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RESEARCH ARTICLE

# In silico approach of bioactive molecule chitosan 501.1 kDa from snail shell as antioxidant and inhibitor of the keap1-nrf2 protein-protein interaction

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## Abstract

**Background:** ROS are created when high levels of oxidative stress occur due to hypercholesterolemia. Nuclear Factor Erythroid 2-related factor (NRF2) controls the expression of antioxidant genes. Kelch-like ECH-associated protein 1 (KEAP1) therapy degrades NRF2. Chitosan 501.1 kDa from snail shells contains bioactive chemicals that can induce NRF2 activity. **Objective**: To evaluate the potential antioxidant activity of the bioactive compound in Mw 501.1 kDa chitosan by targeting KEAP1 and NRF2 proteins in-silico. **Method**: The 3D structures of the bioactive compounds chitosan and control 51M were derived from the PubChem database, and the proteins were derived from the RCSB PDB. The biological activity of chitosan bioactive compounds was predicted using the PASS Online server. Molecular docking was performed using Hex 8.0.0 Cuda with Shape+Electro+DARS and visualised with Discovery Studio. The biological activity of chitosan compounds was predicted as lipotropic and antioxidant. **Result**: The discovery of the bioactive compound chitosan 501.1 kDa interacted strongly with KEAP1. The bioactive compound chitosan also inhibited KEAP1 through residues GLN75 and LEU84 at the 51M-KEAP1 interaction. **Conclusion**: The bioactive compound chitosan 501.1 kDa could inhibit the interaction of KEAP1-NRF2 proteins so that NRF2 could transcribe antioxidant genes. Therefore, may serve as a suitable alternative.

## Introduction

Hypercholesterolemia is a condition when cholesterol levels in the blood exceed the normal threshold. Hypercholesterolemia conditions can cause oxidative stress (SO) due to the highly reactive oxygen species (ROS) levels. Oxidative stress in hypercholesterolemia occurs because of the activity of the KEAP1 protein (Kelch ECH associating protein 1) (Chen *et al*., 2015). KEAP1 is recognised as an important regulator of NRF2 (Nuclear factor-erythroid-2 related factor 2) (Ahtikoski *et al.,* 2019) and as a sensor for noxious stimuli, such as oxidants (Tanji *et al.*, 2013). KEAP1 is also one of the central regulators of NRF2 activity (Wells, 2015). KEAP1 controls NRF2 movement as an inhibitor of NRF2 activity by promoting NRF2 degradation in the ubiquitin-proteasome pathway (Kanansen *et al*., 2013).

In addition, oxidative stress is closely related to hypercholesterolemia (Csonka *et al*., 2016; Chtourou *et al*., 2015). Therefore, synthetic drugs used as antioxidants for treatment targeting KEAP1 have been developed, but synthetic drugs sometimes have adverse side effects and a negative impact on health. However, it is different from the natural ingredients,

i.e. chitosan. In this case, chitosan generally has no significant side effects (Rizzo *et al.,* 2014). Chitosan comes from snail shells (Umar *et al*., 2019).

Moreover, chitosan has pharmacological potentials, such as lowering cholesterol levels (Rizzo *et al.*, 2014), gram-positive and negative antibacterial (Debbabi *et al.*, 2017) and antiulcer (Bhat *et al.*, 2011). For this reason, this study aims to evaluate the antioxidant potential of the bioactive compound chitosan. This study also predicts the activity of chitosan compounds as antioxidants in inhibiting KEAP1 activity using an *in-silico* approach.

## Methods

### *Data retrieval and sample preparation*

The hardware used in the *in silico* study was an HP laptop with the following specifications: Intel® Core i3 processor, 512 GB RAM. The bioactive compound in snail shells in this study was chitosan with molecular weight (MW). SMILES structures and 3D ligands (compounds) chitosan 501.1 with ID 129662530 and ligan (control) 51M with ID 91971266 were derived from the PubChem database. Chitosan compounds in 3D and compound preparation with the Open Babel plugin were integrated with PyRx 8.0. The target proteins (KEAP1 and NRF2) were derived from the RSCB Protein Data Bank with ID 5CGJ. Protein preparation was carried out to remove water molecules and contaminant ligands with Discovery Studio 2019.

### *Biological activity analysis*

Bioactive compounds were predicted using the PASS Online web server. This server contains more than 4000 types of biological activity and is usually used to predict and screen potential bioactive compounds' SMILES or moles. File to indicate physical activity, and the activity threshold (Pa) is set to "more than 0.5". The higher the Pa value, the more accurate the prediction results (Filimonov *et al*., 2014).

