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COMPUTATIONAL INVESTIGATION OF ISORHAMNETIN-3, QUERCETIN-3, AND QUERCETIN FROM *Alstonia scholaris* AS THE POTENTIAL ANTI-INFLAMMATORY AGENTS AGAINST COX-2

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Abstract

Cyclooxygenase-2 (COX-2) plays an essential role in the activation of the inflammatory condition. In numerous types of diseases, including inflammatory-related diseases and cancer, the increased expression of COX-2 is related to the poor prognosis. Therefore, this study aims to evaluate the potency of *Alstonia scholaris* bioactive compounds as a COX-2 inhibitor to develop anti-inflammatory agents. Molecular docking simulation was applied to assess the possibility of each compound of *A. scholaris* as a COX-2 inhibitor. Several bioactive compounds were employed as ligands such as kaempferol, quercetin, isorhamnetin, kaempferol-3-o-β-d-galactopyranoside, quercetin-3-o-β-d-galactopyranoside (hyperoside), and isorhamnetin-3-O-β-d-galactopyranoside. Interestingly, we found three bioactive compounds of *A. scholaris* that may potentially be inhibitory agents of COX-2, namely isorhamnetin-3, quercetin-3, and quercetin. Moreover, the ligand and protein interaction of these three compounds have particular interaction, such as isorhamnetin-3 and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Amide-Pi Stacked, Pi-Alkyl, and Pi-Sigma), quercetin-3 and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Alkyl, and Pi-Sigma), and quercetin and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Pi-Anion, Pi-Alkyl, and Unfavorable Donor-Donor). Further research in evaluating the biological function of these compounds is needed, especially in *in vitro* and *in vivo* experiments.

INTRODUCTION

COX-2 plays a pivotal role in several biological processes. The most well-known process regulated by COX-2 is inflammation [1]. Inflammation is the biological condition of the body's responses to foreign substances such as microbes, viruses, or chemicals [2]. In cancer incidences, COX-2 promotes tumor growth and suppresses tumor immunity [3,4]. Regarding this issue, some therapies, including radiotherapy, chemotherapy, photodynamic, and anti-inflammatory, have been applied to reduce cancer incidence [2,5]. However, combination therapy or new therapy discovery have emerged to find the best way to treat diseases, including herbal medicine.

Nowadays, herbal medicine has gained more attention due to its multiple advantages, such as bioactive-rich compounds, fewer side effects, and being economically easy to obtain. The active compounds from medicinal plants usually possess broad pharmaceutical properties, especially anti-inflammatory effects [6]. Nowadays,

screening bioactive compounds for drug candidates is vital to find new possible content to combat the specific disease. Therefore, broad studies on drug discovery, including *in silico* are mandatorily needed.

The *A. scholaris* has been known as tropical plants from the Apocynaceae family, broadly used as a handicraft source in society. Interestingly, the leaves and barks of *A. scholaris* already proposed to ameliorate several types of diseases, such as anti-diabetic, anti-ulcer, anti-fertility, antioxidant, hepatoprotective, immunomodulatory, anti-cancer, and antimicrobial [7-9]. Importantly, it has been reported that the leaves extract of *A. scholaris* contains a variety of bioactive compounds such as kaempferol, quercetin, isorhamnetin, kaempferol-3-o-β-d-galactopyranoside, quercetin-3-o-β-d-galactopyranoside (hyperoside), and isorhamnetin-3-O-β-d-galactopyranoside [10]. According to the above background, this study aims to evaluate the potency of *A. scholaris* bioactive compounds as COX-2 inhibitors in order to develop anti-inflammatory agents through computational prediction approaches.

MATERIALS AND METHODS

A study conducted by Kaushik et al. (2011) showed the leaves extract of *A. scholaris* contains numerous types of bioactive compounds, namely kaempferol, quercetin, isorhamnetin, kaempferol-3-o-β-d-galactopyranoside, quercetin-3-o-β-d-galactopyranoside (hyperoside), and isorhamnetin-3-O-β-d-galactopyranoside [10]. The bioactive compounds and chemicals properties of A. scholaris used in this study are listed in Table 1.

