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Research Article

Computational study of curcumin as antioxidant and potential inhibitor to abrogate Keap1-Nrf2 interaction

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ABSTRACT

Nrf2 is a kind of transcription factor which is related to antioxidative enzyme synthesis and protecting cell from aging process. Keap1 protein receptor, which acts as a suppressor specifically interacts with Nrf2. Abrogating the interaction between Keap1 and Nrf2 will activate Nrf2 in the cytosol. One of the compound which is suggested as an inhibitor of Keap1-Nrf2 interaction is curcumin. Therefore, this research aims to identify potency of curcumin as antioxidant and an inhibitor in the interaction of Keap1-Nrf2 in order to activate Nrf2. The biological activity prediction of curcumin was analyzed by PASS online server. Then, docking process of curcumin to Keap1 was carried out by Pyrx AutoDock Vina to analyze the binding affinity and compared to Keap1-IQK as a control. Results showed that curcumin has a high ability as an antioxidant and free radical scavenger with Pa score 0.634, and 0.771, respectively. Furthermore, docking process pointed out that IQK had -10.7 Kcal/mol in binding affinity and it was higher than the binding affinity of curcumin about -8.4 Kcal/mol. However, it is suggested that curcumin has the high potential as antioxidant and inhibitor to release *Keap1-Nrf2* complex.

Keywords: Aging, antioxidant, curcumin, Keap1, Nrf2

INTRODUCTION

Curcumin is an herbal compound which is derived from *Curcuma longa* rhizome. It is not only antioxidant but also acts as an electrophilic compound which can trigger the Nrf2/ARE signaling pathway to activate the antioxidative enzyme (Sikora *et al.,* 2010; Shen *et al.,* 2012). The diet of mice treated with 0.2% tetrahydro curcumin at the age of 13 months had significantly longer average lifespan about 11.7% (Kitani *et al.,* 2007). In another study, curcumin also increased the lifespan of *Drosophila* sp. (Shen *et al.,* 2012). Curcumin commonly used as preventing the disease related to aging issue (Sikora *et al.,* 2010). Curcumin induces antioxidant defense by modulating Nrf2. When, curcumin interact with Keap1, Nrf2 is released and translocated into the nucleus where it binds to an antioxidant responsive element in DNA to initiate gene expression of an antioxidant enzyme. That enzyme will reduce free radicals and protect the cell from ROS (Gonzalez-Reyes *et al.,* 2013).

Nowadays, the application of computational or in silico approach has been widely applied for pharmacological studies. Such methods were developed to discover novel molecules which potentially activated protein target (Ekins *et al.,* 2007). Docking process is one kind of analysis in order to f ind small molecules with desired function (Dallakyan *et al.,* 2015). Then, the potential of small molecules can be analyzed based on the biological activity prediction by PASS server. It is indicated by Pa (probability activity) and Pi (probability inactivity) score which varies from 0 to 1. The positive result is determined if Pa is higher than Pi (Paramashivam *et al.,* 2015). Therefore, this study aims to investigate the potential of curcumin as an antioxidant to activate Nrf2 by inhibiting KEAP1-Nrf2 interaction using in silico approach.

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MATERIAL AND METHODS

Ligand preparation: The 3D structure of Curcumin (CID: 969516) was retrieved from PubChem (www.pubchem.ncbi.nlm.nih.gov) in SDF format (Figure 1). Furthermore, it was converted by Open Babel to PDB format for docking preparation.

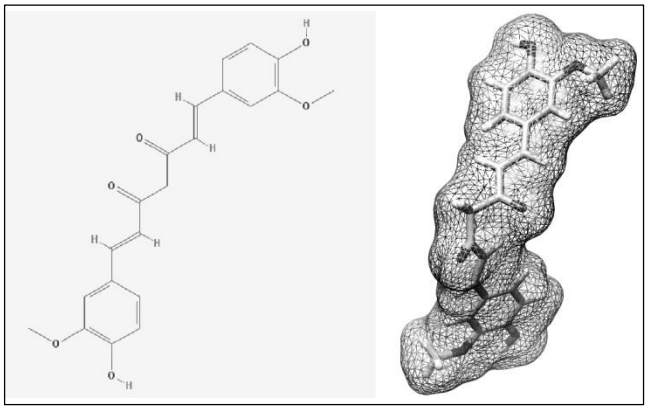

Figure 1: Two and Three-dimensional structure of curcumin

Biological activity analysis: Biological activity includes potential of antioxidant and free radical scavenger of curcumin were predicted by PASS server (http://www.pharmaexpert.ru/passonline/index.php). It is indicated by Pa (Probability activity) score.

