POTENTIAL MOLECULES AGAINST COVID-19 FROM *ANNONA MURICATA*; AN IN-SILICO APPROACH

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ABSTRACT

The Covid-19 pandemic is still a threat to society. The number of patients experiencing fluctuating. The use of herbal medicine is an alternative for the community because it is easy and cheap to use. One of the herbal medicines that is often used is Annona muricata. This study aimed to explore the potential of drugs in Annona muricata plant using the Insilico method. Compounds obtained from KNApSAcK Based on the analysis using SwissADME (Lipinski creteria)criteria. 22 compounds passed the selection. Eight ligands were identified as being able to change the conformation of the initial form of RBD and ACE2 attachment. Eight molecules are able to change the initial conformation, namely Asimilobine, Coreximine, (+)-Stepharine, Coclaurine, Norcorydine, N- Nornuciferinhe, methoxyphenyl)methyl]-1,2,3,4-tetrahydroisoquinolin-5-ol, and Atherosperminine.

Keywords: SARS-CoV-2, Herbal Medicine, Annona *muricata*, in Silico.

ABSTRAK

Pandemi covid 19 sampai saat ini masih menjadi ancaman di masyarakat. Jumlah penderita mengalami fluktuatif. Penggunaan obat herbal menjadi alternatif tersendiri bagi masyarakat karena sifatnya yang mudah dan murah digunakan. Salah satu obat herbal yang sering digunakan adalah *Annona muricata*. Penelitian ini bertujuan untuk menggali potensi obat pada tanaman Annona muricata menggunakan metode Insilico. Senyawa yang diperoleh dari KNApSAcK Berdasarkan analisis menggunakan kriteria SwissADME (Lipinski creteria). 22 senyawa lolos seleksi. Delapan ligan diidentifikasi mampu mengubah konformasi bentuk awal perlekatan RBD dan ACE2. Delapan molekul mampu mengubah konformasi awal, yaitu Asimilobine, Coreximine, (+)-Stepharine, Coclaurine, Norcorydine, N-Nornuciferinhe, methoxyphenyl)methyl]-1,2,3,4-tetrahydroisoquinolin-5-ol, dan Atherosperminine.

Kata kunci: *:* SARS-CoV-2, Obat herbal, Annona *muricata*, in Silico.

INTRODUCTION

At the end of 2019, a new type of virus belonging to the Coronaviridae family has been reported in Wuhan, China (1). This virus spread rapidly to various regions of the world and make it as global pandemic currently. International classification gave the name Sars Cov-2 to this virus. So far, 567 million people have been confirmed positive and 5,38 million people have died in the world (https://covid19.who.int/ accessed 22/07/2022). Sars Cov-2 virus enters cells intermediated with Spike protein, the spike protein consists of sub unit1 (S1) and subunit 2 (S2) (2,3). S1 is responsible for the infection process by binding to the human Angiotensin Converting 2 (ACE 2) receptor, while S2 serves as the initiator of the entry of the virus into host cells (4).

Several pathways from the Sars Cov-2 life cycle are recommended to be treatment targets, these pathways include 1) Attachment of protein S to the host cell receptor which is the Ace-2 receptor, 2) Inhibition of viral proteases, namely viral proteins that play a role in replication, 3) Inhibition of host cell proteases, which are proteins present in the host that play a role in cell replication, this is also used by viruses to multiply, 4) Inhibition of RdRp gene, 5) Inhibition of cell maturation and exocytosis (5).

Herbal medicines are commonly used by the community for alternative medicine to deal with Covid-19 (6). One of them is the *A. muricata* plant (6). However, there are no drugs that are considered to be able to directly treat COVID-19. The results of the analysis show that drugs are immune-boosting (7–9). A. muricata is known to have many active substances to treat viral infections (10). In addition, A. muricata uses to such as fever, pain, respiratory and skin illness, internal and external parasites, bacterial infections, hypertension, inflammation, diabetes, and cancer (11). Therefore, the purpose of this study was to explore the active substance contained in A. muricata to inhibit the attachment of Sars CoV 2 to cell receptors using the insilico method.

