2 signal. Thus, there is an active compound from *Scurrula atropurpurea* which can be an anticancer urological candidate via an Nrf2 signal.

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