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In-silico study of the Effectiveness of *Allium sativum L.* extract as an Angiotensin-Converting Enzyme (ACE) Inhibitor in Hypertension

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Abstract

Over the last decade, the global prevalence of hypertension rate has increased by 5.2% and, in Indonesia, the prevalence rate has increased significantly from 25.8% in 2013 to 34.1% in 2018. Hypertension treatments using blood pressure-lowering drugs, such as angiotensin-converting enzyme (ACE) inhibitors, often cause unpleasant side effects. These side effects increase the interest in using potentially effective natural remedies, such as garlic. This study aimed to determine which organosulfur compounds in garlic can act as an ACE inhibitor to reduce blood pressure in hypertension using a cheminformatics approach. Eighteen organosulfur compounds of Allium sativum L. were screened based on Lipinski's rules and ADMET evaluation. Seven compounds passed the screening and were subjected to QSAR analysis, molecular docking analysis, and molecular dynamics simulations to assess the stability of the protein. The seven compounds then underwent molecular docking and QSAR analysis. Ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide) and S-allylmercaptocysteine (SAMC) were two compounds with better docking values compared to the positive control compound. The QSAR analysis also showed that SAMC had an activity as an ACE inhibitor. The ADMET evaluation showed that Ajoene and SAMC had good absorption and could not penetrate the blood-brain barrier. Molecular dynamics simulation of ACE complexes Ajoene, SAMC, and Captopril ranged from 0.05 to 5.61 Å but exhibited a pattern of synonymous fluctuations for most residues. Based on the simulation data, the organosulfur compounds from garlic, Ajoene, and SAMC are proven to have a mechanism of action as ACE inhibitors to reduce blood pressure in hypertension.

Keywords: ACE inhibitors, cheminformatics, garlic, hypertension

Introduction

Hypertension is a significant risk factor for cardiovascular disease (CVD), a leading cause of morbidity and mortality worldwide.[1] Hypertension management and control are critical to preventing cardiovascular disease and other diseases. Over the last decade, the global prevalence rate has increased by 5.2%, and in Indonesia, the prevalence of hypertension has increased significantly from 25.8% in 2013 to 34.1% in 2018. [2,3]

Hypertension or high blood pressure is defined as having arterial blood pressure above the normal value. According to the Indonesian Ministry of Health, normal blood pressure for systolic blood pressure is less than 120 mmHg and less than 80 mm Hg for diastolic blood pressure.[1] Based on these guidelines, a person is said to have hypertension if the systolic blood pressure is above 140 mmHg and/or the diastolic blood pressure is above 90 mmHg.[3]

Hypertension treatments using blood pressure lowering drugs often cause various unpleasant adverse effects.[4] The most common adverse effect is dry cough due to the accumulation of bradykinin in respiratory tissues due to angiotensin-converting enzyme (ACE) inhibition.[4] It also helps to explain the growing interest in potentially effective natural remedies, such as garlic. Garlic (*Allium sativum L.*) has been used as a spice, food and remedy for centuries. There are several ways to consume garlic for treatment, such as raw garlic, freshly cooked garlic, garlic oil, garlic powder, and aged garlic extract. Research conducted by Reid et al. showed that garlic supplementation to hypertensive patients can reduce blood

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pressure by 10 mmHg.[4] The result might be due to the organosulfur compounds found in garlic can intervene in the pathway that causes hypertension.[4,5]

Although several mechanisms cause hypertension, this research will only focus on the mechanism of organosulfur compounds from garlic as angiotensin-converting enzyme (ACE) inhibitors, which regulate blood pressure through the renin-angiotensin-aldosterone system (RAAS) in the body.[7] The RAAS system, composed of three major components: renin, angiotensin II, and aldosterone, is a critical regulator of blood pressure in our body. Once renin has been released into the blood, it cleaves its target, angiotensinogen, into angiotensin I. Angiotensin I is initially inactive but acts as a precursor to angiotensin II. The conversion of angiotensin I to angiotensin II is catalyzed by an enzyme called angiotensin-converting enzyme (ACE). After angiotensin I is converted into angiotensin II, it affects the kidney, adrenal cortex, arterioles, and brain by binding to angiotensin II type I (AT1) and type II (AT2) receptors to coordinate in regulating blood pressure in the body.[7]

This study uses a cheminformatics approach to determine which organosulfur compounds in garlic can act as an ACE inhibitor to reduce blood pressure in hypertension.