### *Molecular docking analysis*

Molecular docking was performed to determine the interaction of KEAP1 and NRF2 with Hex 8.0.0 Cuda with Shape+Electro+DARS, and the root means square deviation of the value 2. The docking results were visualised with *Discovery Studio 2020* to determine the binding affinity and residues of protein and ligand interactions.

## Results

Chitosan 501.1 kDa was analysed to determine the possible biological activity of antioxidants and free radical scavengers. Chitosan 50.1 kDa was predicted as a lipotropic agent with high probability (Pa=0.797), while all other activities have a relatively lower Pa value, including free radical scavenger (0.397), antioxidant (0.333), and antiobesity (0.331). A detail of the results is presented in Table I.

**Table I: Antioxidant activities prediction using PASS online**

| Compound | Biological activity | Pa | Pi |
|---|---|---|---|
| Compounds Chitosan 501.1 | Lipotropic | 0.797 | 0.002 |
| | Free radical scavenger | 0.397 | 0.018 |
| | Antioxidant | 0.333 | 0.018 |
| Control 51M | Antiobesity | 0.331 | 0.057 |

The molecular docking study was on Chitosan + KEAP1 bioactive compounds, Chitosan + KEAP1 against NRF2, Chitosan + NRF2, and Chitosan + NRF2 against KEAP1. The results of the order of binding affinity from lowest to highest were Chitosan+NRF2-KEAP1 < Chitosan+KEAP1-NRF2 < Chitosan-KEAP1 < Chitosan-NRF2; -451.93 < -387.54 < -276.61 < -229.87 (kcal/mol), with complete data in Table II. This study also found that the binding sites of Chitosan+NRF2-KEAP1 were GLN75, ASP77, LEU76, and GLN337 as conventional hydrogen bonds and LEU76 bond, LEU84 as carbon-hydrogen bond (Table II and Figure 1), while controlling 5M1+KEAP1, 5M1+KEAP1 against NRF2, 5M1+NRF2, and 5M1+NRF2 against KEAP1. The results of the order of binding affinity from lowest to highest were 51M+NRF2-KEAP1 <51M+NRF2 < 51M+KEAP1< 51M+KEAP1-NRF2; -482.12 < -429.09 < -349.86 < -222.86, with complete data in Table II.

This study was different from the control of the bioactive compound chitosan, where the binding site of 5MI+NRF2-KEAP1 was GLN75 as a conventional hydrogen bond, LEU84 as an alkyl bond, and PHE71, ALA72, LEU84 as Pi-Alkyl bond (Table II and Figure 2). Chitosan compounds also had different bonds with 51M except for GLN75 and LEU84. Still, the involvement of GLN75 and LEU84 in the interaction of KEAP1 bioactive compounds and chitosan compounds is predicted to interfere with KEAP1-NRF2 protein-protein interactions so that NRF2 will be activated. Inhibition of KEAP1 causes NRF2 activation and plays a role in the oxidative stress response (Li *et al.*, 2019).

**Table II: Binding affinity and interaction of protein-ligand**

| Complexes | Energy (cal/mol) | Residues | Category (types) | From chemistry | To chemistry |
|---|---|---|---|---|---|
| Chitosan+ KEAP1 | -276.61 | CYS513;GLY564;VAL467;VAL514 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | HIS562;VAL561 | Carbon Hydrogen Bond | H-Donor | H-Acceptor |
| Chitosan+ KEAP1-NRF2 | -387.54 | CYS513;GLY564;VAL467;VAL514 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | HIS562;VAL561 | Carbon Hydrogen Bond | H-Donor | H-Acceptor |
| Chitosan+NRF2 | -229.87 | GLN75;ASP77;LEU76 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | LEU76;LEU84 | Carbon Hydrogen Bond | H-Donor | H-Acceptor |
| Chitosan+NRF2-KEAP1 | -451.93 | GLN75;ASP77;LEU76;GLN337 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | LEU76;LEU84 | Carbon Hydrogen Bond | H-Donor | H-Acceptor |
| 51M+KEAP1 | -349.86 | VAL465;VAL512 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | ILE416 | Pi-lone pair | Lone pair | Pi-Orbitals |
| | | ARG415;ALA556;LEU557;ILE559;VAL606 | Alkyl | Alkyl | Alkyl |
| 51M+KEAP1-NRF2 | -222.86 | VAL465;VAL512 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | ILE416 | Pi-lone pair | Lone pair | Pi-Orbitals |
| | | ARG415;ALA556;LEU557;ILE559;VAL606 | Alkyl | Alkyl | Alkyl |
| 51M+NRF2 | -429.09 | GLN75 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | LEU84 | Alkyl | Alkyl | Alkyl |
| | | PHE71;ALA72;LEU84 | Pi-Alkyl | Pi-Orbitals | Alkyl |
| 51M+NRF2-KEAP1 | -482.12 | GLN75 | Conventional Hydrogen Bond | H-Donor | H-Acceptor |
| | | LEU84 | Alkyl | Alkyl | Alkyl |
| | | PHE71;ALA72;LEU84 | Pi-Alkyl | Pi-Orbitals | Alkyl |

