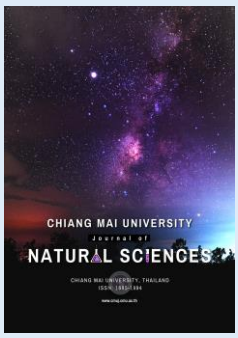

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Research article

Computational Study of Garlic Compounds as Potential Anti-Cancer Agents for the Inhibition of CCR5 and CXCR4

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Abstract Current cancer treatment methods are still inadequate due to the complexity of the cancer progression mechanism, which involves multiple genes, proteins, and signaling pathways. The discovery and validation of novel anticancer compounds remains challenging. Garlic has many medicinal properties that can combat various diseases. Organosulfur present in garlic has been shown to induce apoptosis in cancer cells; however, the underlying mechanism of action of non-organosulfur compounds from garlic in controlling cancer cells remains unclear. The present study aimed to analyze the efficacy of organosulfur and non-organosulfur compounds, including the flavonoid, terpenoid, and saponin groups, as inhibitors of C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4), which play significant roles in the progression of cancer. To determine the interactions between the active compounds of garlic and these receptors (CCR5 and CXCR4), molecular docking was performed using the PyRx v.0.8 software. Amino acid residues were analyzed and visualized using Biovia Discovery Studio and PyMol, respectively. Non-organosulfur compounds exhibited better results than the organosulfur compounds in binding affinity analysis. Tigogenin (from the saponin group) is considered to be a CCR5 inhibitor, while lupeol (from the terpenoid group) is considered to be a CXCR4 inhibitor. In conclusion, our results suggest that garlic compounds could be promising inhibitors of CCR5 and CXCR4, which are highly expressed in cancer. However, further research is needed to validate the in vitro and in vivo activities of garlic compounds for the inhibition of cancer progression.

Keywords: Anticancer agents, CCR5, CXCR4, Garlic, Organosulfur compounds, Non-Organosulfur compounds

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INTRODUCTION

Cancer is the leading cause of death worldwide (Torre et al., 2016). Around 17.2 million cancer cases have been reported globally, with 8.9 million deaths in 2016, and the number of cases further increased by 1.05% in 2018 (Global Burden of Disease Cancer Collaboration, 2018; World Health Organization, 2018). Surprisingly, in the United States, the number of deaths caused by cancer has started to surpass those caused by cardiovascular diseases (Heron and Anderson, 2016). In Indonesia, breast cancer was reported as the leading cause of death among other cancer cases by 16.6%, followed by cervix uteri and lung cancer with a percentage of 9.2% and 8.8%, respectively (The Global Cancer Observatory, 2020).

However, the current cancer treatment strategies remain inadequate (Allemani and Coleman, 2017), and there is a lack of comprehensive evidence for the combination of anticancer drugs and herbal medicines (Cheng et al., 2018; Jermini et al., 2019). Interestingly, most cancer patients now choose complementary and alternative medicine (CAM) to enhance their quality of life, increase their probability of survival, and reduce the side effects of treatment (Chan et al., 2011; Smith et al., 2014; Wu et al., 2016). Drug combinations involve complicated interactions among multiple genes, proteins, and pathways at the pharmacological and pharmacokinetic levels. The drug interactions need to be studied further, as the database of drug-gene–protein interactions is still incomplete (Hu et al., 2016). Therefore, there is an urgent need to discover and validate novel anticancer compounds, which remains a challenge for scientists worldwide.

Garlic (*Allium sativum*) is considered to be one of three top-selling botanical products in United States retail outlets (Wachtel-Galor and Benzie, 2011) and the second most used compound in complementary medicine due to its various medicinal properties (Amagase, 2006.; Varshney and Budoff, 2016). Garlic has low toxicity (Lestari and Rifai, 2019) and possesses anti-inflammatory (Arifah et al., 2020), immunomodulatory (Lestari and Rifa'i, 2019; Lestari et al., 2020), and anticancer properties (Cao et al., 2014). However, many studies using garlic as an anticancer agent have only focused on its organosulfur compounds. Diallyl trisulfide (DATS) is considered the primary anticancer organosulfur compound in garlic (Wang et al., 2010; Shin et al., 2014). Our previous study reported that garlic also contains relatively high levels of flavonoids, terpenoids, and saponins (Balqis et al., 2018). Moreover, the association between the non-organosulfur and organosulfur compounds in garlic have not yet been studied.

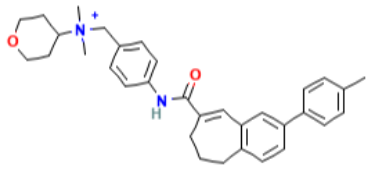
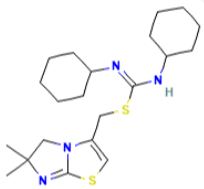
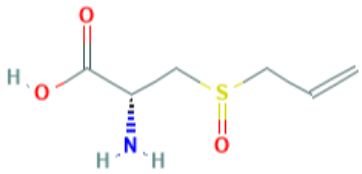
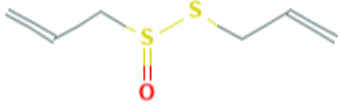
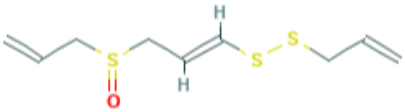
Chemokines and their receptors play critical roles in the inflammatory response mechanisms and help cancer cells migrate to the site of metastasis. C-C chemokine receptor type 5 (CCR5), one of the members of the G protein-coupled receptors (GPCRs) family that binds to multiple ligands, mainly the chemokine (C-C motif) ligand 5 (CCL5), which is highly correlated with immune cell infiltration and promotes tumorigenesis (Jiao et al., 2019; Zhang et al., 2019). In contrast, C-X-C chemokine receptor type 4 (CXCR4) is highly expressed in various human cancer cells. The binding of CXCR4 with its ligand, stromal derived factor-1 (SDF-1), is known to promote the proliferation and survival of cancer cells (Wang et al., 2006; Weitzenfeld and Ben-Baruch, 2014a). To date, the CCR5-CCL5 and CXCR4-SDF-1 pathways have been highlighted due to their tumor-promoting effects. Indeed, the inhibition of these two pathways may aid in the identification of novel anticancer candidates in the future (Weitzenfeld and Ben-Baruch, 2014a; Kalpana et al., 2019). The current study investigated the potential of organosulfur and non-organosulfur compounds to act as inhibitors of CCR5 and CXCR4. We aimed to elucidate the molecular docking mechanisms of organosulfur and non-organosulfur compounds as inhibitor of cancer cell proliferation. We hypothesized that these two major compounds in garlic may synergistically inhibit CCR5 and CXCR4, which may in turn improve the efficacy of cancer treatment.