Methods

The ACE sequences were obtained by searching the NCBI database using RefSeq and *Homo sapiens* filters. Protein modeling of the sequence data will be in FASTA format using the SWISS-MODEL server (https://swissmodel.expasy.org/), and the parameter used for modeling will be QMEANDisCo.[8,9]

The organosulfur compounds of *Allium sativum L.* had been collected from several databases such as IJAH (Indonesia Jamu Herbs) and Herbal DB UI, which were expected to have potential as ACE inhibitors.[10,11] The SMILES structures of these organosulfur compounds were obtained from PubChem, and the SWISSADME (http://www.swissadme.ch/index.php) web server was used to evaluate each ligand's molecular properties and drug-likeness.[12] All drug-likeness parameters in SWISS-ADME were used to filter out organosulfur compounds found in garlic.[13] The ligands rejected by more than two drug-likeness parameters would be excluded from the molecular docking analysis.

The toxicity of the ligands was also analyzed using ADMET lab 2.0 (https://admetmesh.scbdd.com/) web server. Several parameters were analyzed, such as BOILED-EGG, AMES toxicity, skin sensitization, carcinogenicity, and eye irritation.[14,15] The ligands that have passed the toxicity parameter will be utilized for molecular docking prediction.

The QSAR analysis was conducted to screen the bioactivity of the chemical molecules. The analysis was conducted using the Prediction of Activity Spectra for Substances (PASS) tool provided by the Way 2 drug / PASS server (http://www.way2 drug.com/PASSOnline/). The PASS tool provided by this server would be able to predict whether the organosulfur compounds derived from Garlic would have inhibitory activity against the ACE. The output from this server would be a tabulation of data related to the interaction of chemical compounds with biological entities.[16]

The surface area and binding site of the angiotensin-converting enzyme (ACE) would be identified by using the Computed Atlas of Surface Topography of Proteins (CASTp) server (http://sts.bioe.uic.edu/castp). Delaunay triangulation, alpha shape, and discrete flow were the parameters used to measure the surface area and binding site of ACE protein.[17]

Molecular docking of the selected ligands and ACE was performed using the PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) server. There are two parameters evaluated in the molecular docking simulation: docking score and atomic contact energy score (ACE score).[18] The docking score measures the strength of ligand–protein interactions, with higher scores suggesting more favorable interactions and higher affinity.[18] The ACE score evaluates the energy contribution associated with the ligand–protein atomic interactions, with a lower ACE score suggesting a more stable and energetically favorable ligand-protein complex.[18] To validate the molecular docking method, the docking results of organosulfur compounds with ACE had to have a higher docking score and ACE score than the reference compound. In this study, Captopril was used as the reference compound of the ACE inhibitor compared to the organosulfur compounds present in garlic.[4] The results of the molecular docking analysis will be used as input data in the molecular dynamic simulation.

MD simulation of the selected ligands and ACE was performed using the CABSFLEX2 server (http://biocomp.chem.uw.edu.pl/CABSflex2/submit) server.[19] This server runs MD

simulations with parameters such as length of the simulation, time step, temperature, number of cycles, and cycles between trajectory frames with default settings to predict the flexibility of the ACE ligand and molecule.[19] The positions of atoms in proteins are tracked over time, enabling the calculation of root mean square fluctuation (RMSF), which measures the degree of deviation from an average position. The RMSF analysis provides information on the ligand's and enzyme's flexibility and is critical for investigating the interactions between organosulfur compounds and ACE molecules.[18] The expected RMSF threshold value between ligands and proteins in MD simulation would be around 1–3 Å.[18] The conformation between organosulfur compounds and ACE is considered stable in this value range.

Results

The ACE sequence data was obtained from the NCBI database with access codes NP_001171528.1 in FASTA format, and then the data was inputted into the SWISS-MODEL server for modeling simulation. The output of SWISS-MODEL was an ACE protein model with a template of PDB ID 4C2O, with a GMQE value of 0.99, a Global QMEANDisCo of 0.93±0.05, and a sequence identity of 99.83%. The homology modeling between the ACE model and the co-crystal structure 1UZF is shown in Figure 1.

