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VIRTUAL SCREENING OF POTENTIAL HEPATOPROTECTIVE AGENTS FROM *Andrographis panuculata* AGAINST MACROPHAGE INFLAMMATORY PROTEIN-1β

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Abstract

This study aimed to evaluate the bioactive compounds of *A. paniculata* as an inhibitor against the macrophage inflammatory protein-1β (CCL-4) to avoid liver diseases. Multiple bioactive compounds from *A. paniculata* were screen through *in silico* approach. The score of binding affinity and the interaction trends among the ligands and protein were considered to determine potential hepatoprotective agents from those compounds. According to our prediction, there are four compounds of *A. paniculata* which may have potential as an inhibitor to the protein target, CCL-4 such as andrographidine A, bisandrographidine C, anrographidine C, and neoandrographolide. Furthermore, the ligands – residual amino acid interaction are andrographidine A – CCL4 (Van der Waals, Carbon Hydrogen Bond, Conventional Hydrogen Bond, Pi-Pi Stacked, and Pi-Alkyl); bisandrographidine C – CCL4 (Van der Waals, Conventional Hydrogen Bond, Alkyl/ Pi-Alkyl, and Unfavorable Acceptor-Acceptor); anrographidine C – CCL4 (Van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Pi Stacked, and Unfavorable Acceptor-Acceptor); neoandrographolide – CCL4 (Van der Waals, Conventional Hydrogen Bond, and Alkyl/ Pi-Alkyl). In the future, more experiments based on laboratory works is needed mainly to evaluate the specific mechanism of action of these compounds against the CCL4.

INTRODUCTION

The liver plays a pivotal role in the maintenance, performance, and homeostasis of the body [1]. Furthermore, the liver has several responsibilities such as energy provision, nutrient supply, detoxification, and excretion of waste metabolic products [2,3]. This multiple works of the liver and xenobiotic exposure from the environment might become one of the risk factors which lead the liver damage such as hepatitis, cirrhosis, fibrosis, alcohol-related disorders, and cancer [4,5]. Globally, the recent study demonstrated that liver diseases caused approximately two million deaths per year including cirrhosis, viral hepatitis, and carcinoma [6]. Therefore, the research about how to treat and fight liver diseases are importantly necessary.

Recent treatments like synthetic drugs have been applied against liver diseases. However, this modality is not sufficient and is reported to have various side effects [1]. Thus, the safer approaches to combat liver diseases are needed. Nowadays, the trend of exploration of the use of herbal medicine is increased. It has been known for a long time ago that medicinal plants are utilized not only as a food

source but also as a traditional treatment against several types of diseases such as cancer, skin damage, cardiovascular diseases, and liver diseases [7-10].

The *A. paniculata*, king of bitter, is one of an edible and cultivated plants, especially in the tropical area. Besides, the *A. paniculata* is known as a medicinal plant that possesses multiple pharmaceutical advantages such as anti-cancer, anti-allergic, antithrombotic, anti-inflammatory, and anti-viral. A report also showed that this plant could be applied as hepatoprotective and hepato-stimulative agents [11]. Interestingly, *A. paniculata* contains bioactive-rich compounds including andrographolide, neoandrographolide, 14-deoxyandrographolide, 14-deoxy 11,12-didehydroandrographolide, andrographiside, andrograpanin, 14-deoxy-11-hydroxyandrographolide, 14-deoxy-12-hydroxyandrographolide, bisandrographolide A, apigenin, onysilin, andrographidine A, andrographidine C, luteolin, and 3,4-dicaffeoylquinic acid [12]. Thus, in this study, we aimed to evaluate the bioactive compounds of *A. paniculata* as a potential inhibitor against the Macrophage Inflammatory Protein-1β (CCL-4) to avoid liver diseases.

MATERIALS AND METHODS

Multiple bioactive compounds which found in *A. panuculata* were used as potential ligands in docking prediction. In detail, the bioactive compounds used were andrographolide, neoandrographolide, 14-deoxyandrographolide, 14-deoxy 11,12-didehydroandrographolide, andrographiside, andrograpanin, 14-deoxy-11-hydroxyandrographolide, 14-deoxy-12-hydroxyandrographolide, bisandrographolide A, apigenin, onysilin, andrographidine A, andrographidine C, luteolin, and 3,4-dicaffeoylquinic acid (Table 1).