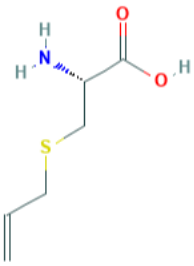
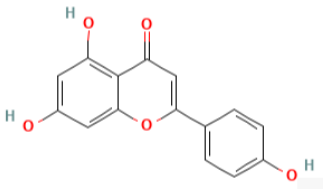
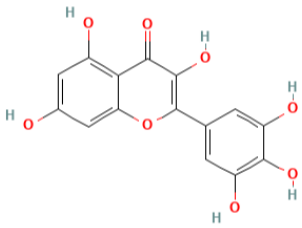
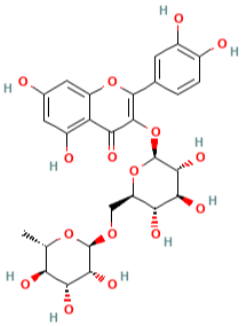
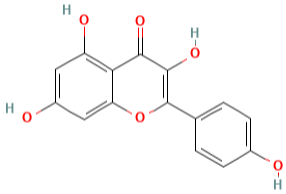
MATERIALS AND METHODS

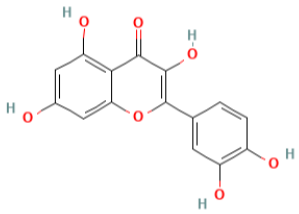
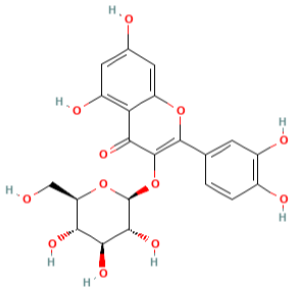
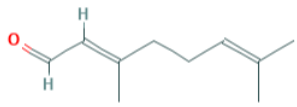
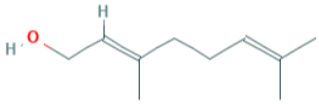
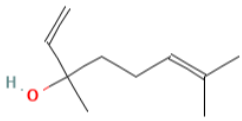
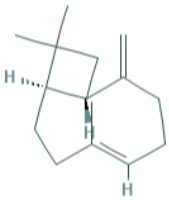
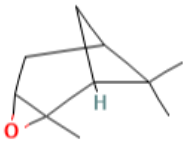
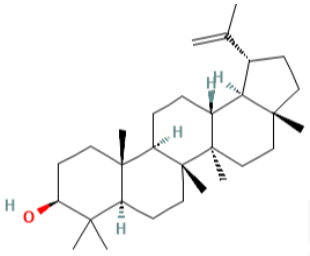
Preparation of proteins and ligands

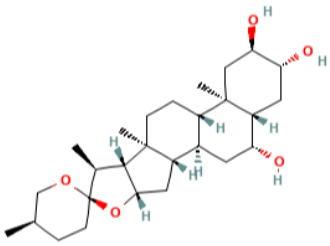
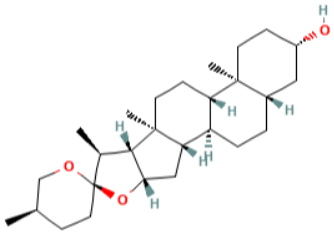
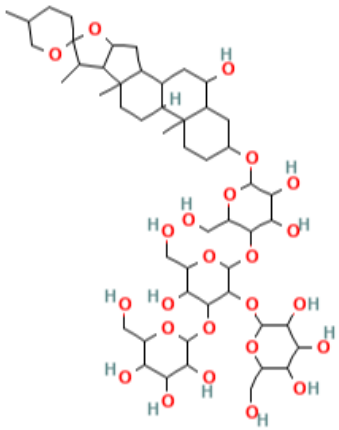
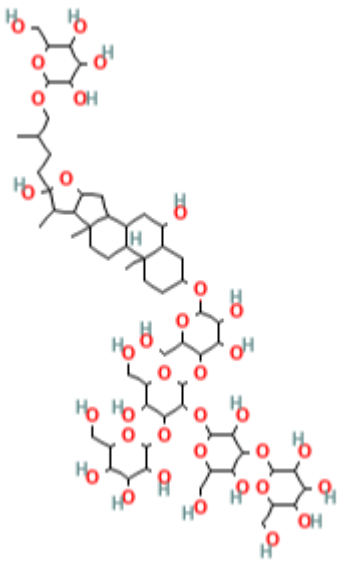
The protein for docking analysis was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) with PDB ID 4MBS for CCR5 and PDB ID 3OE8 for CXCR4. CCR5 protein (4MBS) was used as a protein ligand according to a preliminary experiment conducted by Lau et al. (2015), who used the same protein of CCR5 (4MBS) and performed the molecular docking analysis of chemokine receptor CCR5 with ligand dimethyl-[[4-[[3-(4-methylphenyl) -8, 9-dihydro-7H-benzo[7] annulene-6-carbonyl]amino]phenyl]methyl]-(oxan-4-yl)azanium (TAK-779). In the other hand, the selected CXCR4 was used based on a previous study by García-Cuesta et al. (2019) on the interactive role of increased expression of CXCR4 in tumor progression (Bohn et al., 2009; García-Cuesta et al., 2019). Water and ligands inside the protein were removed using the PyMoL software version 1.7.4.5 Edu (Schrödinger Inc., LLC). Structure of the ligands in the sdf format used for analysis was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Table 1). The three-dimensional structures of organosulfur compounds, flavonoids, and terpenoids were converted into .pdb using PyMOL, while saponins were converted using the Open Babel GUI software version 2.4.1 (O'Boyle et al., 2011). The saponin group was converted using the Open Babel GUI software as the structures needed to be converted into a 3-Dimensional (3D) format first. Drug inhibitor for CCR5 used (TAK-779) (CID 183790) (Lau et al., 2015), while IT1t (CID 25178351) was used as a CXCR4 inhibitor control drug (Debnath et al., 2013).