METHODS OF THE STUDY

Ligant and Protein Sampling

The crystallization structure of spike protein (S) and ACE 2 was obtained through RSCB PDB on https://www.rcsb.org/ with 6zlg code. The active components of *A. muricata* were obtained from the KNApSAcK database on www.knapsackfamily.com/. From the KNApSAcK database, 22 out of 121 chemical compounds were obtained from pubchem and analyzed using the Swiss chemspider ADME physicochemical chemical properties and paying attention to the abbreviation value (12). The compounds were Anonaine (ANO, CSID: 160597), Reticuline (RET, CSID: 439653),

Xylopine (XYL, CSID: 60503), 2,3-Dihydrokhellin (DHY, CSID:179474), Loliolide (LOL, CSID: 100332), Isololiolide (ISL, CSID:11019783), Asimilobine (ASI, CSID: 160875), Coreximine (COR, CSID: 7037179), (+)-Stepharine (STE, CSID:193686), (-)-Isolaureline (ISR, CSID: 44584506), Coclaurine (COC, CSID: 160487), Argentinine (ARG, CSID: 10085878), Norcorydine (NOR, CSID:179491), N-Nornuciferinhe (NOC, CSID:12313579), (z)-3- hexenyl beta-d-glucopyranoside (HEX, CSID:5318045), Vomifoliol (VOM, CSID:5280462), 6,7-Dimethoxy-1-[(4-methoxyphenyl) methyl]-1,2,3,4-tetrahydroisoquinolin- 5-ol (DIM, CSID:157209), Anomurine (ANM, CSID: 157218), Annoionol A (ANL, CSID: 101564134), 1,1,5beta-Trimethyl-6-((E)-3-oxo-1 -butenyl) cyclohexane-3alpha,4beta,6beta-triol (TRI, CSID: 71746439), Annonamine (ANE, CSID: 56929881), and Atherosperminine (ATP, CSID: 96918).

Analysis of Potential Drug Compound

The *A. muricata*'s compounds were selected using SwissADME physicochemical properties, including molecular weight, Log P value, and number of H-bond donor, H-bond acceptor, rotatable bond, and Total Polar Surface Area (TPSA) (12) through smiles. From this analysis, 22 compounds that passed the selection were obtained.

Molecular Docking

The protein obtained from RSCB PDB was then prepared using Discovery studio version 16 (13) to remove ligands and water. The ligands were prepared by minimizing energy and converting to .pdb (protein data bank) format using open bable which was integrated into PyRx 8.0.(14). Docking is carried out using the Hex 8.0.0 program, with Shape+Electro+DARS to determine the type of correlation and some standard parameters in the software. Protein-ligand result visualized using Biovia Discovery Studio (13). Analysis of the shape and structure of the receptor-ligand using Pymol.

RESULTS AND DISCUSSION

Analysis of sources Ligans compounds from KNApSAcK obtained as many as 121 compounds. The compounds were selected using Swiss ADME so that only 22 compounds remained. Lipinski's roles (15) and swiss ADME radar (12) make it possible to analyze a substance into a drug. Based on these provisions, it can be analyzed 22 chemical components from 121 components that meet these criteria, among the criteria in question are molecular weight not exceeding 500 kD, log P≤ 5, H-bond donors 5, Hbond acceptors 10 correlated with 90% of drugs used. Topological polar surface area (TPSA) has a value of < 100 A^o which means it has good

permeability and bioactivity properties. ADME radar analysis based on lipophilicity: XLOGP3 between − 0.7 and + 5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds. Based on the selection results, the largest molecule was found in COR, 329, 4 g/mol while the smallest molecule was found in LOL and ISL, 196.2 g/mol. The size of the molecule indicates the ability of a substance to enter cells and other body tissues, including brain tissue (16). The results of the analysis of compounds based on Lipinski criteria can be seen in full in table 1.

