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The Significance of Serine of NTD-NPC1L1 for Cholesterol Binding with Compounds in Traditional Spices

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***Abstract*— It has been proposed that Niemann-Pick C1 like-1 (NPC1L1) is an intermediate membrane protein that facilitates cholesterol absorption at enterocytes, yet it is still being unknown mechanism. The study aimed to evaluate the pharmacokinetics of anti-cholesterol in traditional spices and investigate the interactions between anti-cholesterol compounds and NPC1L1. This research analyzed binding pocket, Admet, drug-likeness, the interactions, and binding affinities by DoGSiteScorer and Depth, AdmetSAR tools, and MOE.2009 software, respectively. The interactions and binding affinities were determined by molecular docking between NPC1L1 and 18 ligands derived from spices such as Cinnamon, Bay leaf, coriander, Garlic, Red Onion, Tumeric, Indosenian Chilli Pepper. Inhibitors were docked with NPC1L1 (PDB ID: 3QNT), and a comparison was made between the results of Ezetimibe, a prescribed NPC1L1 inhibitor. The Lipinski Rule of Five aids in identifying drug-like compounds and those that are not. As an octanol–water partition coefficient log P not greater than 5, all ligand including Ezetimibe has a higher affinity for the aqueous phase. 11 out of 18 inhibitors were well absorbed and distributed by forecasting oral bioavailability. Quercetin, Curcumin, and 6-Gingerol from onion, turmeric, and ginger are the potential to inhibit cholesterol absorption in the small intestine. These three highest binding energy ligands (-14.0320 to -12.3998 kcal/mol) had high binding affinities as Ezetimibe (-15.5075 kcal/mol). High binding affinities of Ezetimibe and these ligands interact at almost in the exact locations of the N-terminal domain. Through Ser_102 of N-terminal domain NPC1L1 binding with the ligands, we suggest that traditional spices of three ligands could interfere with cholesterol absorption at the early stages.**

***Keywords*— Molecular docking; cholesterol inhibitor; hypercholesterolemia; N-terminal domain; NPC1L1.**

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I. INTRODUCTION

Cholesterol is a substrate for bile acids, vitamin D, and steroid hormones and an essential component of cell membranes [1]. The interrelation of the metabolic pathway between de novo production, intestinal absorption, and sterol excretion determines total cholesterol levels [2] **.** Also, more than 25 enzymes are required to synthesize cholesterol from acetyl coenzyme-A in a three-stage process in the cytoplasm and endoplasmic reticulum (ER) [3]. The sequences of enzymatic reactions through the mevalonate system converted acetyl-CoA to cholesterol in the endoplasmic reticulum. By converting HMG-CoA to mevalonate, the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase played an essential role in regulating this chain of reactions [4].

Cholesterol can also be obtained through ingestion of dietaries [5]. The Nieman-Pick type C1-like 1 (NPC1L1) protein absorbed dietary cholesterol across the small intestine [6]. Then released as chylomicron, from which cholesterol enters the liver [2]. Since few cells can metabolize cholesterol, secretion through the biliary is crucial to maintain homeostasis [7]. At the hepatocyte level, the heterodimer of the two-binding cassette (ABC) half-transporters G5 and G8 (ABCG5/G8) plays an essential role in hepatobiliary cholesterol secretion [7].

Despite cholesterol's crucial role, studies reported that high plasma cholesterol levels lead to serious illnesses, including atherosclerotic cardiovascular disease and stroke [3]. Pharmacological treatments have been demonstrated for hypercholesterolemia. Sets of studies encompassing 20 years, 1980 to 2010, in the United States, Canada, and Europe indicated that statin treatment and lifestyle intervention reduced total cholesterol levels [8].