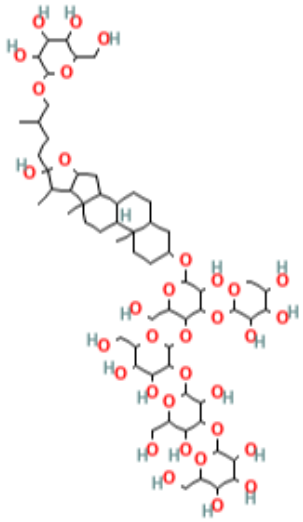
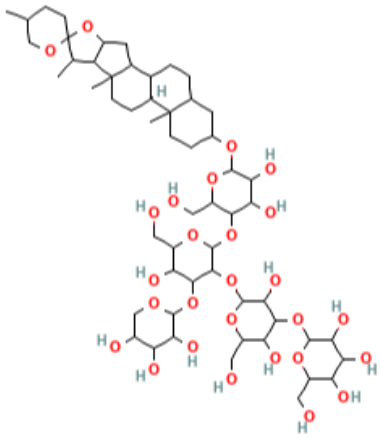
Table 1. List of ligand and protein for molecular docking.

No.	Ligands	2D Structure
1	CCR5 inhibitor (CID 183790)	
2	CXCR4 inhibitor (CID 25178351)	
3	Alliin (CID 87310)	
4	Allicin (CID 65036)	
5	Ajoene (CID 5386591)	

No.	Ligands	2D Structure
6	Allyl Methyl Trisulfide (CID 61926)	
7	Diallyl Trisulfide (CID 16315)	
8	S-allylcystein (CID 9793905)	
9	Apigenin (CID 5280443)	
10	Myricetin (CID 5281672)	
11	Rutin (CID 5280805)	
12	Kaempferol (CID 5280863)	

No.	Ligands	2D Structure
13	Quercetin (CID 5280343)	
14	Isoquercitrin (CID 5280804)	
15	Citral (CID 638011)	
16	Geraniol (CID 637566)	
17	Linalool (CID 6549)	
18	Caryophyllene (CID 5281515)	
19	α-Pinene (CID 91508)	
20	Lupeol (CID 259846)	

No.	Ligands	2D Structure
21	Agigenin (CID 44566818)	
22	Tigogenin (CID 99516)	
23	Isoeruboside (CID 194485)	
24	Sativoside B1 (CID 14464368)	

No.	Ligands	2D Structure
25	Sativoside R1 (CID 131752731)	
26	Sativoside R2 (CID 3474285)	

Molecular docking of ligand-protein

The interactions between the ligands and proteins were analyzed using Autodock Vina with PyRx v.0.8 (https://pyrx.sourceforge.io) (Dallakyan and Olson, 2015; Trott and Olson, 2010). Before docking, the Open Babel GUI was used to minimize the ligands with the aim of minimizing the energy of these compounds; thus, the protein-ligand interaction distance results are accurate to the level of quantum chemical calculations (Mirzaei et al., 2015). Docking was performed by binding the ligand to the active site of the protein. The center of active site for each protein was obtained by reverse docking using drug inhibitor first, TAK-779 for CCR5, and IT1t for CXCR4. Reverse docking using drug inhibitor as ligand with the aim to discover alternate drug candidate for protein which involved in specific pathway of disease. Reverse docking is utilized to identify the potential targets from the vast number of proteins/receptors by examining their known ligands or crystal structures (Huang et al., 2018; Pertami et al., 2021). Reverse docking initiated with docking between protein and drug inhibitor using blind docking. After the drug inhibitor attached to the active site of protein, then continuing to be docking with active compounds from garlic. Reverse docking using each drug inhibitor resulted the center of CCR5 active site was X: 157.9064, Y: 106.5949, Z: 19.4361, with dimensions at X: 24.8522, Y: 13.0475, Z: 15.0708 Å, while the center of CXCR4 was at X: 48.6176, Y: -0.1687, Z: 17.2453, with the dimensions of active site as X: 26.3700, Y: 20.8968, and Z: 27.9578 Å. Binding affinity values in kcal/mol were analyzed as a result of the bonding strength between the ligand and the protein. Visualization of docking and bonding interactions was performed using BIOVIA Discovery Studio (Dassault Systèmes BIOVIA, 2015).

Protein interactions and networks

Analysis of protein interactions with CCR5 and CXCR4 was performed using the Biological General Repository for Interaction Datasets (BioGRID) database (https://thebiogrid.org/). Protein networks were analyzed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (http://string-db.org/) (Szklarczyk et al., 2015). Protein interactions by BioGRID help to determine the proteins that interacted with CCR5 which involved in cancer progression via various mechanisms or pathways. Meanwhile, STRING was used to determine the proteins (decided by researchers) that were involved in specific pathways, such as the cancer pathway, together with the list of genes in those pathways.

RESULTS

Molecular docking analysis

The binding affinity value is indicated based on the Gibbs free energy (ΔG) between the protein ligands. ΔG is negative when the system reaches equilibrium at a constant temperature and pressure (Du et al., 2016). The bonding strength of the protein-ligand is determined by the magnitude of the negative ΔG; thus, it could be considered to measure the stability of any given protein-ligand complex or the binding affinity of a ligand to a given acceptor alternatively (Gilson and Zhou, 2007)

Table 2. Binding affinity between active compounds from Garlic over CCR5 and CXCR4.