Several drugs are recommended for treatment of Sars Cov 2 which work by inhibiting the attachment of spike protein to ACE2 (16–20). This research is supposed to find Penelitian ini bertujuan untuk mencari the effect of bioactive compounds to inhibit the attachment of receptor binding dose.. Thus, 22 ligands may be used as drugs. To prove this, a docking analysis was carried out with Hex 8.0.

The docking was carried out in two stages, the first stage is by docking the bioactive components with RBD, the second is done by attaching the Ligan+RBD complex with ACE2. The results of the docking analysis show that the highest attachment energy is found in XYL with -258 kcal/mol, followed by
the second one is DIM, -256.1 kcal/mol. The highest energy was shown by the LOL compound with -181.4 kcal/mol (Table 2.). Low energy indicates a strong attachment between one molecule to another, however, the correct attachment position will favor inhibition (19,21).

Table 1. Bioactive components in *A. muricata* as a drug candidate

Characteristics	ANO	RET	XIL	DHY	LOL	ISL	ASI	COR	STE	ISR	COC
Molecular weight (g/mol)	265,3	329,4	295,9	262,26	196,2	196,2	267,3	327,4	297,4	309,4	285,3
MLOGP	2,83	1,75	-0,51	0,49	1,49	1,49	2,43	1,75	1,81	2,72	1,84
H-Bond Donor	1	2	1	0	1	1	2	2	1	0	3
H-bond acceptors	3	5	1	5	3	3	3	5	4	4	4
Rotateable Bonds	0	4	5	2	0	0	1	2	2	2	3
TPSA (Å²)	30,49	62,16	21,51	57,9	46,53	46,53	41,49	62,16	47,56	30,93	61,72

Characteristics	ARG	NOR	NOC	HEX	VOM	DIM	ANM	ANL	TRI	ANE	ATP
Molecular weight (g/mol)	295,4	327,4	281,4	262,3	224,3	329,4	343,4	230,3	242,3	296,4	309,4
MLOGP	3,13	1,75	2,66	-1,02	1,14	1,75	1,98	1,49	0,38	-0,79	4,11
H-Bond Donor	1	2	1	4	2	2	1	3	3	1	0
H-bond acceptors	4	3	3	6	3	5	5	3	4	1	5
Rotateable Bonds	3	5	2	6	2	5	6	3	2	2	3
TPSA (Å²)	32,7	59,95	30,49	99,38	57,53	59,95	48,95	60,69	77,76	29,46	21,7

Table 2. Interacting residues in S Protein with ACE 2 Protein in *A. muricata* compounds

Ligan	Binding Energy (kcal/mol)	
	Ligan –RBD	(Ligan+RBD) ACE 2
RBD-ACE 2	-1224.8	
ANO	-222.5	-1072.3
RET	-222.4	-1067.7
XYL	-258.1	-1067.7
DHY	-220.4	-1097.2
LOL	-181.4	-1066.5
ISL	-191.2	-1064.2
ASI	-219.6	-972
COR	-253.3	-973.7
STE	-218.0	-972
ISR	-240.8	-1098.8
COC	-245.4	-1037.2
ARG	-231.4	-1154.3

Ligan	Binding Energy (kcal/mol)	
	Ligan –RBD	(Ligan+RBD) ACE 2
COC	-245.4	-1037.2
ARG	-231.4	-1154.3
NOR	-249.1	-996.9
NOC	-235.8	-994.4
HEX	-213.7	-1160.7
VOM	-203.3	-1100.6
DIM	-256.1	-991.0
ANO	-250.3	-1111.2
ANN	-193.4	-1069.6
TRI	-198.2	-1050.3
ANM	-224.9	-1117.0
ATP	-251.6	-949.9

The attachment points of RBD with ACE2 (6zlg) are located at several points including K417, G446, Y449, Y453, L455, F456, A475, G476, E484, Y486, N487, Y489, F490, Q493, G496, Q498, T500, N501, Y502, and Y505 (Table 3). Two ligands namely ARG (GLY496, TRY453, and LYS417), and ANM (PHE456 and TRY473) are located at the initial attachment of RBD-ACE2.