Accession of target protein: The 3D structure of protein receptor Keap1 (PDBID: 4IQK) which binds to IQK ligand was collected from Protein Data Bank (PDB) (www.rscb.org/pdb/home/home.do). It was opened by PyMol to separate from the ligand and another water molecule. This optimization was essential for docking preparation (Figure 2).

Molecular docking analysis: Curcumin was docked to Keap1 receptor in a specific site similar to IQK as ligand reference. Docking process was carried out by Pyrx AutoDock Vina. It was aimed to calculate binding affinity score of the curcumin-Keap1 complex.

Molecular interaction analysis: The molecular interaction was analyzed by Lig and Scout to visualize and identify interaction residues in the binding domain of curcumin and Keap1 receptor. Potential inhibition of curcumin was indicated by molecular interaction of Keap1 which interacts to curcumin.

RESULTSAND DISCUSSION

The biological activity of the herbal compound was analyzed by PASS online server with Structure Activity Relationship

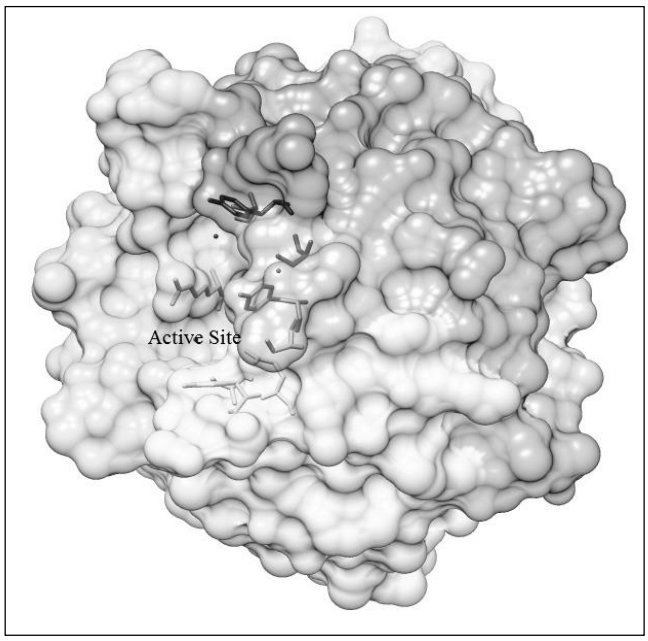

Figure 2: Prepared model of Keap1 as protein target

(SAR). It is determined by score of Pa (probability activity) and Pi (probability inactivity) with values varing from 0 to 1 (Paramashivam *et al.,* 2015). Curcumin was analyzed in order to find out the possible biological activity of antioxidant and free radical scavenger. The analysis shows that curcumin obviously determines as free radical scavenger which is indicated by Pa score 0.771. Moreover, curcumin was also highly potential as an antioxidant agent with Pa score of 0.634 (Table 1). Curcumin was proved to be as antioxidant and free radical scavenger. Moreever, curcumin inhibit the DNA damage, protein denaturation and lipid peroxidation. (Savcun *et al.,* 2013; Menon *et al.,* 2007).

Table 1: Biological activity prediction of curcumin

Biological Activity	Probability Activity (Pa)
AntioxidantFree radical scavenger	0.634
	0.771

Those Pa and Pi scores considered to be measurement tools to predict active or inactive compound respectively. The minimum standard of reliable value is 0.3. Biological activity with Pa > 0.7 indicates that the result of in silico assay will be relatively similar to laboratory assay, while Pa > 0.3 indicates that those compounds are potentially in silico approach (Benchabane *et al.,* 2009).