Ligand Name	Binding Affinity (kcal/mol)	
	CCR5	**CXCR4**
CCR5 inhibitor	-10.8	-
CXCR4 inhibitor	-	-8.6
Organosulfur		
Alliin	-4.9	-5.2
Allicin	-4.0	-4.1
Ajoene	-4.6	-4.7
Allyl Methyl Trisulfide	-3.4	-3.6
Diallyl Trisulfide	-3.9	-4.0
S-Allylcystein	-5.0	-4.6
$\bar{X}$ ***Organosulfur***	**-3.65**	**-4.37**
Flavonoid		
Apigenin	-8.1	-8.2
Myricetin	-7.0	-8.0
Rutin	-8.6	-7.5
Kaempferol	-6.9	-8.2
Quercetin	-7.8	-8.0
Isoquercitrin	-8.9	-8.0
$\bar{X}$ ***Flavonoid***	**-7.8**	**-7.98**
Terpenoid		
Citral	-5.9	-5.7
Geraniol	-5.8	-5.6
Linalool	-5.7	-5.5
Caryophyllene	-6.7	-7.1
α-Pinene	-6.4	-5.4
Lupeol	-8.7	-8.7
$\bar{X}$ ***Terpenoid***	**-6.53**	**-6.33**
Saponins		
Agigenin	-7.7	-8.2
Tigogenin	-9.4	-6.5
Isoeruboside	-8.6	-8.3
Sativoside B1	-8.3	-7.0
Sativoside R1	-7.8	-7.2
Sativoside R2	-8.3	-8.5
$\bar{X}$ ***Saponins***	**-8.35**	**-7.61**

The docking study results between garlic active compounds with CCR5 and CXCR4 revealed that organosulfur family compounds had higher binding affinity values than the flavonoid, terpenoid, and saponin family groups (Table 2). CCR5-ligand docking (Figure 1) showed that the binding affinity value of S-allylcystein was the lowest among all the organosulfur compounds (–5.0 kcal/mol), but it still higher than isoquercitrin from flavonoid group, lupeol from terpenoid group, and tigogenin from saponins group (–8.9, –8.7, and –9.4 kcal/mol). A lower binding affinity value indicated that the binding of the protein-ligand was more stable. This docking study indicated that saponins are more effective as CCR5 inhibitors than organosulfur family groups.

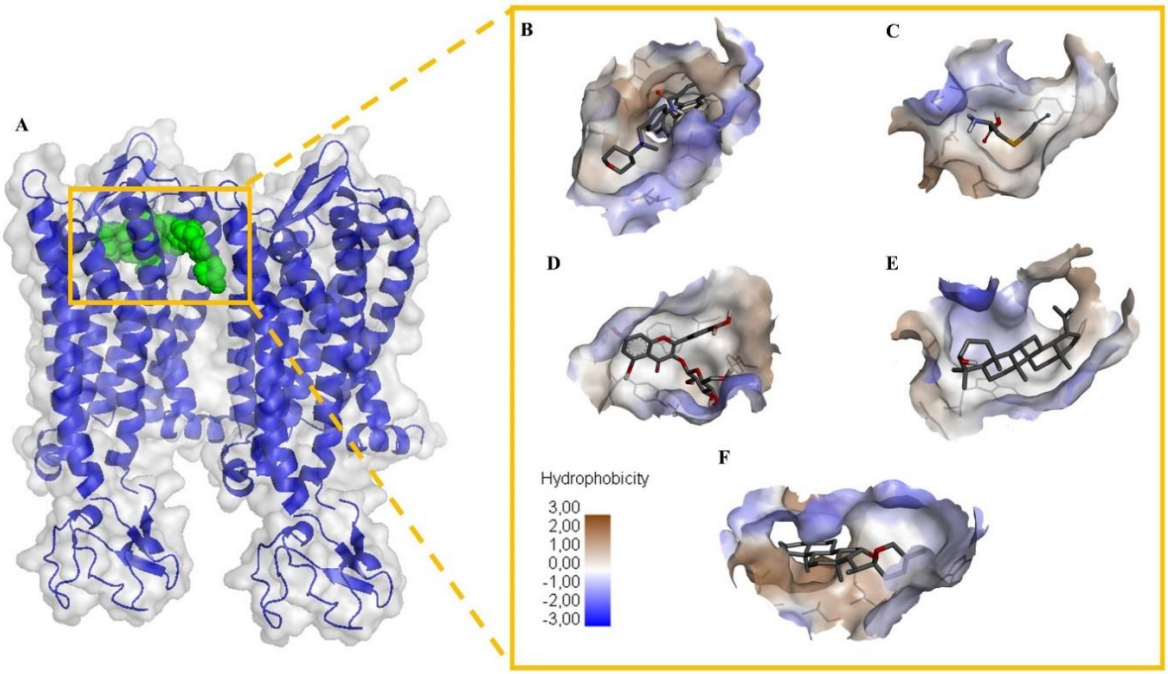


Figure 1. Visualization of molecular docking between active compounds found in garlic and protein CCR5. (A) Position of the ligand in the active site of the protein. The green sphere as ligand and surface blue cartoon as protein; docking of (B) CCR5 inhibitor known as TAK-9 (C) S-allylcystein (D) isoquercitrin (E) lupeol and (F) tigogenin. The yellow box contained interaction between compounds and CCR5 based on the hydrophobicity on the protein surface and amino acid residues for each compound. Visualization presented based on the lowest binding affinity value for each family compound in CCR5 docking. CCR5, C-C chemokine receptor type 5.

On the other hand, docking of the CXCR4-ligand (Figure 2) resulted in lupeol as one of the terpenoid family groups with the lowest binding affinity value (–8.7 kcal/mol). The binding affinity value of lupeol was also lower than that of the CXCR4 inhibitor (–8.6 kcal/mol), which indicates that lupeol could be more effective as a CXCR4 inhibitor. Meanwhile, molecular docking in CXCR4 with an organosulfur family group showed a higher binding affinity value than that of the non-organosulfur compound. Organosulfur compounds might be less stable when bound to CCR5 and CXCR4 (Table 2). rather than non-organosulfur compounds.

