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Research Article

GC-MS Analysis and In-silico Molecular Docking study of Skin Fruit Arabica Coffee (*Coffea arabica* L.) Methanol Extract as Mosquito Repellent

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ABSTRACT
Malaria is an endemic disease that is still a problem globally, especially in countries with tropical and subtropical climates. People generally use mosquito repellents from synthetic materials, but they still cause effects and toxicity. Using active compounds from plants is an alternative to developing mosquito repellents. The 30 active compounds from the GC-MS analysis of methanol extract of Arabica coffee skin fruit and we selected the highest percentage compound, namely n-Hexadecanoic acid, Caffeine, Hexadecanoic acid methyl ester, 3-O-Methyl-d-glucose and desulphosinigrin. The highest compounds were carried out by molecular docking with Odorant binding protein 1 (OBP1) as the protein target and N, N-Diethyl-3-methylbenzamide (DEET) as the native ligand. Desulphosinigrin has the highest binding affinity, which is -6.2 Kcal/mol, close to the native ligand DEET. It can be concluded that the active compound desulphosinigrin has the potential as a repellent. This study concludes that active compounds from the methanol extract of Arabica coffee skin have the potential as a repellent.

Key words: *Anopheles sp; Coffea arabica L; Coffee Skin Fruit; Malaria; Repellent.*

Introduction

Malaria is a disease caused by the bite of female *Anopheles* sp, and it is still a problem in the world, especially in tropical and subtropical countries. According to World Health Organization (2019), 299 billion cases are infected by malaria. Indonesia is the highest country, especially in Papua, exceeding 75% have been reported (Alim et al., 2020). Malaria can be prevented by controlling the malaria vector, repellent, biological control, and environmental management (WHO, 2011). Preventing malaria transmission is done with vector control (*Anopheles sp*) and has been used as a mosquito repellent made from the synthetic ingredient N, N-Diethyl-3-methylbenzamide (DEET). However, it still has side effects (Kartini et al., 2020).

Female mosquitoes bite humans because of a response to odour molecules emanating from human skin and sweat. Odorant Binding Protein (OBP1) is expressed by special cells excreted into the lymphatic fluid in the olfactory dendrite (White et al., 2009). Murphy et al. (2013) reported that natural repellent 6-methyl-5-heptene-2-one (6-MH) binds to OBP1 at the

same site of N, N-Diethyl-3-methylbenzamide (DEET), as a native ligand.

The skin fruit of Arabica coffee has many active compounds, including flavonoids, polyphenols, tannins, caffeine, and chlorogenic acid. The active compounds have potential effects as anti-inflammatory and antibacterial (Masruri et al., 2019). The polyphenols found in the arabica coffee skin fruit are used in health as antioxidants and stress oxidative (Geremu et al., 2016). Studying the active compounds of methanol extract from skin fruit arabica coffee and analysis of virtual screenings is the first step to discovering structure-based drugs with knowledge of ligand interactions. This study aims to determine the potential active component found in the methanol extract of skin fruit arabica coffee, with the goal of determining how effective it is as a mosquito repellent.

Materials and Methods

Equipment and materials for extraction of skin fruit of Arabica coffee include methanol 98%, blenders, and analytical balances. The molecular docking study uses tools and materials, including Computers (Lenovo Ideapad), Microsoft Windows 10 Home 64-bit, AutoDock Vina 1.1.2, Open Babel, and BIOVIA Discovery Studio. 2020 and LigPlot+. Arabica Coffee Fruit Skin, The 3D structure of the OBP1 protein (PDB ID 6LU7) and the 3D structure of the GC-MS compound results were downloaded from the PubChem Compound page (https://pubchem.ncbi.nlm.nih.gov/).

Collection and extraction of samples

Arabica coffee (*Coffea arabica* L.) was collected from Coffee plantation farmers in Jambi (Sumatra Island, Indonesia). The plant carried out determinations at the Laboratorium Biology Research of Ahmad Dahlan University with 186/Lab.Bio/B/VI/2021. Fruit Arabica coffee was washed with clean water, then skin fruit and coffee beans were separated. The skin of arabica coffee is dried for seven days and extraction with methanol for five days. The filtrate from the extraction is evaporated using a rotary evaporator.

GC-MS Analysis

The methanol extract of skin fruit arabica coffee was analyzed by GC-MS using the Thermo scientific trace 1310 gas chromatography and single quadrupole mass spectrometer. The operating conditions of the column are as follows: ion source temperature 200ºC and interface temperature 250ºC. The temperature of a column oven is 60ºC, and the injection temperature is 260ºC. The carrying gas used is helium (99.99%); the total flow is 50 ml/ minute, and the column flow is 3 ml/ minute. The whole time running the GC-MS program is 60 minutes.