To validate the model given by SWISS-MODEL, we compared it to PDB ID 1UZF, an ACE with Captopril in its structure, as shown in Figure 1. The homology modeling results show the RMSD value of the model, and 1UZF is 0.35 Å. The similarity between them is 99.5%, suggesting that the ACE model given by SWISS-MODEL closely resembles the 1UZF model.

From the three herbal medicine databases, 56 chemical substances were retrieved. This study combined the data and removed the non-organosulfur compounds. The three databases yielded eighteen organosulfur compounds, as

Table 1 The Eighteen Organosulfur Compounds are Mostly in Garlic (*Allium Sativum* L.).

Compound Name	SMILES Structure	PUBCHEM ID
Diallyl sulfide	C=CCSCC=C	11617
Diallyl disulfide	C=CCSSCC=C	16590
Diallyl trisulfide	C=CCSSSCC=C	16315
Alliin	C=CCS(=O)CC(C(=O)O)N	87310
Ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide)	C=CCSSC=CCS(=O)CC=C	5386591
Allicin	C=CCSS(=O)CC=C	65036
Methyl propyl disulfide	CCCSSC	16592
Methylselenocysteine	C[Se]CC(C(=O)O)N	147004
Allyl methyl disulfide	CSSCC=C	62434
S-Methyl-L-cysteine sulfoxide	CS(=O)CC(C(=O)O)N	182092
Allyl methyl sulfide	CSCC=C	66282
S-Methylthiocysteine	CSSCC(C(=O)O)N	3080775
S-allylmercaptocysteine (SAMC)	C=CCSSCC(C(=O)O)N	9794159
y-L-Glutamyl-S-allyl-L-cysteine	C=CCSCC(C(=O)O)NC(=O)CCC(C(=O)O)N	11346811
y-L-Glutamyl-S-1-propenyl-L-cysteine	CC=CSCC(C(=O)O)NC(=O)CCC(C(=O)O)N	87289205
Methyl propyl disulfide	CCCSSC	16592
S-1-Propenyl-L-cysteine	CC=CS(=O)CC(C(=O)[O-])[NH3+]	90657185
2-vinyl-4H-1,3-dithiin	C=CC1SCC=CS1	133337

Figure 1 The Homology Modelling Between ACE model generated from SWISS-MODEL (gray) and PDB ID: 1UZF (violet). The captopril ligand originated from 1UZF (green)

listed in Table 1.

The eighteen compounds were then analyzed for their physicochemical properties and drug-likeness using the SWISS-ADME server, and the compounds that received a rejection of more than two drug-likeness parameters, as shown in Table 2, would be eliminated for molecular docking analysis. As a result, two compounds, γ-L-Glutamyl-S-allyl-L-cysteine and γ-L-Glutamyl-S-1-propenyl -L-cysteine, are eliminated.

The compounds that had passed the selection would be analyzed for toxicity. The combined ADMET and Boiled-Egg analysis showed that seven compounds in Table 3 were safe to use as lead compounds for ACE inhibitors.

Furthermore, we conducted a QSAR study to investigate whether any of the seven compounds had the potential to become ACE inhibitors. The results of the QSAR analysis showed that three organosulfur compounds from garlic had the potential as ACE inhibitors, as shown in Table 4. Among the three compounds, SAMC has the highest bioactivity as ACE inhibitors, indicating that SAMC can potentially be an inhibitor of the ACE protein.