The other ligands that attach close to key positions are among RET, DHY, VOM, and ANL. This position allows bioactive compounds that can inhibit, some close positions are also possible to inhibit attachment (22). One of the disadvantages of docking is that it only uses the active part of the compound. In real conditions, the active protein is integrated with the protein complex, so that the attachment to the complex is not possible. The binding inhibition ability is also due to the position of the protein-ligand attachment (23).

To find out the results, a second docking was carried out, namely attaching the results of the docking complex (Ligan+RBD) to the ACE2 protein and to determine the position of the attachment, visualization was carried out using the Pymol program (24). the position of attachment of the ligand to the RBD showed shows a variety of positions (Fig. 1). The ligands screened at the key position of attachment between RBD and ACE2, the position was close to the attachment of RBD-ACE 2 between amino acids 400-502 used for further analyses. Based on this confirmation, the compounds were further selected into 17 compounds they are RET, DHY, LOL, ASI, COR, STE, COC, ARG,

NOR, NOC, HEX, VOM, DIM, ANM, ANL, ANE, and ATP (Table 2).

Based on the analysis of the 17 substrates used, it was found that 8 components were able to change the conformation of RBD-ACE2, the conformation is presented in Fig. 2. The docking analysis was continued by Ligan+RBD complex with ACE 2 docking. The results of the docking showed that the lowest energy was found in Atherosperminine (ATP), but the conformation of the attachment of the Ligan+RBD complex did not change. The conformational differences can be seen in 6 other compounds, namely ASI, COR, STE, COC, NOR, NOC, DIM, & ATP (Fig. 2).

The most striking conformational changes were in COR, NOR and DIM, the RBD attachment conformation had a different position compared to RBD-ACE2 (Fig. 2). Because docking is done randomly, the docking position of COR, NOR and DIM, may have many perception (25).

The random docking process of tillers looks for the lowest energy for attachment, so that if the attachment occurs at a position that is in accordance with the original position, it is possible that the energy generated from the docking may be lower than the energy in the proper position. Attachment may also occur at sites where other proteins are attached. So that in actual conditions the attachment is not possible.

Table 3. *A. muricata* potential value analyses of active compounds as medical substance. *ES = Electrostatic; HB = Hydrogen Bond; HP = Hydrophobic; UF = Unfavorable bond; VW = Van der Waals; OT = Other Bond*

RET		DHY		LOL		ASI		COR		STE		COC		ARG		NOR	
LYS378	HB	THR376	HB	SER459	HB	SER443	HB	ASN448	HB	ASN450	HB	THR345	HB	GLY496	HB	ASP442	HB
SER375	HB	ASP405	HB	GLU471	HB	ARG509	ES	ARG509	HB	THR345	HB	LEU441	HP	ARG403	HB; ES	THR345	HB
THR376	HB	ASP405	HB	ARG457	HP	ASP442	ES	THR345	HB	LEU441	HB	TRP436	HP	TYR453	OT	B:LEU441:O - :LIG1	OT
VAL407	HB	VAL407	HP	LYS458	HP	SP442;SER4	HP	LEU441	HB	SER443	HB	TRP436	HP	LYS417	HP	LEU441	HP
ILE410	HB	LYS378	HP	ARG457	HP	LEU441	HP	THR345	HB	LEU441	OT	ASN343;ALA344	HP			LYS444	HP
VAL407	HB	VAL407	HP	LYS458	HP	LYS444	HP	ASN448	HB	LEU441	HP	PHE374	HP			ARG346	HP
LYS378	ES	ARG408	HP	LYS458	HP	ARG346	HP	LEU441	HP	LYS444	HP	TRP436	HP			LEU441	HP
TYR508	HP	TYR508	HP					LEU441	HP	LYS444	HP					TYR451	HP
VAL407	HP	ARG408	HP					LEU441	HP	LEU441	HP					LEU441	HP
ARG408	HP	VAL407	HP					LYS444	HP								
ALA411	HP																
ALA435	HP																
VAL407	HP																
LYS378	HP																
TYR508	HP																
VAL407	HP																
ARG408	HP																