Statins are well-known medications that help people with hypercholesterolemia lower their cholesterol levels. By inhibiting HMG-CoA reductase, a rate-limiting enzyme

involved in cholesterol biosynthesis, the lowering-cholesterol mechanism interferes with cholesterol synthesis [9]. Blocking the HMG-CoA reductase simultaneously lowers the cholesterol synthesis and intermediates cholesterol syntheses such as ubiquinone, geranylgeranyl pyrophosphate (GGPP), and farnesyl pyrophosphate (FPP) [10]. Due to the fundamental function of these intermediates, inhibition of their synthesis causes cell dysfunction and excuses statin's disruptive effect.

Statin-associated muscular symptoms (SAMS), which include fatigue, discomfort, muscle tenderness, and convulsions, are the most common side effects [11] . A study of 642 patients showed that more than 577 patients experienced statin-associated musculoskeletal symptoms [12]. The inability to tolerate statins due to muscular discomfort contributes to the uncontrolled cholesterol level [12]. Several studies also revealed that cardiovascular events are significantly recurrent despite patients being administered statins at high doses. In addition, in order to investigate additional lipid-modifying medications, high doses were used, which raised safety concerns [13].

To address these difficulties, Ezetimibe was developed as a novel medicine to treat hypercholesterolemia. Unlike statins, whose target is the HMG-CoA-R enzyme acting early in the cholesterol biosynthesis pathway, when it binds to the NPC1L1 receptor, Ezetimibe, a well-known cholesterol-lowering medication, reduces cholesterol absorption in the small intestine [14]. NPC1L1 is a polytopic transmembrane protein that functions as a cholesterol importer and regulates cholesterol absorption in the intestine [15]. NPC1L1 acts crucially in dietary and biliary cholesterol absorption by directly binding its N-terminal domain (NTD) to cholesterol. Cholesterol uptake is initiated by the formation and endocytosis of NPC1L1-flotillin-cholesterol membrane microdomain promoted by cholesterol. According to the findings, depletion of NTD's cholesterol-binding ability reduces the development of NPC1L1-flotillin-cholesterol membrane microdomains, impairing NPC1L1 endocytosis and cholesterol adsorption [16].

Previous studies in mice have reported that NPC1L1 played an essential role in intestinal cholesterol, and Ezetimibe has been proved to significantly reduce 70% intestinal cholesterol absorption by binding to the NPC1L1 [6]. However, a recent study in 2020 showed that Ezetimibe reduces cholesterol transport through occluding the tunnel in NPC1L1, which connects NTD to SSD, rather than competing with cholesterol binding (sterol sensing domain), consequently restraining the internalization of NPC1L1 [17]. Ezetimibe has now been commonly administered to lower blood cholesterol [18], also has efficacy and safety in reducing the risk of myocardial infarction and stroke, as well as cardiovascular mortality and risk of cancer [18], can lower LDL-cholesterol by 20 to 25% among patients treated with statins [19].

Although statins and Ezetimibe demonstrate different mechanisms in reducing cholesterol levels, both of them are drugs prescribed to treat hypercholesterolemia and coronary heart disease patients. However, consuming dietary containing cholesterol inhibitory constituents is recently considered a favorable additional lipid-lowering method.

In recent years, identifications of pharmacologically effective and low side effects bioactive compounds isolated from natural resources, particularly herbs and spices, to be used as curative medication and the food industry have shown significant growth. Research projects and scientific reviews have been conducted and documented to investigate the pharmacological properties of medicinal plants and their opportunity as phytomedicine [20]. Spices are one among four groups of MAPs, medicinal and aromatic plants, providing “medicines” to humans to counter diseases, maintain health, or cure illnesses [21] [22]. Since spices have been used as one of the primary ingredients in most cuisines in Indonesia [23], we focused our research on anti-cholesterol compounds in cooking spices.