Before the molecular docking simulation between seven compounds and ACE was carried out, the position of the binding pocket of the protein was analyzed. The calculation result

Table 2 SWISS-ADME Drug-Likeness Analysis of Organosulfur Compounds in Garlic

Compound Name	Druglikeness				
	Lipinski	Ghose	Veber	Egan	Muege
Diallyl sulfide	Yes	No	Yes	Yes	Yes
Diallyl disulfide	Yes	No	Yes	Yes	No
Diallyl trisulfide	Yes	No	Yes	Yes	No
Alliin	Yes	Yes	Yes	Yes	No
Ajoene	Yes	Yes	Yes	Yes	Yes
Allicin	Yes	No	Yes	Yes	No
Methyl propyl disulfide	Yes	No	Yes	Yes	No
Methyl selenocysteine	Yes	No	Yes	Yes	No
Allyl methyl disulfide	Yes	No	Yes	Yes	No
S-Methyl-L-cysteine sulfoxide	Yes	No	Yes	Yes	No
Allyl methyl sulfide	Yes	No	Yes	Yes	No
S-Methylthiocysteine	Yes	No	Yes	Yes	No
S-allylmercaptocysteine (SAMC)	Yes	Yes	Yes	Yes	No
γ-L-Glutamyl-S-allyl-L-cysteine	Yes	Yes	No	No	No
γ-L-Glutamyl-S-1-propenyl-L-cysteine	Yes	Yes	No	No	No
Methyl propyl disulfide	Yes	No	Yes	Yes	No
S-1-Propenyl-L-cysteine	Yes	No	Yes	Yes	No
2-vinyl-4H-1,3-dithiin	Yes	No	Yes	Yes	No

Table 3 ADMET Analysis of Organosulfur Compounds That are Present in Garlic. The output Value of ADMET Analysis is in the Range of 0 to 1 and is Converted into the Following Categories 0-0.1 (---); 0.1-0.3 (--); 0.3 - 0.5 (-);0.5-0.7 (+); 0.7 - 0.9(++); 0.9-1.0(+++).

Compound Name	ADMET Analysis						
	BBB permeable	HIA	LC_{50}	AMES Toxicity	Skin Sensitization	Carcinogenicity	Eye irritation
Alliin	—	+++	3.515	+	–	–	–
Ajoene	—	+++	5.774	+++	+++	++	+++
Methyl selenocysteine	—	+++	3.259	++	-	–	–
S-Methyl-L-cysteine sulfoxide	—	+++	2.756	–	–	++	—
S-Methylthiocysteine	—	+++	3.785	–	+	–	–
S-allylmercaptocysteine (SAMC)	—	+++	4.259	–	++	–	–
S-1-Propenyl-L-cysteine	—	+++	3.972	+	-	+	—

from the CASTp server showed the surface area and volume of the binding pocket of the ACE protein, as shown in Figure 2.

In molecular docking analysis, Captopril was used as the reference compound of the ACE inhibitor since it had been approved as a drug to treat hypertension. The PatchDock output results in Table 5 showed that two garlic-derived compounds, Ajoene and SAMC, have a higher docking value than the reference compound.

In addition to the docking analysis, the researchers also analyzed and visualized the interactions between the ligands and proteins of the seven organosulfur compounds against the captopril molecule. The results are presented in Table 6 and Figure 3.

The molecular dynamics simulation of Ajoene with ACE protein showed a stable conformation because most of the ACE protein residues had RMSF values between the RMSF thresholds of 1–3 Å. Similarly, SAMC showed a stable conformation because it only had a slight residue of ACE protein with an RMSF value falling above the threshold value. The results of the RMSF graph of the two compounds can be seen in Figure 4.

Discussion

According to the 2022 global burden report, hypertension is the leading cause of cardiovascular disease and premature death

Table 4 The PASS Results from Organosulfur Compounds in Garlic

Compound	ACE inhibitor bioactivity
Alliin	No
Ajoene	No
Methyl selenocysteine	No
S-Methyl-L-cysteine sulfoxide	Yes; Pa = 0.060, Pi = 0.038
S-Methylthiocysteine	Yes, Pa = 0.095, Pi = 0.017
S-allylmercaptocysteine (SAMC)	Yes, Pa = 0.104, Pi = 0.015
S-1-Propenyl-L-cysteine	No