Molecular Docking as further analysis was used to evaluate the potential of curcumin as Keap1 inhibitor. Docking process was conducted on the active site of Keap1 receptors which bind to IQK ligand. IQK is a reference

inhibitor used as a control for comparison to curcumin. It utilizes to investigate the potential of curcumin to inhibit the interaction of Keap1 and Nrf2.

Nrf2 (NF-E2-Related Factor 2) is a transcription factor which interacts to its specific repressor, Keap1 (Motohashi *et al.,* 2004). Nrf2 in responding to cytotoxic stress is well defined. The main role of Nrf2 is involved in oxidative stress response and aging issue. Nrf2 degradation is initiated by Keap1 interaction. (Bruns *et al.,* 2015).

Based on Table 2, the results pointed out that IQK efficiently inhibited Keap1 receptor with minimum binding affinity score about -10.7 Kcal/mol that compared to curcumin which only had -8.4 kcal/mol. However, it suggests that curcumin potentially acts as an inhibitor of Keap1 because it also has a binding affinity score which is relatively low. In addition, the result of docking process also showed molecular interaction while curcumin binds to substrate binding domain of Keap1 in the SER508A, ALA556A,

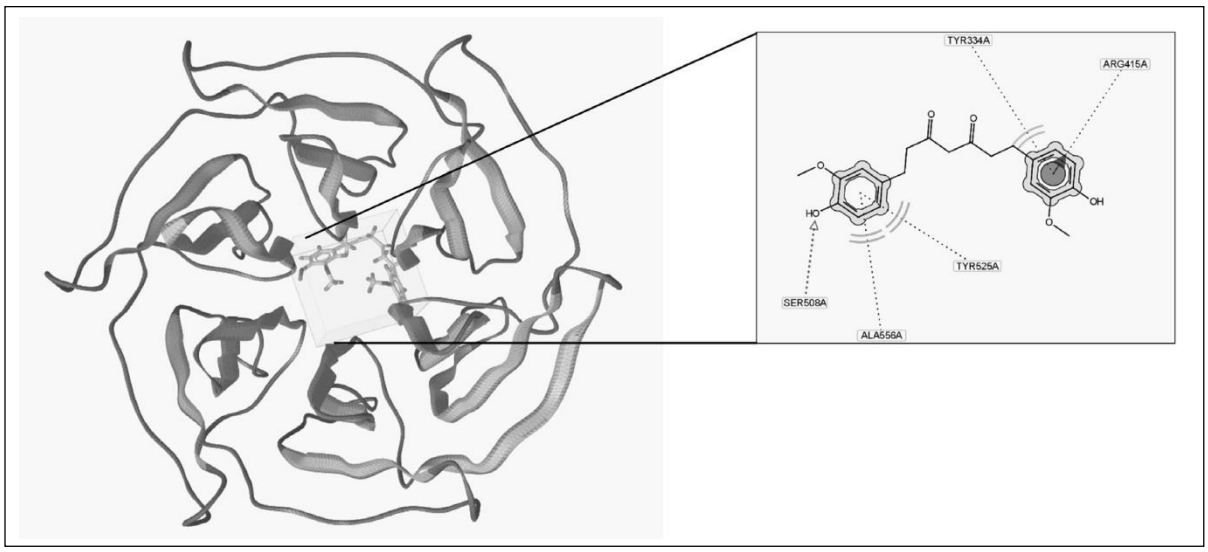

Figure 3: Molecular interaction of curcumin with Keap1 protein receptor (blue) on substrate binding domain (SER508A, ALA556A, TYR525A, TYR334A and ARG415A)