Table 1. *A. panuculata* bioactive compounds chemicals properties according to Lipinski's rule of five [13]

No	Bioactive Compounds	CID	Molecular Mass (Dalton)	Hydrogen Bond Donor	Hydrogen Bond Acceptors	High Lipophilicity (LogP)	Molar Refractivity
1.	Andrographolide	5318517	350	3	5	3.90	102.00
2.	Neoandrographolide	9848024	480	4	8	5.01	134.67
3.	14-Deoxyandrographolide	11624161	334	2	4	4.20	101.13
4.	14-Deoxy 11,12-didehydroandrographolide	5708351	332	2	4	3.98	98.90
5.	Andrographiside	44593583	512	6	10	4.42	136.37
6.	Andrograpanin	11666871	318	1	3	4.50	100.25
7.	14-Deoxy-11-hydroxyandrographolide	91884987	350	2	5	4.08	101.81
8.	14-Deoxy-12-hydroxyandrographolide	38350572	350	3	5	3.91	101.95
9.	Bisandrographolide A	12000062	312	5	6	-0.05	77.14
10.	Apigenin	5280443	270	3	5	0.95	61.89
11.	Onysilin	42608095	300	1	5	2.65	73.40
12.	Andrographidine A	13963762	462	4	10	3.15	107.88
13.	Andrographidine C	5318484	460	4	10	2.93	105.60
14.	Luteolin	5280445	286	4	6	0.83	63.43
15.	3,4-Dicaffeoylquinic acid	5281780	312	5	6	-0.05	77.14

The 2D structure of ligands was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Then, each ligand was evaluated according to Lipinski's rule of five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) to verify the properties of the drug-like compound [13]. Furthermore, the 3D protein structure of CCL-4 was built through SWISS-MODEL (https://swissmodel.expasy.org/) by inserting the protein sequence of the target protein. Finally, the protein was then docked with energy minimized-ligands via PyRx as previous protocols [14-16].

RESULTS AND DISCUSSION

The C-C chemokine receptor type 5 (CCR5) is the receptor for ligands such as CCL2, CCL4, or CCL5 [17]. The interaction and activation of the CCR5 signaling cascade

mediate the inflammation incidence. Several studies showed CCL4 or CCR5 was highly up-regulated in liver fibrosis. This evidence indicates that CCL4 or CCR5 can be targeted as a biomarker to reduce liver fibrosis [17,18]. In this study, we evaluated several bioactive compounds from *A. panuculata* against the CCL4 to disrupt the interaction of CCL4 with its receptor, CCR5. Further, we postulated the interaction of ligands and CCL4 could change the conformation of the protein complex; in turn, CCL4 failed to bind with CCR5 properly.

Molecular docking prediction showed there are four compounds of *A. paniculata*, which may have potential as an inhibitor to a target protein, CCL-4. In this present study, we found andrographidine A, bisandrographidine C, anrographidine C, and neoandrographolide have the greatest binding affinity score compared to other compounds. Interestingly, we also found several chemicals interaction among the ligand and residual amino acids (Table 2). In detail, the interaction is andrographidine A – CCL4 (Van der Waals, Carbon Hydrogen Bond, Conventional Hydrogen Bond, Pi-Pi Stacked, and Pi-Alkyl); bisandrographidine C – CCL4 (Van der Waals, Conventional Hydrogen Bond, Alkyl/ Pi-Alkyl, and Unfavorable Acceptor-Acceptor); anrographidine C – CCL4 (Van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Pi Stacked, and Unfavorable Acceptor-Acceptor); neoandrographolide – CCL4 (Van der Waals, Conventional Hydrogen Bond, and Alkyl/ Pi-Alkyl) (Figure 1). The binding affinity or binding free energy is the crucial factor to evaluate the interaction between two or more biomolecules. More negative the binding free energy score, indicates the favourable interaction [19,20].

Table 2. Binding affinity score among ligands and target protein, CCL4.

No.	Ligands	Binding Affinity	Ligand - Amino Acid Interaction
1.	Andrographidine A	-6.8 kcal/mol	**Van der Waals:** LEU B:43, ARG B:41, ALA B:40, THR B:39, TYR B:38, MET A:26, ARG B:69, SER B:70.
			Carbon Hydrogen Bond: GLY A:27, SER A:28
			Conventional Hydrogen Bond: ASP A:29
			Pi-Pi Stacked: PHE B:47
			Pi-Alkyl: PRO B:44
2.	Bisandrographidine C	-6.8 kcal/mol	**Van der Waals:** ASP A:29, GLN B:72, ALA B:33, THR A:32, GLY A:27
			Conventional Hydrogen Bond: SER A:56, SER A:28
			Alkyl/ Pi-Alkyl: PHE B:36, LEU A:57, ALA A:33, TYR A:52, PRO A:30
			Unfavorable Acceptor-Acceptor: PRO A:31, SER A:55
3.	Anrographidine C	-6.6 kcal/mol	**Van der Waals:** SER B:70, PRO B:25, GLY B:27, THR B:39, LEU B:43, LYS B:42, ASN B:46
			Conventional Hydrogen Bond: ARG B:69
			Carbon Hydrogen Bond: SER A:28, ARG B:41, PRO B:44
			Pi-Pi Stacked: PHE B:47
			Unfavorable Acceptor-Acceptor: MET A:26
4.	Neoandrographolide	-6.6 kcal/mol	**Van der Waals:** LYS B:42, ARG B:69, PRO B:25, THR B:39
			Conventional Hydrogen Bond: MET B:26, GLY B:27, SER B:28, ARG B:41
			Alkyl/ Pi-Alkyl: LEU B:43, PRO B:44, PHE B:47

A. paniculata contains a broad spectrum of compounds including diterpenes, lactones, and flavonoids. These bioactive compounds have the ability as an antioxidant, it can reduce and remove the free radical species such as superoxide radicals and hydrogen peroxide [3]. Another research showed mice induced-paracetamol treated by *A. paniculata* and *Swertia chirayita* extract could reduce the serum level of glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, alkaline phosphatase, and lipid peroxides which were known as parameters of the hepatic disorder [7].