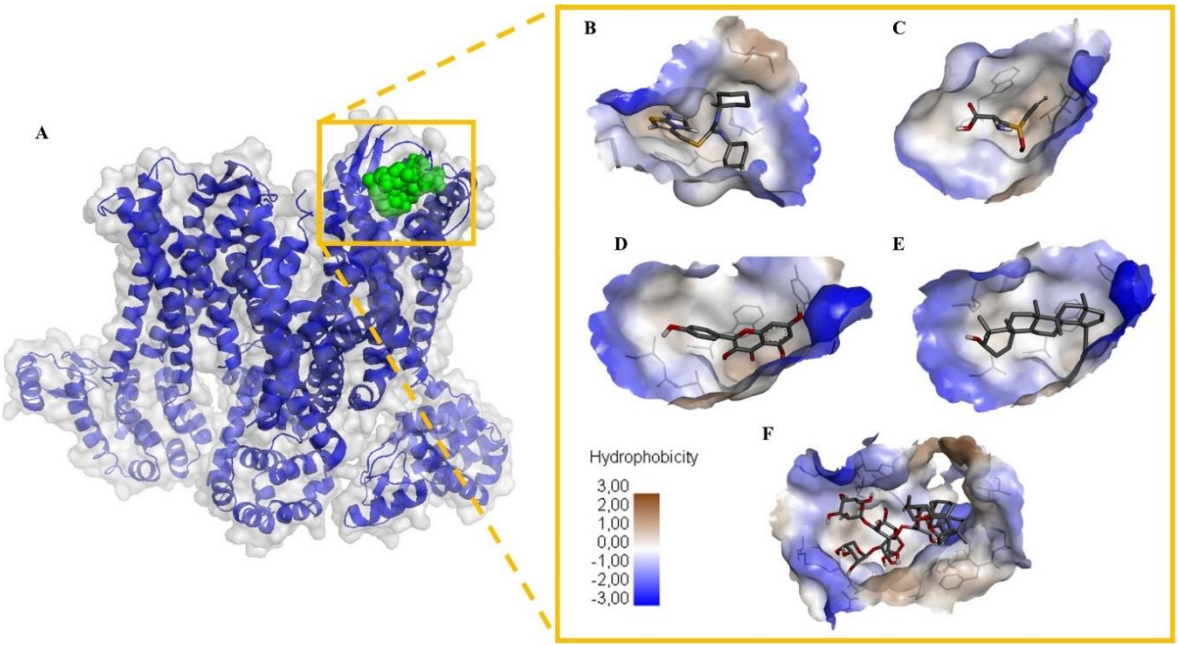


Figure 2. Visualization of molecular docking between active compounds found in garlic and protein CXCR4. (A) Position of the ligand in the active site of the protein. The green sphere as ligand and surface blue cartoon as protein; docking of (B) CXCR4 inhibitor known as IT1t (C) alliin (D) kaempferol (E) lupeol and (F) sativoside R2. Yellow box contained interaction between compounds and CXCR4 based on the hydrophobicity on the protein surface and amino acid residues for each compound. Visualization presented based on the lowest binding affinity value for each family compound in CXCR4 docking. CXCR4, C-X-C chemokine receptor type 4.

Protein interactions and networks analysis

Protein interaction analysis from BioGRID revealed CCR5 interactions with other proteins (Figure 3), especially evidence of the interaction between CCR5 and CXCR4 (Table 3). More than 15 proteins interact with CCR5. Based on BioGRID analysis showed that CCR5 and CXCR4 were involved in various cancer signaling pathways together with the Janus kinase (JAK), signal transducer and activator of transcription (STAT), JAK2, STA3, and STAT5 pathways.

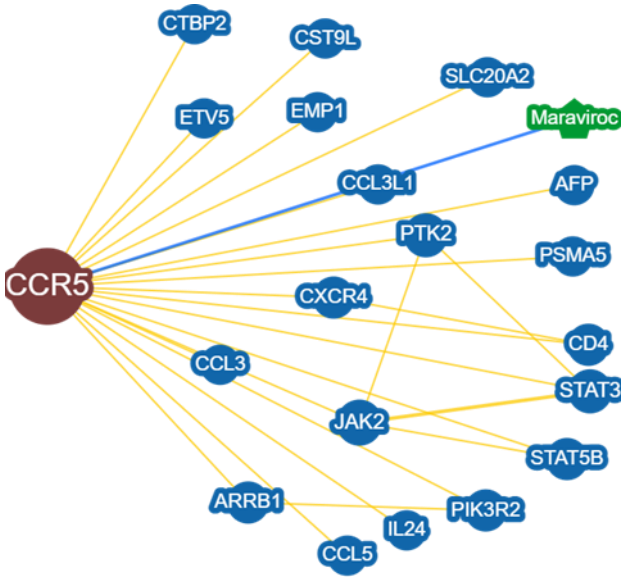


Figure 3. Protein interactions provided by BioGRID database.

Table 3. Protein interactions and evidence of interaction with CCR5.

Protein	Evidence	References
AFP	Affinity Capture-Western	(Atemezem et al., 2002)
ARRB1	Reconstituted Complex	(Huttenrauch et al., 2002)
CCL3	Reconstituted Complex	(Navenot et al., 2001)
CCL3L1	Reconstituted Complex	(Miyakawa et al., 2002; Struyf et al., 2001)
CCL5	Reconstituted Complex Affinity Capture-Western	(Proudfoot et al., 2001; Struyf et al., 2001) (Slimani et al., 2003)
CD4	Affinity Capture-Western	(Xiao et al., 2000)
CST9L	Two-Hybrid	(Wang et al., 2011)
CTBP2	Two-Hybrid	(Wang et al., 2011)
CXCR4	Reconstituted Complex	(Agrawal et al., 2002)
EMP1	Two-Hybrid	(Luck et al., 2020)
ETV5	Two-Hybrid	(Wang et al., 2011)
IL24	Two-Hybrid	(Wang et al., 2011)
JAK2	Affinity Capture-Western	(Wong et al., 2001)
PIK3R2	Affinity Capture-Western	(Mellado et al., 2001)
PSMA5	Two-Hybrid Affinity Capture-Western	(Fernandis et al., 2002)
PTK2	Affinity Capture-Western	(Cicala et al., 1999)
SLC20A2	Two-Hybrid	(Sokolina et al., 2017)
STAT3	Affinity Capture-Western	(Mellado et al., 2001)
STAT5B	Affinity Capture-Western	(Mellado et al., 2001)

Further analysis using the String Database revealed that CXCR4 is involved in the cancer pathway (red bulb) and interacts with CCR5 in the chemokine signaling pathway. Chemokines and their receptors have been reported that play both anticancer and pro-cancer roles. The CCR5 and CXCR4 pathways are involved in the development of cancer (Weitzenfeld and Ben-Baruch, 2014b). The inhibitory mechanisms of these chemokine receptors might have implications for cell growth in cancer progression.