Table 1. Physicochemical properties of *A. scholaris* bioactive compounds according to Lipinsky's principles [11]

No.	Bioactive Compounds	CID	Molecular Mass (Dalton)	Hydrogen Bond Donor	Hydrogen Bond Acceptors	High Lipophilicity (LogP)	Molar Refractivity
1.	Kaempferol	5280863	286	4	6	0.64	62.82
2.	Quercetin	5280343	302	5	7	0.52	64.36
3.	Isorhamnetin	5281654	316	4	7	1.04	68.98
4.	Kaempferol-3-O-β-d-galactopyranoside	5282149	448	7	11	1.15	97.24
5.	Quercetin-3-O-β-d-galactopyranoside (Hyperoside)	5281643	464	8	12	1.03	98.79
6.	Isorhamnetin-3-O-β-d-galactopyranoside	5318645	478	7	12	1.55	103.40

The 2D structure of compounds was retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Each compound of *A. scholaris* then evaluated based on Lipinsky's principles (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) [11]. After that, the 3D structure of COX-2 was built through the SWISS-MODEL (https://swissmodel.expasy.org/). Both ligands and protein structures were prepared and optimized prior to molecular docking with PyRx 8.0. software.

More specifically, the docking dimension of each ligand to target protein are the following X: 47.1115 Angstrom, Y: 50.7964 Angstrom, and Z: 65.2031 Angstrom. This study's molecular docking and visualization procedure were based on our previous study [12-14].

RESULTS AND DISCUSSION

Inhibiting the activation of COX-2 can suppress inflammation activity. This basic theory is widely used as a treatment strategy against multiple types of diseases, including inflammatory-related diseases and cancer [2,3]. As a regulatory master of inflammation, the COX-2 is usually synthetically inhibited by nonsteroidal anti-inflammatory drugs to suppress their activation outcomes [2]. There is a lot of evidence showing the adverse effect of inflammation, especially in the case of chronic inflammation. A report also showed that the increase of inflammation rate is related to the poor prognosis in some types of cancer [3]. In this study, we are concerned about the inhibition mechanism of COX-2. Therefore we can reduce and block the activation of inflammation.

The leaves extract of *A. scholaris* has been known as a medication agent to treat several diseases, such as diabetes, inflammatory-related diseases, and cancer [15,16]. In this present study, we found three compounds of *A. scholaris*, namely isorhamnetin-3, quercetin-3, and quercetin which have the most significant binding affinity score after molecular docking simulation (Table 2).

Table 2. The binding affinity score and interaction properties of the ligand-target protein

No.	Ligands	Binding Affinity	Ligand - Amino Acid Interaction
1.	Isorhamnetin-3	-7.5 kcal/mol	**Van der Walls:** LEU B:9, LEU B:170, THR B:183, GLN B:181, THR B:172, THR B:87, LEU:84, PHE:23
			Conventional Hydrogen Bond: LYS B:171, SER B:80, PRO B:166
			Amide-Pi Stacked: GLY B:169
			Pi-Alkyl: ILE B:83
			Pi-Sigma: LEU B:20
2.	Quercetin-3	-7.4 kcal/mol	**Van der Walls:** MET B:17, LEU B:9, PRO B:166, LEU B:170, LYS B:171, THR B:183, THR B:172, THR B:87, LEU B:84
			Conventional Hydrogen Bond: THR B: 182, SER B:80
			Carbon Hydrogen Bond: GLY B:169
			Pi-Alkyl: ILE B:83
			Pi-Sigma: LEU B:20
3.	Quercetin	-7.1 kcal/mol	**Van der Walls:** PRO B:15, PHE B:184, ILE B:16, THR B:183, MET B:86, ASP B:88, ASN B:91, PRO B:93
			Conventional Hydrogen Bond: THR B:87, ASP B:92
			Pi-Anion: GLU B:89
			Pi-Alkyl: PRO B:149
			Unfavorable Donor-Donor: THR B:185