Several studies proved that particular spices show bioactivity to lower cholesterol levels, such as onion containing quercetin, which has been significantly proven to lower serum and hepatic cholesterol in mice [24] [25]. Furthermore, turmeric containing Curcumin has been reported to lower LDL cholesterol in patients with cardiovascular risk [26]. Cinnamon is revealed to increase the HDL-C levels; and reduce fasting blood sugar levels, insulin, and bodyweight of mice due to its high cinnamaldehyde [27]. A previous study also showed that red pepper contains capsaicin and is capable of reducing cholesterol intestinal absorption and elevation of either cholesterol or triglyceride excretion in feces [28]. However, the cholesterol-lowering activities of Indonesian spices and herbs have not been studied thoroughly. Therefore, we conducted screenings on those dietary and spices that are the potential to inhibit cholesterol absorption in the small intestine are shown in Table 1. The proposed research aims to study the mechanisms of these cholesterol inhibitory compounds underlying cholesterol absorption in the small intestine targeting NPC1L1 binding interaction through molecular docking and evaluating their pharmacokinetic properties.

II. Materials and Method

A. Drug-Likeness Prediction (Lipinski’s Filter)

The rule's physicochemical parameters are as follows: 1) the number of hydrogen bond donors must be between 0 and 5; 2) the number of hydrogen bond acceptors must not exceed 10. The less aqueous phase partitioned into the lipid membrane by passive diffusion, the more hydrogen bonds there are. 3) A molecular weight of less than 500 is required. Because the concentration at the surface of the intestinal epithelium is lowered, the higher the molecular weight (MW), the lower the chemical absorption. 4) The logarithm of the octanol-water partition coefficient (LogP) must be less than or equal to five. Lipophilicity has been linked to LogP. 4) The logarithm of the octanol-water partition coefficient (LogP) has to be less than or equal to 5. LogP is linked to lipophilicity, which has been shown to have a major impact on absorption and permeability. High lipophilicity inhibits absorption via reducing molecule solubility in water. 5) The number of rotatable bonds must be less than ten [29] [30].

B. ADMET Prediction

This study determined the physicochemical properties of cholesterol-inhibitory compounds and control ligands using

Toxicity prediction and the ADMET descriptors algorithm. To predict ADMET parameters, AdmetSAR version 1.0 was used. Using the pKCSM server, ADMET characteristics such as cytochrome P450 2D6, human intestinal absorption (HIA), aqueous solubility, blood-brain barrier (BBB), and hepatotoxicity were calculated. In Toxicity prediction, Ames mutagenicity, aerobic biodegradability, developmental toxicity potentials, carcinogenicity, skin irritancy, and skin sensitization were used to estimate the toxicity parameter [31]. Lipinski's rule of five is well-known as a practical approach for determining drug-like characteristics and screening small compounds for druggability [32].

C. Molecular Docking

1) Binding Pocket preparation: The Protein Data Bank (PDB) database provided NPC1L1 (PDB ID: 3QNT) whose binding pockets were investigated. The investigation was performed using DoGSiteScorer [33], the DEPTH server , and Site Finder MOE 2009.10 software [34] [35]. DoGSiteScorer revealed the features of the potential detected pockets and the value of a surface area, volume, and druggability assessment score [33]. DEPTH is a user-friendly and suitable tool to detect drug-docking sites [34].

2) Preparation of Standard Ligand and NPC1L1 Structure: The NPC1L1 protein was stripped of water molecules and natural ligands (PDB ID: 3QNT). The current forcefield was employed as a partial charges' technique in the preparation of NPC1L1, and numerous required parameters such as hydrogen and lone pairs, H and LP, gradient 0.05, and forcefield partial charges computation were adjusted using MOE 2009.10 software [34] [35]. Ezetimibe was chosen as a positive ligand structure because earlier research has shown that it can bind to NPC1L1 [14]. Ezetimibe's ligand structure was acquired from the PubChem database and the ligand was optimized using the MOE2009.10 software [36].