NOC		HEX		VOM		DIM		ANO		ANN		ANM		ATP	
LEU441	HB	TRP436	HB	ASP467	HB	ASP442	HB	PHE515	HB	PHE456	HB	ASP405	HB	ILE468	HB
THR345	HB	SER373	HB	SER469	HB	ASP442	HB	ASP428	HB	GLU471	HB	LYS378	HB	ILE468	HB
ARG509	ES	ASN343	HB	PRO491	HP	ASN343	HB	ASP428	HB	LYS458	HP	VAL407	HP	ASN354	HB
ASP442	ES	ASN343	HB	ARG457	HP	ASN343	HB	LEU517	HB	ARG457	HP	VAL407	HP	ALA352	HP
ARG346	HP	TRP436	HP	LYS458	HP	ALA344	HB	PRO426	HP	LYS458	HP	TYR508	HP	ALA352:	HP
LEU441	HP	LEU368	HP			ARG346	ES	LEU518	HP	TYR473	HP	TYR508	HP	TYR351	HP
LYS444	HP	PHE342	HP			THR345	HP	PHE429	HP			TYR508	HP	ALA348	HP
		PHE374	HP			LEU441	HP	PHE464	HP					ALA348	HP
		PHE374	HP			TYR451	HP							ALA352	HP
														ALA348	HP
														ILE468	HP
														ALA352	HP

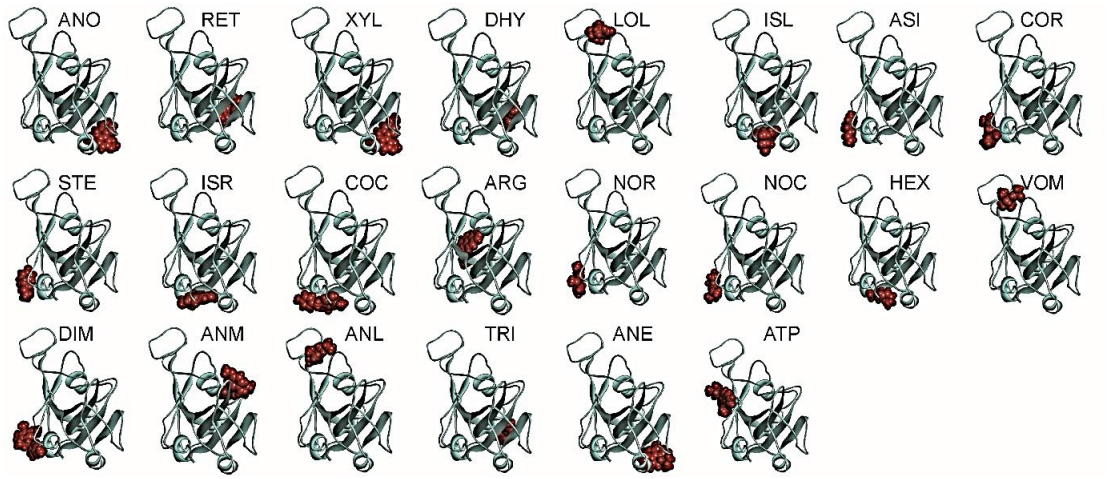

Fig. 1. Position RBD with several ligand compounds, anonaine (ANO), Reticuline (RET), Xylopine (XYL), 2,3-Dihydrokhellin (DHY), Loliolide (LOL), Isololiolide (ISL), Asimilobine (ASI), Coreximine (COR), (+)-Stepharine (STE), (-)-Isolaureline (ISR), Coclaurine (COC), Argentinine (ARG), Norcorydine (NOR), N-Nornuciferinhe (NOC), (z)-3- hexenyl beta-d-glucopyranoside (HEX), Vomifoliol (VOM), 6,7-Dimethoxy-1-[(4-methoxyphenyl)methyl]-1,2,3,4-tetrahydroisoquinolin- 5-ol (DIM), Anomurine (ANM), Annoionol A (ANL), 1,1,5beta-Trimethyl-6-((E)-3-oxo-1 -butenyl) cyclohexane-3alpha,4beta,6beta-triol (TRI), Annonamine (ANE), and Atherosperminine (ATP).