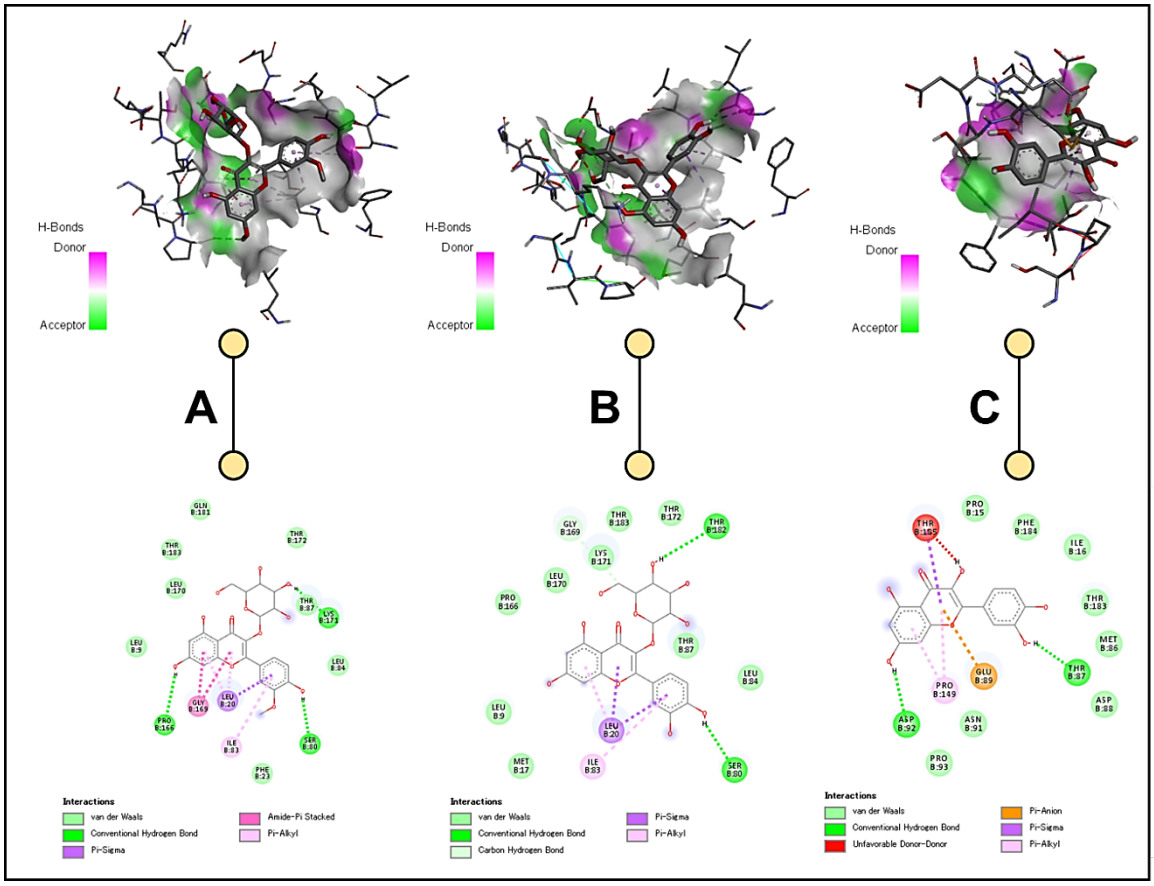

Figure 1. Data visualization of ligand-protein interaction. **A)**. Isorhamnetin-3 – COX-2 interaction, **B)**. Quercetin-3 - COX-2 interaction, and **C)**. Quercetin - COX-2 interaction.

On the other hand, we also evaluate the ligand-protein interaction pattern of these three compounds to the target protein, COX-2. The results show each ligand-protein complex has particular interaction, namely isorhamnetin-3 and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Amide-Pi Stacked, Pi-Alkyl, and Pi-Sigma), quercetin-3 and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Alkyl, and Pi-Sigma), and quercetin and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Pi-Anion, Pi-Alkyl, and Unfavorable Donor-Donor) (Figure 1). Binding free energy and chemical bonds in ligand-protein interaction are important parameters in drug design or discovery. These indicators are used to determine whether the ligand can be proposed as a drug or not. The lower score of binding free energy is considered the greatest favourable ligand-protein interaction, and the chemical bonds pattern is important to maintain the interaction stability [17,18].

CONCLUSION

In this study, we found three bioactive compounds of *A. scholaris,* which may act as inhibitory agents of COX-2, namely isorhamnetin-3, quercetin-3, and quercetin. Moreover, the ligand and protein interaction of these three compounds have particular interaction, such as isorhamnetin-3 and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Amide-Pi Stacked, Pi-Alkyl, and Pi-Sigma), quercetin-3 and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Alkyl, and Pi-Sigma), and quercetin and COX-2 protein interaction (Van der Walls, Conventional Hydrogen Bond, Pi-Anion, Pi-Alkyl, and Unfavorable Donor-Donor). Further research in evaluating the biological function of these compounds is needed, especially in *in vitro* and *in vivo* experiments.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this study.

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