TABLE I
SPICES AND THEIR CHOLESTEROL INHIBITORY COMPOUNDS

Sources	Name of Compounds	References
Cinnamon	Cinnamaldehyde	[27]
Bay leaf (*Laurus nobilis)*	1,8-cineole	[37], [38]
coriander (*Coriandrum sativum)*	Linalool	[39], [40]
Garlic (*Allium sativum*)	S-allyl cysteine (SAC	[41]
	S-ethylcysteine (SEC)	[42]
	S-propylcysteine (SPC)	[42]
Red Onion	Quercetin	[43]
Turmeric (*Curcuma longa*)	Curcumin	[44]
Indonesian Chilli Pepper (*Capsicum Annuum Linn*)	Capsaicin	[45], [46] [47]
	Dihydrocapsaicin,	
	Nordihydrocapsaicin	
Black pepper (*Piper nigrum*)	Piperine	[48]
lemon grass (*Cymbopogon citratus)*	Citral	[49]
Ginger (*Zingiber officinale)*	6-Gingerol	[50], [51]

3) Docking: The interaction of proteins and ligands is used in molecular docking as a method to determine the preferred orientation of the two molecules. The MOE2009.10 program was used to complete this procedure, which included optimization of numerous parameters such as the triangle matcher with 2500000 iterations for placement, 100 repetitions for the initial retain, and force field refining, as well as one-time rescoring. dG London [35] [36].

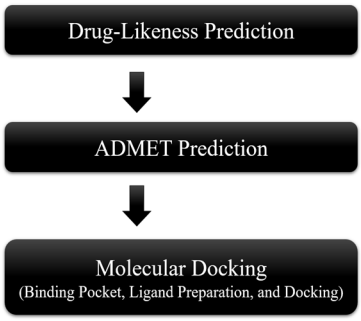

Fig. 1 Research Method Flow Chart

III. RESULTS AND DISCUSSION

A. Drug-Likeness Prediction

A promising drug candidate must be well absorbed and distributed throughout the system. By forecasting oral bioavailability, Lipinski's rule of five is often used to design and develop prospective medications [52]. Lipinski's rule of five states that When the H-bond donors are between 0 and 5, a drug or candidate molecule is expected to be maximally absorbed or penetrated, the number of H-bond acceptors must not exceed 10, and the molecular weight must not be less than 500, and the computed Log P (cLog P) must be larger than 5 [53]. In correlation with drug permeability and flexibility, two more restrictions have been added by advanced research; topological polar surface area (PSA) should be not greater than 140 À, and rotatable bonds should be less than 10 [54]. To evaluate the drug-likeness properties, 19 compounds were filtered using AdmetSAR) to meet Lipinski's rule of five.

Lipinski's five-tool rule determines molecular weight and lipophilicity (expressed as LogP), hydrogen bond donors and acceptors, and rotatable bonds. Table 2 depicts the drug-likeness properties of 19 compounds of spices predicted using AdmetSAR. The molecular weight of compound (2) saponin was way bigger than 500. Thus, it was poorly absorbed. A negative LogP value implies that the compound has a higher affinity for the aqueous phase, meaning that compounds have a higher affinity for the aqueous phase (2), (5), (6), (7), (8), (9), and (10) (in yellow-marked-compounds) have a higher solubility in aqueous. However, the green-marked compounds are ones with higher TPSA (Topological Polar Surface Area) and are associated with lower membrane permeability. 15 out of 19 screened compounds complied due to their high bioavailability. They are projected to function as an orally delivered drug-like molecule according to Lipinski's rule of five. Supplementary are shown in Table 3 details the ADMET analysis of fourteen metabolites.

B. ADMET Prediction

Compounds expressing optimal absorption level, excellent solubility, optimum BBB penetrability, non-hepatotoxicity,

and non-inhibitory properties were preferred as promising druggable compounds [55]. Table 3 shows the detail of the ADMET analysis of fourteen ligands. To investigate the ligands' absorption performance in the small intestine, we assessed the HIA and Caco-2 permeability. There are 6 out of 14 ligands showed good intestinal absorption. Ligands (1), (3-4), (16-18), and control were significantly absorbed, while (1), (5-15) were poorly absorbed in the human small intestine (HIA). The greater the HIA score, the better adsorption in the intestine, where an orally administered drug's major absorption occurs [54]. In this study, we found that compound (3), 1,8-cineole, has the greatest HIA score of all fourteen compounds and control. The capacity to traverse monolayers of the human colon cancer cell line Caco-2 permeability is used to assess human intestine medication absorption, Caco-2.