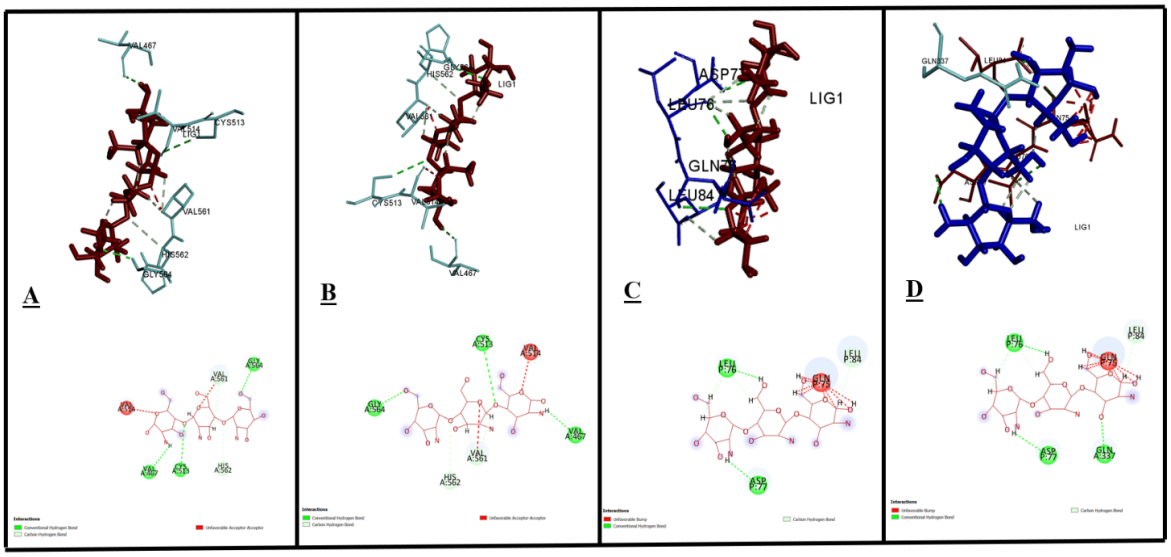


**Figure 1: The interaction of A. Chitosan+KEAP1, B. Chitosan+ KEAP1-NRF2, C. Chitosan+NRF2, and D. Chitosan+NRF2-KEAP1, shown in the 3D (above) and 2D (below) diagrams**