Molecular Docking Studies

The 3D structure of the receptor Odorant Binding Protein 1 (OBP1) has been downloaded from PDB ID 3N7H. The validation method used redocking with binding the origin ligand N, N-Diethyl-3-methylbenzamide (DEET) to determine the dimensions and position of the grid box according to the active site. Ligand preparation can be obtained from databases Pubchem page in format .sdf. The evaluation of the suitability of pharmacological drugs orally by Lipinski's five rules. The test must comply with the rules: the molecular weight of 500 Daltons, the log is not more than five, the donor is not more than five, the number of hydrogen acceptors is more than ten, and the molar activity is between 40-130. Molecular docking studies were performed using AutoDock Vina software with a grid box size of 30x30x30 A, and the positions were x: 14, 123, y: 4,313, and z: 14,093. The binding affinity value of the docking was recorded, and the structure of the docking result in each ligand was visualized to determine the interaction between protein and ligand.

Results and Discussion

GC-MS analysis.

The Gas Chromatograph-Mass spectrophotometry (GC-MS) identified 30 compounds active from the methanol extract of skin fruit arabica coffee Figure 1 and Table 1. The results of the GC-MS chromatogram presented that Desulphosinigrin has the highest percentage compound in this extract. The other active compound was Caffeine, n-Hexadecanoic acid, Hexadecanoic acid-methyl ester and 3-O-Methyl-d -glucose. Based on the previous study, Coffee Pulp Extracts (CPE) from skin fruit of arabica coffee, with preparation washing, removing, drying, and blending, had active compounds of quinic acid, malic acid, chlorogenic acid, and caffeine. The most prominent in CPE were chlorogenic acid and caffeine (Duangjai et al., 2016).

Coffee pulp or coffee husk has the potential as a substrate in bioprocesses and has been used for biogas, fish feed, enzyme production, aromatherapy, and mushroom production (Pandey et al., 2000). Pulp of Coffea arabica also contains pectin that can be used to manufacture gels (Reichenbach et al., 2020). Many studies have reported using the chemical content of the Pulp of coffee arabica in the health sector. Coffee pulp extracts have potential as antimicrobials against *S. aureus, S. epidermidis, P. aeruginosa, and E. coli* (Duangjai et al., 2016).

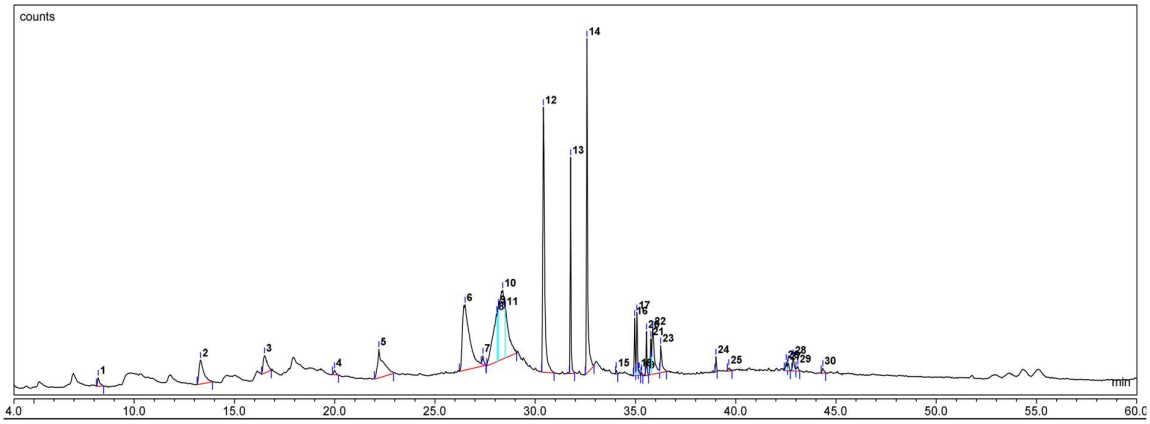


Figure 1. Chromatogram of GC-MS analysis of the methanol extract of arabica coffee fruit skin.

Molecular Docking Studies

In the in-silico studies, molecular docking was used to analyze the active compound of coffee fruit skin extract as the active compound has the potential as a mosquito repellent, Anopheles sp. Repellent is an alternative insecticide that can protect the skin from mosquito bites. As we know, repellents are made of synthetic chemicals that cause allergies and respiratory problems. The N-Diethyl-3-methylbenzamide (DEET) was reported to have the ability to inhibit A. gambiae mosquitoes with an inhibition rate of 88–7%–92.5% (Nathan et al., 2005). DEET interacts directly with the olfactory receptor (*odorant receptor*) in Anopheles sp. The Odorant binding protein 1 (OBP 1) is the alternative structure-based design of mosquito repellent, which is more effective because it targets the olfactory ligand. Odorant-binding proteins (OBPs), which influence the chemosensory system and behavioral responses, are essential to understanding the population structure and developing effective control measures against this vector. The OBP1 is host-specific and attracts oviposition by malaria vectors. The perception of odor in insects occurs mainly in the olfactory senses. In this

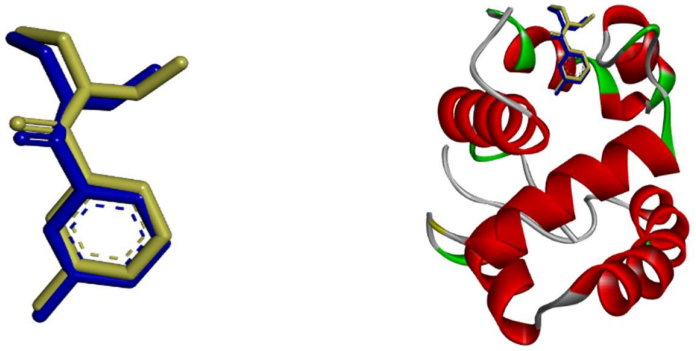
specialized structure, OBP1 expressed by specialized supporting cells is secreted into the lymph fluid surrounding the olfactory dendrites (Murphy et al., 2013).