A compound with Papp more than $8x10^{-6}$ cm/s is considered to have a high Caco2-2 permeability value [56]. All ligands were not able to present high Caco-2 permeability. However, ligands (3-4) and (13-17) and control are considered to have moderately good Caco-2 permeability. To estimate the level of drug distribution, volume distribution and BBB permeability are assessed. The volume of distribution (Vd) is a measurement of the ratio of the total amount of drug in the body to the drug's plasma concentration at a given time. It suggested the tendency of drug movement between the plasma and tissue compartments. A high Vd-drug requires a higher dose of the drug in order to meet a stated plasma concentration due to its propensity to leave the plasma. Hydrophilic molecules prefer to persist in the bloodstream to distribute to adipose containing an abundance of lipid and therefore have a lower Vd.

TABLE II
DRUG-LIKENESS PREDICTION BY LIPINSKI'S RULE OF FIVE

No	Ligands	Molecular weight	Log P	TPSA	Rotatable bonds	HB Donors	HB Acceptors
1	Cinnamaldehyde	132.16	1.90	17.07	2	0	1
2	Saponin	1223.35	-2.67	422.05	14	15	27
3	1,8-Cineole	154.25	2.74	9.23	0	0	1
4	Linalool	154.25	2.97	20.23	4	1	1
5	S-Allylcysteine	161.22	-2.07	88.62	5	2	3
6	S-ethylcysteine	149.21	-2.35	88.62	4	2	3
7	S-propylcysteine	163.24	-1.82	88.62	5	2	3
8	Gamma-glutamyl-s-Allylcysteine	290.34	-2.84	155.02	11	4	6
9	Gamma-glutamyl-s-methylcysteine	264.30	-3.48	155.02	9	4	6
10	Gamma-glutamyl-s-Propylcysteine	292.35	-2.59	155.02	11	4	6
11	Quercetin	302.24	1.54	131.36	1	5	7
12	Curcumin	368.38	3.20	93.06	8	2	6
13	Capsaicin	305.41	3.58	58.56	10	2	3
14	Dihydrocapsaicin	307.43	4.44	58.56	11	2	3
15	Nordihydrocapsaicin	293.40	3.23	58.56	10	2	3
16	Piperine	285.34	3.46	38.77	4	0	3
17	Citral	152.23	3.03	17.07	4	0	1
18	6-Gingerol	294.39	2.76	66.76	10	2	4
C	Ezetimibe	409.43	4.89	60.77	6	2	3

When Vd is less than 1 L/kg (log Vd -0.15), it is considered lower, and when it is more significant than 2.81 L/kg (Log Vd>0.45), it is considered higher [56]. The Vd value for all ligands was less than 0.71 L/kg, except for compound (11). Most medications are unable to enter the brain because of the blood-brain barrier (BBB) [57]. Cytochrome P450 is a crucial enzyme in drug metabolism and is prevalent in the liver. CYP1A2, CYP2C9, and CYP3A4 are three primary P450 isoforms regulating drug metabolism in the small intestine [58].

This study depicted that ligand (3-7) and (16-17) acted as a non-inhibitory compound of CYP1A2, CYP2C9, and CYP3A4. Whereas ligands (1) and (11) did not inhibit CYP2C9 and CYP3A4, and ligands (13-15) and (18) only exhibited non-inhibitory compounds of CYP3A4. Our control ligand, Ezetimibe, showed the non-inhibitory property to CYP1A2 only. AMES toxicity test is utilized to investigate mutagenicity in compounds [54].

The test ligands demonstrated non-mutagenic properties proved by a negative AMES toxicity test, similar to Ezetimibe. LD50 dose in a rat model derived from admetSAR revealed the lethality dose of a toxin. The lower dose of LD50, the more lethal the compound and vice versa. We found in this study that compound no.4 (linalool) was the most toxic among the test ligands, indicated by the lowest LD50 of 1.740. Compound no.16 (piperine) had the highest LD50 dose among all the test ligands and 0.64 points higher than the control ezetimibe (2.81 versus 2.17, respectively).