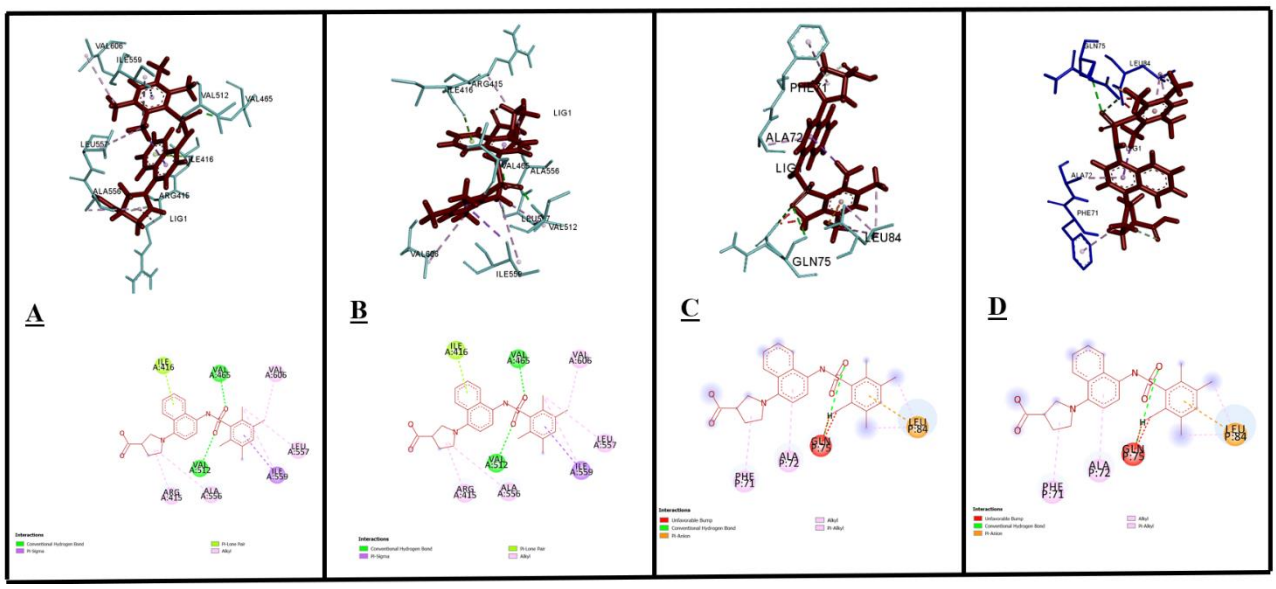


**Figure 2: The interaction of A. 51M+KEAP1, B. 51M+KEAP1-NRF2, C. 51M+NRF2, and D. 51M+NRF2-KEAP1 are shown in the 3D (above) and 2D (below) diagrams**

## Discussion

The pathogenicity of hypercholesterolemia is related to oxidative stress. Free radicals are molecules or atoms with one or more unpaired electrons in their outer orbital, making them highly reactive species (Biswas *et al.*, 2017; Cocuzza *et al.,* 2007) The body has a natural antioxidant defense system to fight ROS, i.e., SOD (Leung *et al.*, 2019; Meng *et al.*, 2018). SOD is an endogenous antioxidant with a role in oxidative stress.

Antioxidants are also a crucial part of the diet for hypercholesterolemia (Niki, 2012). However, endogenous antioxidants (SOD) in the body are limited in number to counteract free radicals, so the body cannot inhibit the rate of oxidative stress (Ayunda *et al.,* 2019), but other antioxidant parameters are found in hypercholesterolemia conditions along with increased oxidative stress. Here, SOD is activated during the response to oxidative stress via the KEAP1/NRF2 pathway (Niedzielska *et al.,* 2016; Zhuang *et al.,* 2017). KEAP1 plays a role in NRF2 activity. NRF2 is released from the KEAP1 molecule and acts as a transcription factor for genes encoding other antioxidant enzymes.

This study found six specific amino acid residues and low affinity in Chitosan+NRF2-KEAP1, compared to control 51M+NRF2-KEAP1, which found five specific amino acid residues and lower affinity compared to the bioactive compound chitosan. Thus, chitosan bioactive compounds had stronger interactions compared to 51M. Chitosan has also been proven as a lipotropic, antioxidant, and free radical scavenger (Umarudin *et al.*, 2022). Because the structure of chitosan has three hydrogens: C−2 ($NH_2$), C−3 (OH) and C−6 (OH) (Liping *et al.,* 2020); (Kim *et al.,* 2006), they play an important role in free radical scavenging activity, thus forming more stable molecules. Another research also revealed that chitosan could significantly increase the activity of the antioxidant enzyme superoxide dismutase (SOD) (Li *et al.,* 2022; Marianti & Mahatmanti, 2018) so that chitosan can regulate the activity of antioxidant enzymes and decrease lipid peroxidation. The molecular weight (MW) of chitosan also has a role, where MW lower than 501.1 kDa, such as 2.2 kDa, influences the free radical scavenging capacity (Chang *et al.,* 2018), meaning that the greater the molecular weight of chitosan, the stronger it is in scavenging free radicals compared to the MW of chitosan, with low molecular weight (Avelelas *et al*., 2019). Hence, the greater the molecular weight, the more hydroxyl and $NH_2$ groups function as strong free radical scavengers.

In this study, it is hoped that the interaction of chitosan with KEAP1 can interfere with the KEAP1/NRF2 pathway so that NRF2 can be activated. Candidates for the bioactive compound chitosan from snail shells are preferred because they have few side effects. Previous studies have also shown that inhibition of KEAP1 can inhibit diseases caused by oxidative stress, such as hyper cholesterol and diabetes.

## Conclusion

The 501.1 kDa chitosan compound contained in snail shells has been predicted to have the potential as an antioxidant agent because it has the potential to inhibit KEAP1 protein activity. KEAP1 can be inhibited via the GLN75 dan LEU84 residue with a very low binding affinity. This research is expected to predict the possibility of chitosan compounds with Mw 501.1 kDa potential as antioxidants in the treatment of hypercholesterolemia, so it is necessary to do further in vivo studies on animal modelling trials of high-fat diet models.

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## Abbreviations

Oxidative stress (SO); Reactive oxygen species (ROS) Kelch ECH associating protein 1 (KEAP1 protein); Nuclear factor-erythroid-2 related factor 2 (NRF2); Molecular weight (MW); superoxide dismutase (SOD).

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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