DISCUSSION

Research on the discovery of novel compounds as anticancer candidates is promising and challenging. CCR5 and CXCR4 inhibitors may be promising candidates for cancer therapy based on their critical function during cancer progression (Weitzenfeld and Ben-Baruch, 2014a). Garlic possesses anticancer properties and regulates the phosphoinositide 3-kinase/serine-threonine kinase (PI3K/Akt) and JNK, B cell lymphoma-2 (Bcl2), and caspase 3 signaling pathways to induce apoptosis (Wang et al., 2010; Shin et al., 2014). However, its upstream mechanism of action, which involves the main receptor in cancer, remains unclear. Moreover, most garlic utilization is focused on organosulfur compounds. Herein, we compared the anticancer mechanisms of organosulfur and non-organosulfur compounds from garlic (Table 2) using an in-silico approach.

Surprisingly, the organosulfur compounds showed weaker binding capability than non-organosulfur compounds with CCR5 or CXCR4 (Table 2). Our molecular docking results demonstrated that tigogenin from the saponin group had a lower binding affinity than other compounds (except the CCR5 inhibitor or IT1t). Gibb's free energy measures the amount of free energy generated from the binding affinity. The more negative the Gibb's free energy value, the greater the interaction between the ligand and the protein

(Atho'illah et al., 2021; Pertami et al., 2021). The key residues of CCR5 are Tyr37 and Trp86, with the latter being an important residue for the inhibition of CCR5 (Lin et al., 2019). Interestingly, our present study demonstrated that both organosulfur and non-organosulfur compounds have the same binding site as the CCR5 inhibitor at Tyr37, Trp86, and Gln280 (Figure 4). Tigogenin interacts with CCR5 through van der Waals interactions (Supplementary Table 1), which provides a major driving force for the binding of CCR5 (Lin et al., 2019). Meanwhile, another study reported that Gln280 plays a key role in the intermolecular interaction between CCL5 and CCR5, and the mutation of Gln280 significantly reduced the activity of CCR5 significantly (Tamamis and Floudas, 2014a, 2014b). The CCR5 binding cavity is mainly hydrophobic as various non-polar amino acids, including Trp86, influence the ligand-dependent CCR5 mechanism (Salmas et al., 2015). In cancer progression, CCR5 manipulates the chemokine networks to support tumor growth by leading them to the metastatic regions of cancer cell homing and increases the proinflammatory pro-metastatic immune phenotype, accelerates the DNA repair, ensures the survival of aberrant cells, and increases the resistance to DNA damage (Aldinucci et al., 2020). Therefore, blockade of the CCL5/CCR5 axis by counteracting the garlic constituents may inhibit the progression of cancer.

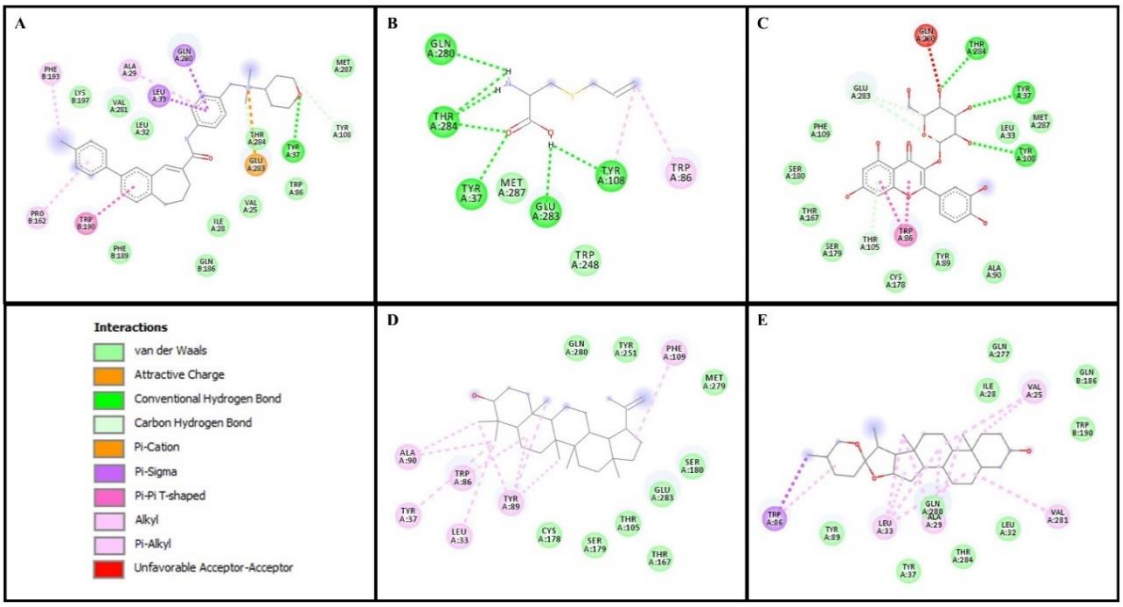


Figure 4. Amino acid residues and interaction types from molecular docking between CCR5 and ligands. (A) CCR5 inhibitor (TAK-79) (B) S-allylcystein (C) Isoquercitrin (D) lupeol and (E) tigogenin.