The other two ligands that also had higher LD50 doses compared to the Ezetimibe were compound no.1 (cinnamaldehyde) and no.11 (quercetin) of 2.48 and 2.47, respectively. The total clearance of ligands (3), (13-15), and (18) is higher than that of (1), (4-7), (11-12), (16-17), and control. For toxicity prediction, of all fourteen ligands, only ligands (13,14,16) and control were determined to likely yield hepatotoxicity. Of all six well-adsorbed ligands in the small intestine, only 4 had relatively good Caco-2 permeability, three ligands could cross BBB, six ligands had a lower Vd, and ligand (18) inhibited CYP1A2 and CYP2C9.

C. Molecular Docking

Molecular docking techniques were applied in this study to determine the interaction between NPC1L1 and fifteen molecules using MOE.2009 software. The interactions between these compounds and target proteins with homologous active site amino acid residues are depicted in

Table 4. The results showing that the higher binding energy affinity ligands have a hydrogen bond with NPC1L1 in Table 4 may provide compelling evidence that ligands bind near the N terminus of NPC1L1.

TABLE III
ADMET PREDICTION OF FOURTEEN LIGANDS AND CONTROL

		Ligands														
	Parameters	1	3	4	5	6	7	11	12	13	14	15	16	17	18	Control
Absorption	Water solubility (log mol/L)	-2.175	-2.63	-2.612	-2.888	-2.887	-2.888	-2.925	-4.01	-4.185	-4.321	-3.919	-3.464	-3.377	-3.164	-5.288
	Caco2 permeability (log Papp 10-6 cm/s)	1.634	1.485	1.493	0.704	0.65	0.7	-0.229	-0.093	1.364	1.375	1.357	1.596	1.504	0.94	1.641
	Intestinal absorption (% absorbed)	95.015	96.505	93.163	79.971	81.604	79.978	77.207	82.19	90.075	89.568	1.357	94.444	95.317	92.416	91.013
Distribution	VDss (Human, log L/Kg)	0.266	0.491	0.152	-0.561	-0.56	-0.562	1.559	-0.215	0.391	0.435	0.393	0.158	0.166	0.524	-0.436
	BBB Permeability (logBB)	0.436	0.368	0.598	-0.277	-0.326	-0.283	-1.098	-0.562	-0.241	-0.268	-0.225	-0.102	0.626	-0.77	-0.017
Metabolism	CYP1A2 inhibitor	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No
	CYP2C9 inhibitor	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes
	CYP3A4 inhibitor	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	Yes
Excretion	Renal OCT2 substrate clearance	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No
	Total Clearance (logml/min/kg)	0.203	1.009	0.446	0.591	0.544	0.591	0.407	-0.002	1.298	1.245	1.215	0.232	0.376	1.339	-0.334
Toxicity	Ames Toxicity	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Hepatotoxicity	No	No	No	No	No	No	No	No	Yes	Yes	No	Yes	No	No	Yes
	Rat Oral Toxicity (LD50)	1.88	2.01	1.704	2.02	2.008	2.021	2.471	1.833	2.065	2.091	2.067	2.811	1.815	1.958	2.172