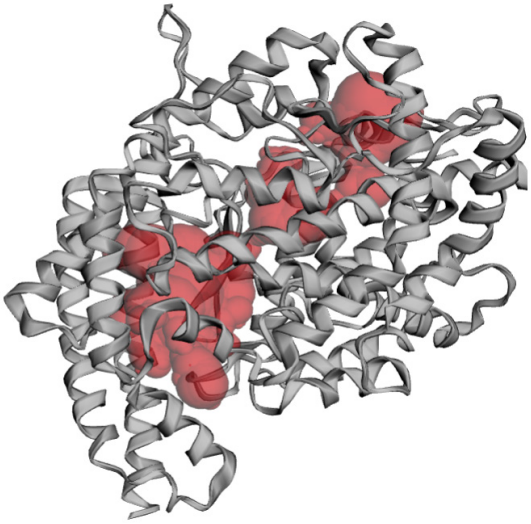


Figure 2 The ACE Model Generated from SWISS-MODEL server. The red Color Shows the Binding Pocket of ACE

worldwide. It is estimated that the number of hypertension cases has increased significantly in low and middle-income countries (LMICs).[1] However, global mean blood pressure (BP) has remained constant or decreased slightly over the past four decades due to the widespread use of antihypertensive drugs. Although antihypertensive medications can lower blood pressure, they frequently have unpleasant unpleasant adverse effects.[4] As a result, people are looking for alternative treatments for hypertension, using natural remedies like garlic (*Allium sativum L.*).

In this study, we will try to explain the mechanism of action of the active compounds in garlic in lowering blood pressure, one of which is by inhibiting the action of ACE in regulating blood pressure in the body. A virtual screening will be conducted to determine whether organosulfur lead compounds from garlic can reduce blood pressure in hypertensive patients. Therefore, this study aims to determine whether garlic's organosulfur compounds can reduce blood pressure in hypertensive patients by inhibiting ACE proteins, similar to captopril compounds.

L-cysteine sulfoxides and γ-glutamyl-L-cysteine peptides are garlic's two main organosulfur compounds.[6] Alliinase is released when garlic is crushed or chopped, which catalyzes the formation of Allicin from S-allyl-L-cysteine sulfoxide (Alliin), and Allicin degrades quickly into a variety of organosulfur compounds.[6]

The QSAR analysis was performed to screen the bioactivity of the organosulfur compounds from garlic by using the Prediction of Activity Spectra for Substances (PASS) tool. Its algorithm predicts the probability of activity (Pa) and the probability of inactivity (Pi) of the organosulfur compounds from garlic as ACE inhibitors based on their known structure-activity relationships database.[16] The Pa and Pi values provide an indication of the likelihood of a compound exhibiting or lacking a specific activity, respectively, as predicted by the PASS algorithm.[16] By default, the Pa=Pi value is set as the PASS threshold, so all compounds with Pa>Pi are suggested to have biological activity.[16] The average prediction accuracy is estimated to be about 95% accurate.[16] The QSAR analysis results

Table 5 The Molecular Docking Prediction of Selected Ligands and ACE Using The PatchDock Web Server

Ligand	Docking Score	Atomic Contact Energy score (ACE score)
Captopril	3642	-34.72
Ajoene*	4452	-159.33
Alliin	3132	-48.53
Methyl Selenocysteine	2756	-66.99
S-1-Propenyl-L-cysteine	3134	-48.53
S-allylmercaptocysteine (SAMC)*	3704	-99.93
S-Methyl-L-cysteine sulfoxide	2750	-146.86
S-Methylthiocysteine	2944	-78.44