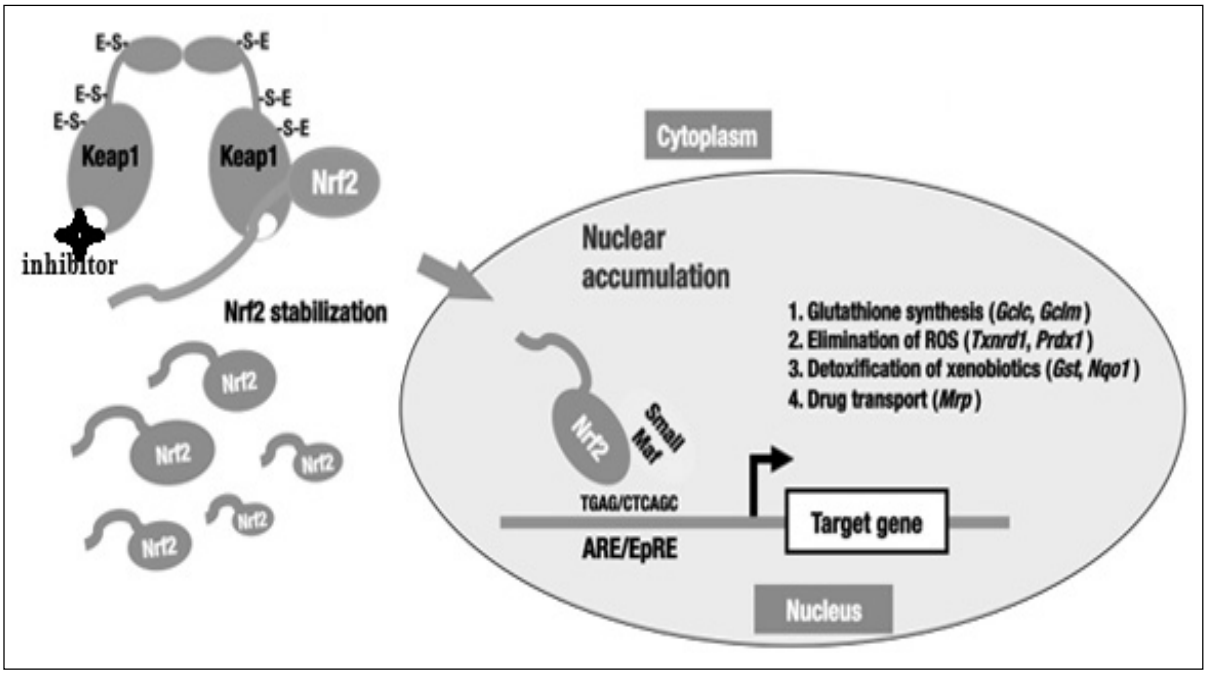

Figure 4: Mechanism of Keap1 inhibition for activating Nrf2 (Jockers *et al.,* 2016)

Table 2: Binding affinity score of IQK and curcumin to Keap1 receptor

Protein receptor	Ligand	Binding affinity (Kcal/mol)
Keap1(PDBID: 4IQK)	IQK	-10.7
	Curcumin	-8.4

TYR525A, TYR334A and ARG415A (Figure 3). It indicated that curcumin could act as IQK when inhibit the Keap1 protein in the substrate binding domain.

For inhibiting aging process, curcumin activates Nrf2 which is already in the cytoplasm and remain bound to Keap1 receptor. Curcumin interacts to Keap1, Nrf2 is released and is translocated into nucleus where it binds to an antioxidant responsive element in DNA to initiate gene expression. Nrf2-regulated gene classified into phase II xenobiotic-metabolizing, an antioxidant enzyme, DNA repair enzymes and anti-inflammatory enzyme (Figure 4) (Jockers *et al.*, 2016). That enzyme will reduce free radicals and protect the cell from ROS for increasing the ability of the cell to repair (Gonzalez-Reyes *et al.*, 2013).

CONCLUSION

It could be concluded that curcumin has highly biological activity as an antioxidant and free radical scavenger in the computational study. Moreover, curcumin also strongly binding to the active site of Keap1 and interact to the substrate binding domain of Keap1 in SER508A, ALA556A, TYR525A, TYR334A and ARG415A. It suggested that curcumin is highly potential as an inhibitor of Keap1 to activate for Nrf2 protein.

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