Based on the results of the analysis of the ligands that are able to change the conformation, it is found that the COR has the lowest energy, which is 973.7 kcal/mol. This result can certainly be lower considering that COR attachment is not present on the active site of ACE2.

NOR and DIM also have low attachment energy values, namely -996.9 kcal/mol and -991 kcal/mol. The interpretation of the results was also the same as the COR, the results may be higher if attached to the initial attachment of ACE2-RBD.

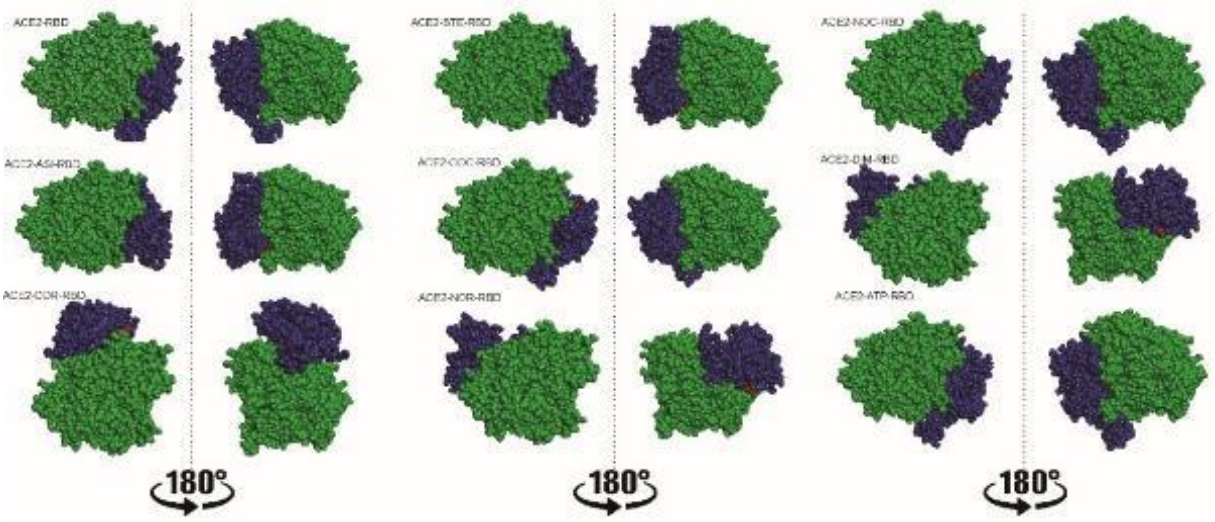

Fig. 2. 3D view of the docking result between Ligand and ACE 2 on bioactive components

While other compounds such as breast milk, STE, COC, & ATP showed actual results. The energy of breast milk shows a value of -972 kcal/mol, COC and STE shows a value of -1-37.2 kcal/mol, COC shows a value of -1037.2 kcal/mol and ATO shows a value of -949.9 kcal/mol, the values resulting from the seven bioactive molecules. showed a lower average compared to other compounds (see table 2). This indicates that there is a correlation between the energy produced and the conformation of the resulting attachment.

CONCLUSION AND SUGGESTION

From the exploratory analysis of the potential of natural materials, 22 of the 121 ligands were obtained, 17 had bonds that were close to the initial binding of Protein S with ACE 2. And of the 8 compounds, 3 compounds were found which were strongly suspected to be inhibitors of the attachment of protein S and ACE 2. These compounds are COR, NCR and DIM. As a suggestion, based on insilico analysis, this analysis can be continued with in vitro and in vivo analysis.

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