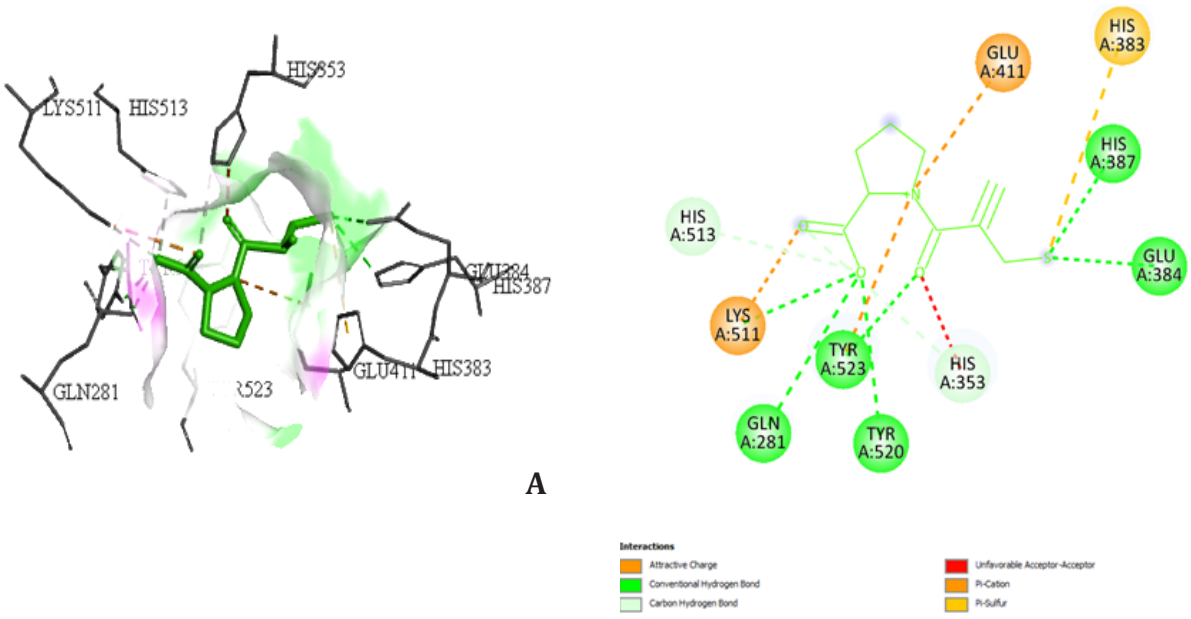
* The most favorable docking score and ACE score

Table 6 The Organosulfur Compound Interaction with ACE

Compound Name	Organosulfur Compound Interaction with ACE			
	Hydrogen Bonds	Salt Bridges	Hydrophobic Interactions	Total interactions
Captopril	GLN281	HIS353, LYS511, HIS513	PHE457	5
Ajoene*	HIS353, HIS513	GLU384, GLU411	VAL379	5
Alliin	HIS353, GLU384, HIS387, GLU411, HIS513	GLU411	ALA354, VAL380	8
Methyl Selenocysteine	HIS353, ALA354, GLU384	HIS383, HIS387		5
S-1-Propenyl-L-cysteine	ALA356, HIS387, GLU411	HIS387	TYR520, TYR523	6
SAMC*	ALA356, GLU384, HIS387, GLU411	HIS383, HIS387	PHE457, TYR523, PHE527	9
S-Methyl-L-cysteine sulfoxide	HIS353, ALA354, ALA356, HIS513	GLU384, HIS387, GLU411		7
S-Methylthiocysteine	HIS353, GLU411, HIS513, TYR523	HIS383, HIS387		6

in Table 4 show that three compounds have the potential to be ACE inhibitors.

Furthermore, we used molecular docking to investigate how these organosulfur compounds form complexes with ACE proteins. Molecular docking was used to perform virtual screening of the active compounds from garlic, to rank the results, and to propose structural hypotheses about how these ligands inhibit ACE proteins, which is very useful in lead optimization of these organosulfur compounds. Although Ajoene had no activity against the ACE protein in QSAR analysis compared to SAMC, the docking simulation results showed that Ajoene had a