Furthermore, our present study demonstrated that lupeol from the terpenoid group showed the lowest binding affinity compared to other compounds (except the CXCR4 inhibitor) through van der Waals interactions (Supplementary Table 2). Interestingly, both organosulfur and non-organosulfur compounds have the same binding site as the CXCR4 inhibitor at Asp97, Leu41, Trp94, Ala98, and His113 (Figure 5). A previous study by Das et al. (2015) showed that these amino acid residues might have important roles in CXCR4, with Asp97 serving a critical role in cell fusion and is considered to be important for the binding of the CXCR4 inhibitor (Das et al., 2015). CXCR4 interaction with SDF-1 leads to the activation of several transduction signaling and their downstream pathways, which are involved in cell survival, proliferation, migration, adhesion, and chemotaxis (Xu et al., 2015). Notably, CXCR4 has either negligible or absent expression in normal cells, while it is overexpressed in the tumor cells close to normal cells (Chen et al., 2014). As mentioned earlier, the CXCR4/SDF-1 axis promotes tumor growth and attracts immune cells to the tumor sites; therefore, the blockade of this axis may ameliorate cancer metastasis (Mishra et al., 2016; Song et al., 2021). Our molecular docking results suggest that garlic compounds are bound to several key amino acids of CXCR4 and may have a beneficial impact in delaying the CXCR4 downstream mechanism during cancer progression.

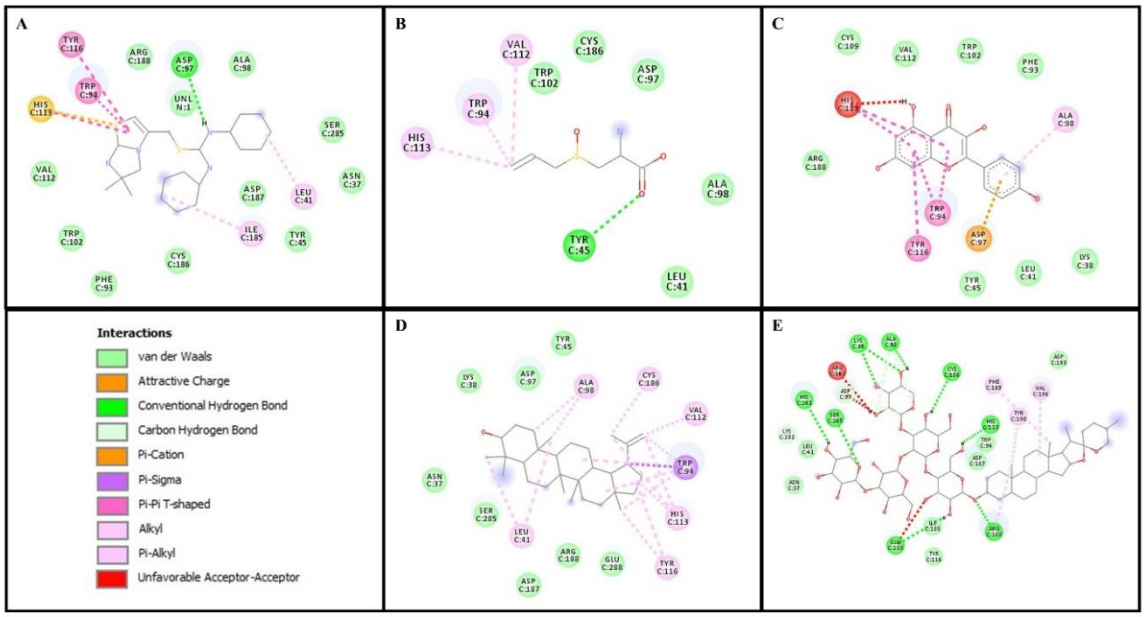


Figure 5. Amino acid residue and interaction from molecular docking between CXCR4 and ligands. (A) CXCR4 inhibitor (IT1t) (B) alliin (C) kaempferol (D) lupeol and (F) Sativoside R2.

The research study showed that all garlic compounds including organosulfur, terpenoids, flavonoids, and saponins groups can attach to target proteins with varying binding affinity values. Garlic is rich of organosulfur compounds (OSC) which classified into water-soluble organosulfur and oil-soluble organosulfur. Even though the mechanism of organosulfur compounds as anticancer is still unclear, previous research revealed that sulfur acts as a component in some amino acids and Fe-S clusters for enzyme activity. As we know, Fe-s plays an important role for origin of life such as DNA, RNA, and acetyl-CoA (Zeng et al., 2017). Omar & Al-Wabel (2010) reported that organosulfur compounds are involved in several metabolizing enzymes due to their structure, which act as activate or detoxify in cancer signaling. These findings suggested that organosulfur compounds might be involved as anticancer by modulating the activity of both of CCR5 and CXCR4 in cancer progression.

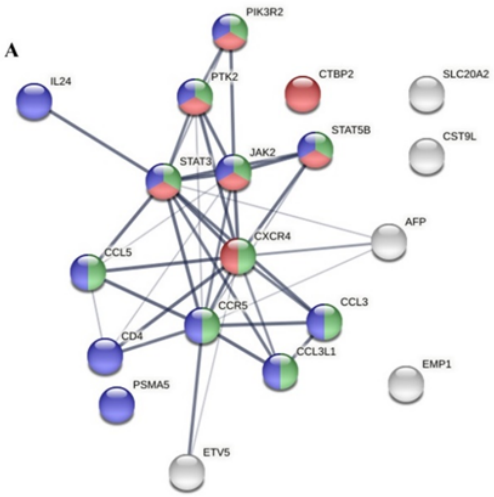
Free radicals are unstable molecules, very reactive, and cause DNA damage. Radicals-linked damage on DNA and proteins plays a significant role in cancer development (Pourahmad et al., 2016). Flavonoids are phytochemical compounds found in plants and polyphenols with diphenylpropane (C6-C3-C6) skeleton (Pasetto et al., 2014). Flavonoids characterized by the existence of functional hydroxyl groups. The structures mediate antioxidant activity by scavenging free radicals and/or by chelating metal ions. These functional hydroxyl groups especially the B ring hydroxyl configuration could be radical scavengers by donating the hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals by forming flavonoids radical which are relatively more stable (Kumar & Pandey, 2013). On the other hand, flavonoids group has been reported of their ability to inhibit the human immunodeficiency virus (HIV) by affecting cluster of differentiation 4 (CD4) and co-receptors CCR5 and CXCR4 (Pasetto et al., 2014). The inhibitory activity of flavonoids over CCR5 and CXCR4 in HIV suggested that flavonoids might be able as an anticancer due to the inhibition role over CCR5 and CXCR4 in cancer progression.