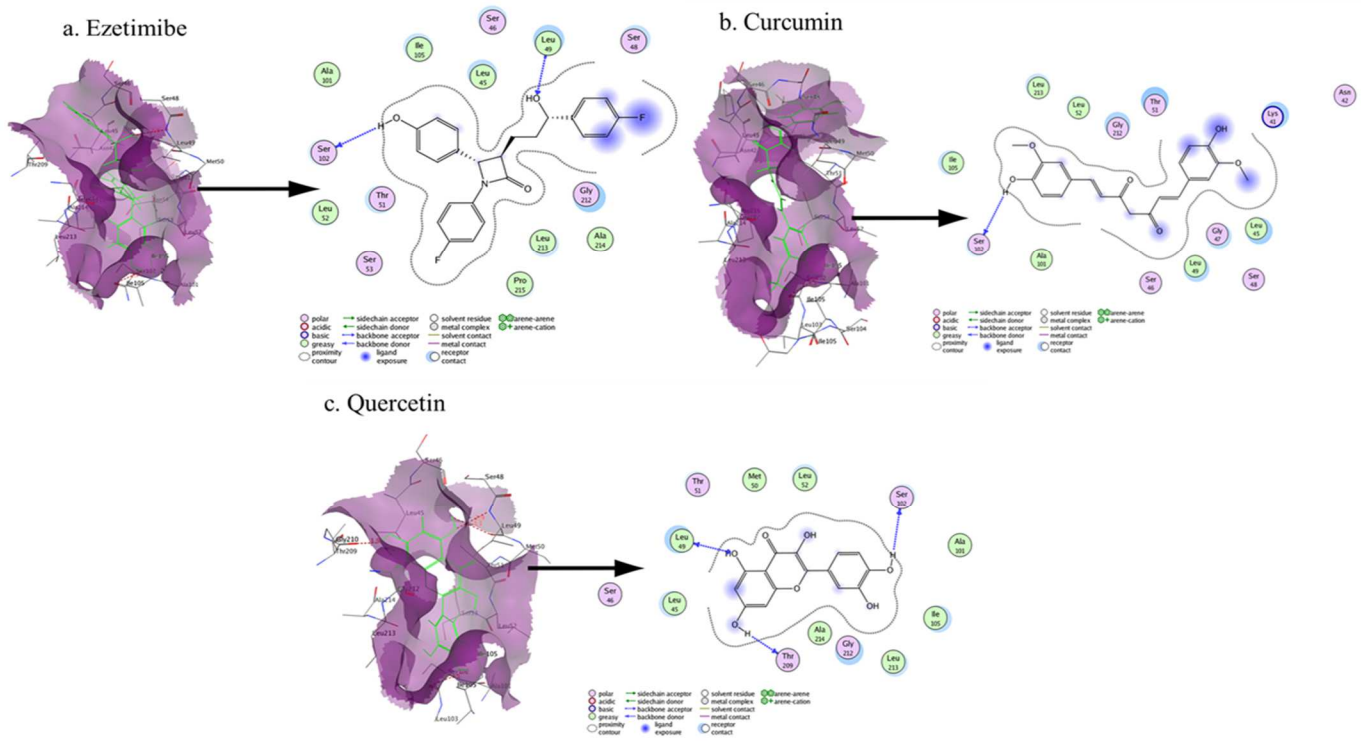

Fig. 2 Docking of Three Lowest Binding Energy Compounds With 3QNT Protein

TABLE IV
SPICES AND THEIR CHOLESTEROL INHIBITORY COMPOUNDS ANALYSIS

No	Ligand	Binding energy	Residues involved	
			Hydrogen bond (Distance A^0)	Hydrophobic interaction
1	Cinnamaldehyde	-7.2197	-	-
3	1,8-Cineole	-7.8122	LEU_52 (3.4)	-
4	Linalool	-8.9817	LEU_213 (1.7)	-
5	S-Allylcysteine	-9.9760	SER_98 (2.0), GLU_38 (2.2), LYS_94 (2.8)	-
6	S-Ethylcysteine	-9.6814	SER_98 (2.0), GLU_38 (2.2), LYS_94 (2.8)	-
7	S-Propylcysteine	-9.9379	SER_98 (2.0), GLU_38 (2.2), LYS_94 (2.8)	-
11	Quercetin	-14.0320	SER_48 (2.9), LEU_49 (3.1), SER_102 (3.1), THR_209 (1.9)	-
12	Curcumin	-13.4514	SER_102 (3.3)	-
13	Capsaicin	-11.4263	-	-
14	Dihydrocapsaicin	-11.3797	SER_102 (3.0)	-
15	Nordihydrocapsaicin	-11.0796	-	-
16	Piperine	-10.8380	-	-
17	Citral	-8.4666	-	-
18	6-Gingerol	-12.3998	LEU_49 (2.9), SER_48 (3.3), MET_50 (2.1)	-
Control	Ezetimibe	-15.5075	LEU_49 (2.9), SER_102 (2.7)	-