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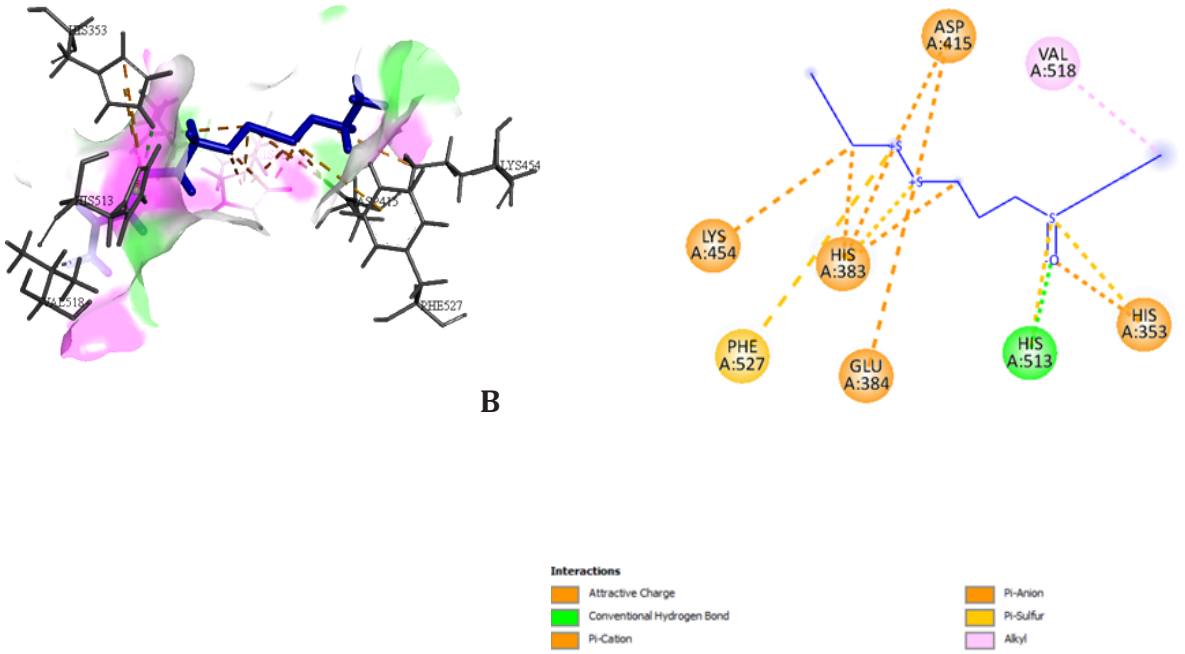

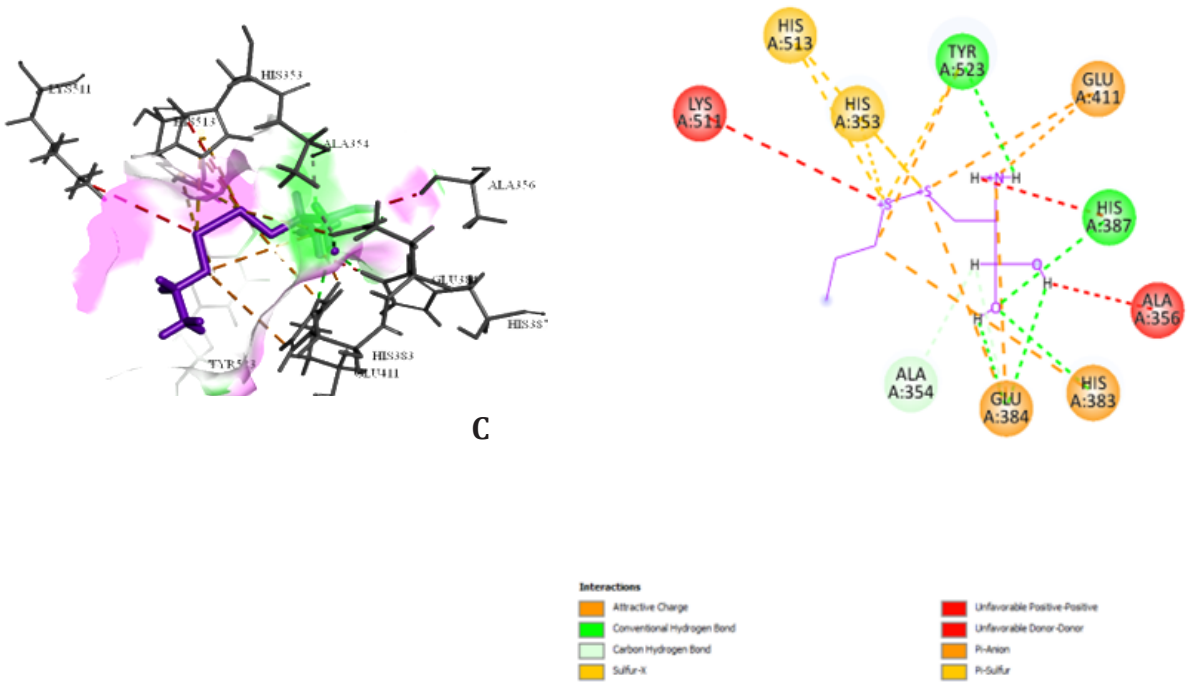

Figure 3 Molecular interaction between ACE and (A)captopril (yellow); (B) Ajoene (blue); and (C)SAMC (violet) in its binding site. Figures were visualize using Discovery Studio 2021