Furthermore, another plant polyphenol which have antioxidant activity is terpenoids. Terpenoids consist with hydrocarbon chain made up of isoprene structural units. Monoterpenoid (C10), sesquiterpenoid (C15), diterpenoid (C20), triterpenoid (C30), tetraterpenoid (C40), polyterpenoid (C>40), etc. In addition to terpene hydrocarbons, terpenoids are synthesized through mevalonic acid (MVA) and 2C-methyl-D-erythritol-4-phosphate (MEP) biochemical pathway. Both pathways provide terpenes skeletons, which results in functionalized and physiologically active secondary metabolites (Bergman et al., 2019). For example, geraniol induces tumor cell apoptosis and inhibits Akt signaling pathway exhibit an anticancer effect (Kim et al., 2011). Interestingly, lupeol which showed

the strongest inhibitory towards CXCR4 could act as multi-target agent which involve in the several important pathways, including PI3K/Akt signaling pathway (Saleem, 2009; Liu et al., 2013). Another study reported that the isolated terpenoid inhibit JAK2 phosphorylation through interaction with FERM-SH2 domain of JAK2 resulting in STAT3 inhibition (Bailly, 2020). These effects are necessary to delay cancer progression, which further supported by our networking analysis.

Garlic also contains with high levels of terpenoids, flavonoids, steroidal saponins, and so on. Steroidal saponins are widely distributed among monocotyledonous families. Saponin is structurally made up from distinct components consisting of an isoprenoid unit and sugar residue oligosaccharides as glycone part. Saponins group especially steroidal saponins contain aglycone part at the former, while glycone at the end of chain. Saponins consist of a hydrophilic sugar moiety and a hydrophobic genin called sapogenin (Elekofehinti et al., 2021). The steroidal sapogenins contain a cholestane nucleus, and side-chain cyclization may occur, yielding furostanes, furosprostanes, and spirostanes. Tigogenin, a spirostane-tipe sapogenin, which possess both tetrahydropyran and tetrahydrofuran rings joined at C-22 bearing saccharides chains displayed antitumor activities in cancer several cell lines (Simmons-Boyce and Tinto, 2007; Juang and Liang, 2020).

In addition, our network analysis revealed that both CCR5 and CXCR4 share the same pathway via the cancer pathway, chemokine signaling pathway, and immune system (Figure. 6). These three pathways play pivotal roles in cancer progression. CCR5 is responsible for the establishment of the cancer microenvironment in various cancer types. CCR5 manipulates the immune cells to become a pro-metastatic immune phenotype to build a protective microenvironment, leading to cancer cell growth, survival, migration, and invasion during metastasis (Jiao et al., 2019; Aldinucci et al., 2020; Pervaiz et al., 2021). CXCR4 regulates cell division via JAK2/3 in the PI3K/Akt signaling pathway (Abdelouahab et al., 2017). Targeting CCR5 and CXCR4 upstream in the cancer pathway might be an effective strategy for treating cancer.



B

Description	False discovery rate	Genes
Pathway cancer	1.31×10^{-05}	STAT3, STAT5B, PTK2, JAK2, PIK3R2, CTBP2, CXCR4
Chemokine signaling pathway	5.89×10^{-14}	CCR5, CXCR4, CCL3L1, CCL3, CCL5, JAK2, STAT3, STAT5B, PTK2, PIK3R2
Immune system	1.14×10^{-06}	CCR5, CCL5, CCL3L1, CCL3, CD4, STAT3, STAT5B, JAK2, PTK2, PIK3R2, IL24, PSMA5

Figure 6. Result of CCR5 and CXCR4 networks. (A) Protein networks based on String Database, Pathway cancer was marked with red color, Chemokine signaling pathway with green color, and Immune system with blue color. (B) False discovery and identification of genes that engaged for each pathway. CCR5, C-C chemokine receptor type 5; CXCR4, C-X-C chemokine receptor type 4.

Therefore, organosulfur compounds have been reported to block the CCR5 and CXCR4 signaling pathways, leading to apoptosis and delayed cancer proliferation (Wang et al., 2010; Shin et al., 2014; Guan et al., 2015; Song et al., 2015; Lee et al., 2018). A previous study reported that tigogenin has a weak anti-proliferative activity; in contrast, tigogenin synthesis to its analogs or derivatives might transform tigogenin to be a more potent and selective anticancer candidate (Li et al., 2018; Michalak et al., 2020). Numerous studies have shown that lupeol induces apoptosis in cancer cells by inhibiting the PI3K/Akt pathway

and suppressing STAT3 activity. In this scenario, based on our molecular docking results, the non-organosulfur in garlic was identified as a promising candidate to interfere with CCR5 and CXCR4 signaling pathways for improving the treatment to treat or possibly improve the quality of life of cancer patients.

CONCLUSION

The results of the present study suggest that the non-organosulfur compounds from garlic might act as potential CCR5 or CXCR4 inhibitors, with better efficacies than the organosulfur compounds. Tigogenin from the saponin group was predicted to be a CCR5 inhibitor, while lupeol from the terpenoid group was predicted to be a CXCR4 inhibitor. These garlic compounds may be used to regulate both the CCR5 and CXCR4 signaling pathways to inhibit the proliferation, migration, and survival of cancer cells. However, further in vitro and in vivo studies should be conducted to validate the activities of garlic compounds and determine their efficacies to be used as primary compounds for the prevention of cancer progression.

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AUTHOR CONTRIBUTIONS

Balqis Balqis designed and conducting the experiments, perform analysis and interpretation, and wrote the manuscript. Betty Lukiati and Mohamad Amin assisted in conducting the experiments and make critical review. Siti Nur Arifah and Mochammad Fitri Atho'illah assisted in data collection and processing, data analysis or interpretation, and writing manuscript. Nashi Widodo designed and supervised all of the experiments and make critical review of the manuscript. All authors have read and approved of the final manuscript.

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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