The closest homolog of NPC1L1 in the sterol-sensing domain (SSD)-containing proteins suggests that cholesterol binds to NPC1L1's N-terminal region, according to a recent study [59]. Molecular docking analysis showed a correlation between hydrophobicity and polarity of NPC1L1 and the hydrogen bond of the ligand. Ligands have lower binding energy and likely bind to the polar amino acids such as serin and the hydrophobic amino acids at the pocket in Table 4. This finding implies that hydrophobicity and polarity of the pockets determine the interaction between ligand and pocket and follows the previous research [35]. Hydrophobic interactions and hydrogen bonding are essential factors in determining the binding affinity and stability of protein-ligand complexes in molecular docking [60] [61]. Finding the best affinity with the lowest binding energy among the test compounds was important. To better understand the molecule binding interactions, a molecular docking model was conducted at the active site of the receptor (PDB ID: 3QNT) between the receptor-ligands interaction. The binding affinities of fifteen compounds range from −7.2197 to −15.5075 kcal/mol; meanwhile, the binding affinity for Ezetimibe (control ligand) was −15.5075 kcal/mol. Three ligands with better binding affinities were selected for in-depth molecular docking analysis in Fig. 1. The control ligand displayed the strongest binding affinity to receptor quercetin, and the other four lowest-binding energy compounds such as Curcumin, 6-gingerol, capsaicin, and dihydrocapsaicin could be highly selective cholesterol inhibitors in theory.

According to previous research, Ezetimibe binds to an extracellular site distinct from where cholesterol binds to block conformational changes in NPC1L1 that are necessary for cholesterol translocation across the membrane. Ezetimibe binding to NPC1L1 required a loop in the MLD, and Phe 532 and Met 543 appear to be crucial contributors [62]. However, our findings show that Ser_102 and Leu_49 are two amino acids in ligand-NPC1L1 interaction. In line with the specific amino acid binding obtained from ezetimibe-NPC1L1 interaction, other ligands show that Ser-102 is dominant in interacting with the ligand Fig. 1. Additionally, the ligands attach to the tunnel's center and prevent NPC1L1 from absorbing cholesterol [17].

The binding is formed on the active site via H-bonding. Quercetin formed four hydrogen bonds with SER_48, Leu_49, Ser_102, and Thr_209. 6-gingerol formed three hydrogen bond interactions with Leu_49 (2.9), Ser_48 (3.3), Met_50 (2.1). Curcumin formed a single hydrogen bond with Ser_102. Dihydrocapsaicin formed one hydrogen bond interaction with Ser_102. SER of NPC1L1 is almost always present at the ligands with low-affinity binding. Every ligand with a low affinity for binding is expected to interact with Ser of NPC1L1. Moreover, Ser was also involved in the gating mechanism of the cholesterol-binding site of the N-terminal domain of NPC1L1, which contributed to the conformational shift of the NPC1L1 catalytic site [63]. Taken together, Ser 102 of NPC1L1 considered to play an important function in binding ligands to the NPC1L1 domain.

IV. CONCLUSION

In conclusion, Quercetin, Curcumin, and 6-Gingerol from onion, turmeric, and ginger are the potential to inhibit cholesterol absorption in the small intestine. These three highest binding energy ligands (-14.0320 to -12.3998 kcal/mol) had high binding affinities as Ezetimibe (-15.5075 kcal/mol). High binding affinities of Ezetimibe and these ligands interact at almost in the exact locations of the N-terminal domain. Through Ser_102 of N-terminal domain NPC1L1 binding with the ligands, we suggest that the three ligands from traditional spices could interfere the cholesterol absorption at the early stages.

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