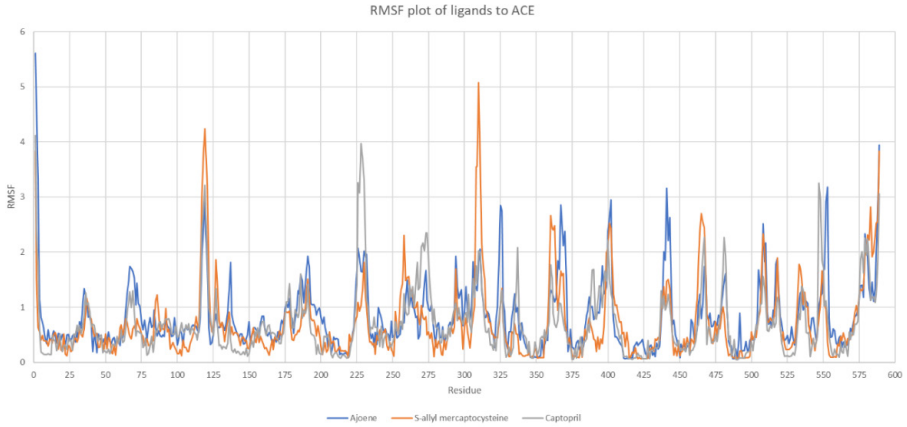

Figure 4 RMSF plot of Ajoene (blue), SAMC (orange) and Captopril (grey) with an Angiotensin-Converting Enzyme (ACE)

higher docking score than Captopril and SAMC.

Based on the docking simulation results, MD simulations were carried out to analyze the dynamic interactions between ACE and ajoene, SAMC, and Captopril. Figure 2 depicts a superimposed graphical representation of the residue-based Root Mean Square Fluctuations (RMSF) analysis of 625 residues in the ACE protein complexes to Ajoene, SAMC, and Captopril. The RMSF value was used to calculate the magnitude of each residue's fluctuations. The RMSF of the three complexes varies greatly, ranging from 0.05 to 5.61 Å. Although the interaction between ACE and ajoene, SAMC, and Captopril has a wide range of fluctuations, the superimposition of the RMSF values of the Ajoene, SAMC, and Captopril complexes onto the ACE protein also exhibits a pattern of synonymous fluctuations for the majority of the residues. This suggests that Ajoene and SAMC can bind to the ACE protein's binding pocket via a mechanism similar to Captopril.

The outcomes of this molecular docking simulation are consistent with the outcomes of the randomized controlled trials. Garlic preparations reduced systolic blood pressure (SBP) by 8.7 mm Hg and diastolic blood pressure (DBP) by 6.1 mm Hg compared to medications at standard dose, which reduced SBP by 9.1 mm Hg and DBP by 5.5 mm Hg.[4] Ajoene is one of garlic's most important natural compounds derived from Allicin. Ajoene is rapidly metabolized and excreted from the body, even after large amounts of garlic (up to 25 grams) or 60 mg of pure Allicin are consumed (in tablet preparations).[20] Meanwhile, SAMC is metabolized and excreted from the body for a longer time than Ajoene. These observations showed that these compounds might play a key role in the biological activity of garlic.[20]

The limitations of this study arose from the fact that it was not conducted directly on individual patients but instead relied on existing data in the database and the results of other people's meta-analyses. However, the results of our in-silico analysis align with the data from other researchers' meta-analyses, which strengthens our hypothesis that there are organosulfur compounds in garlic, namely Ajoene and SAMC, have the exact mechanism of action as antihypertensive drugs, and this can be a suggestion for more in-depth molecular research.

In concusion, that garlic consumption effectively lowers blood pressure with minimal side effects in people with hypertension. Organosulfur compounds from garlic, Ajoene, and SAMC have a mechanism of action as ACE inhibitors to regulate blood pressure, which is similar to antihypertensive drugs.

Furthermore, the findings in this study could be a valuable source of information for the development of natural-based antihypertensive drugs.

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