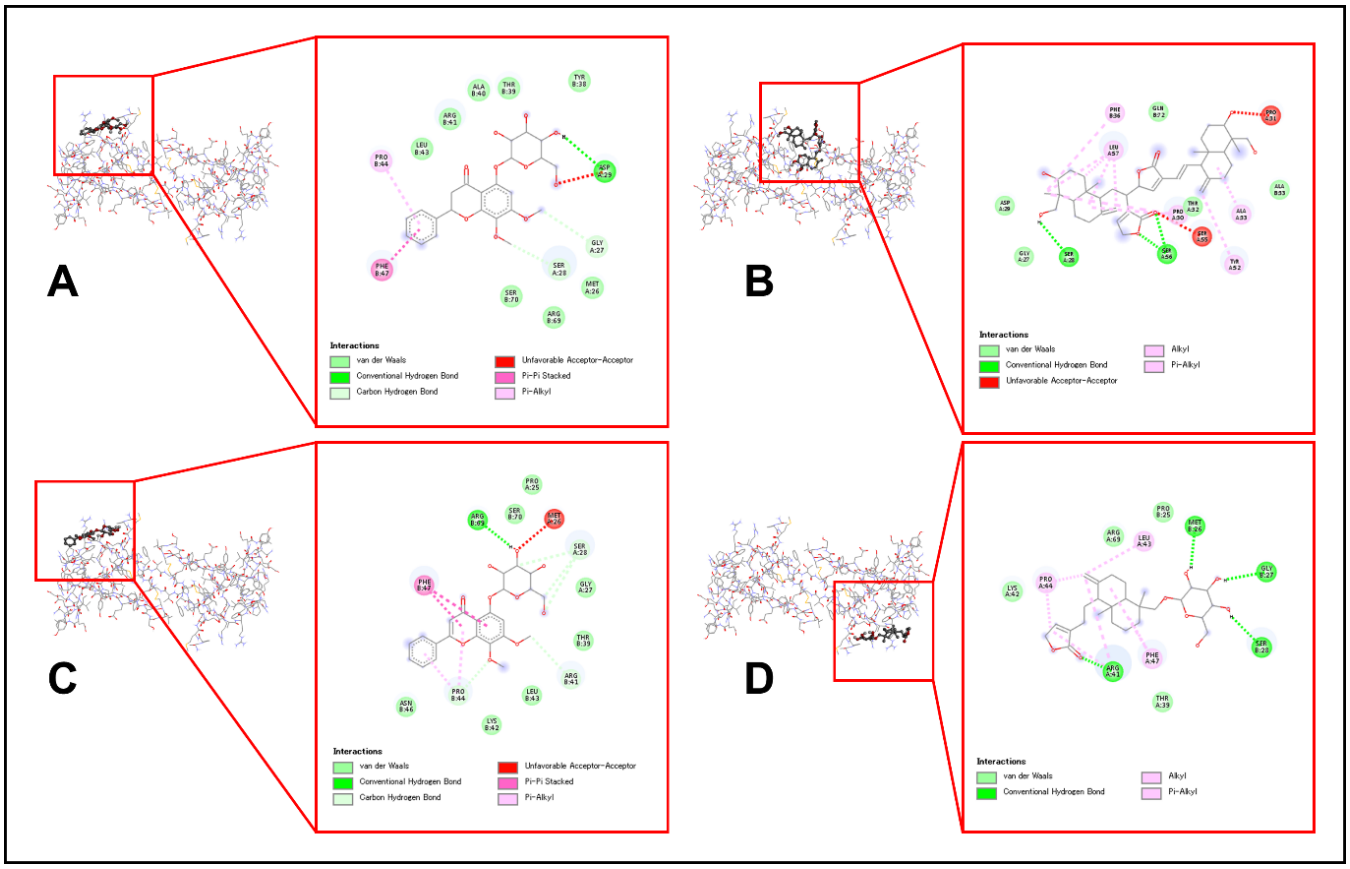

Figure 1. Data visualization of four ligands with the greatest binding affinity score against CCL-4 protein. The figure showed there are different interactions of ligands on certain target protein binding site. A). Andrographidine A - CCL4 interaction; B). Bisandrographidine C - CCL4 interaction; C). Anrographidine C - CCL4 interaction; and D). Neoandrographolide - CCL4 interaction

CONCLUSION

In this present study, we found four potential bioactive compounds from *A. paniculata* against target protein, CCL4. These compounds are andrographidine A, bisandrographidine C, anrographidine C, and neoandrographolide which were selected based on the predicted binding affinity score. In the future, more experiment based on laboratory works is needed especially to evaluate the specific mechanism of action of these compounds against the CCL4.

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CONFLICT OF INTEREST

There is no conflict of interest in this work.

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