Table 1. Active compounds of the methanol extract of arabica coffee fruit skin.

Name	Chemical formula	Molecular weight	Retention time (min)	Relative Area (%)
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	8.21	0.59
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	13.31	3.68
Isosorbide Dinitrate	$C_6H_8N_2O_8$	236	16.5	2.21
l-Gala-l-ido-octonic lactone	$C_8H_{14}O_8$	238	19.99	0.31
Melezitose	$C_{18}H_{32}O_{16}$	504	22.2	5.34
Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	26.5	15.49
Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	27.39	0.42
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	28.09	6.63
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	28.16	2.03
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	28.38	11.57
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	28.51	5.63
Caffeine	$C_8H_{10}N_4O_2$	194	30.41	14.82
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	31.77	5.49
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	32.58	12.38
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	34.04	0.12
Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294	34.96	1.38
11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	35.07	1.78
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	35.18	0.18
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	$C_{28}H_{44}O_4$	444	35.3	0.09
Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	35.55	1.14
Linoelaidic acid	$C_{18}H_{32}O_2$	280	35.76	1.21
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	35.86	3.47
Octadecanoic acid	$C_{18}H_{36}O_2$	284	36.26	1.47
Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	39.01	0.42
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	39.65	0.22
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	42.51	0.40
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	42.62	0.29
1H-Cyclopropa [3,4]	$C_{26}H_{36}O_8$	476	42.86	0.71
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	43.1	0.27
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	44.35	0.27

Table 2 showed that the five highest compound structures (https://pubchem.ncbi.nlm.nih.gov) were docked to the OBP1 protein target and identified the amino acid interactions. The validation method was confirmed by re-docking the OBP1 structure to its original ligand, DEET. The redocking process is carried out to test appropriate and efficient docking procedures or steps (Shivanika et

al., 2022). The re-docking process on DEET compounds against the OBP1 protein showed a Root-Mean-Square Deviation (RMSD) value of 0.498. This RMSD value indicates that the redocking process can be used to perform molecular docking of other test ligands. According to Muleme et al. (2009), a program that can produce the same pose in the redocking process with an RMSD value at the best conformation of 1.5–2 can be said to be successful. Figure 2 presents DEET and co-crystals, which are located on the same side and attached to the binding site, and the binding site shows the location of the best redocking pose displayed on the red binding site.



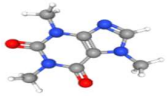
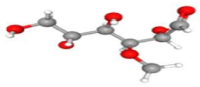
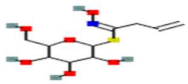
a b

Figure 2. Redocking co-crystal DEET into the binding site of the OBP1 protein. The DEET co-crystal conformation is shown in yellow carbon (a) and the best-redocked pose (b).

AutoDock Vina program performed the molecular docking of OBP1 protein PDB ID 3N7H with the five highest compounds from the methanol extract of Arabica coffee fruit skin and compared it to DEET. The molecular docking results are shown in Table 3, and there is the value of binding affinity and interactions between the test compound and amino acids on the active site of the OBP1 protein.

DEET is a native ligand of OPB1 and has a binding affinity value of -7.5 Kcal/mol. Desulphosinigrin has the lowest binding affinity with OBP1 compared to the other four compounds (-6.2 Kcal/mol) (Table 3). The binding affinity of Desulphosinigrin is close to the native ligand of OBP1 (-7,5 Kcal/mol). A binding affinity value indicates a better ability to bind and inhibit protein activity. In this study, Desulphosinigrin has the potential to inhibit the OBP1 protein compare to the other active compounds. Hydrogen bonding and hydrophobic interactions simultaneously can also increase the stability of the ligand at the binding site and increase binding affinity and drug efficacy (Varma et al., 2010). The interaction between DEET with amino acids on the protein's active site only shows hydrophobic interactions, and no hydrogen bonds are formed. Meanwhile, Desulphosinigrin, Hexadecanoic acid compounds, and methyl esters showed more stable interaction with the presence of hydrogen bonds in the amino acid. Desulphosinigrin has hydrogen bonds with Phe123, and Hexadecanoic acid-methyl ester has hydrogen bonds with Ser79 and 3-O-Methyl-d-glucose compounds, which formed hydrogen bonds with the amino acid residues Leu73, Ala88, and Trp114 Figure 3.

Table 2. The highest percentage active compound structure of the methanol extract of pulp *Coffea arabica* L. is coffee skin.

Name	Canonical SMILES	Structure
n-Hexadecanoic acid	CCCCCCCCCCCCCCCC(=O)O	
Caffeine	CN1C=NC2=C1C(=O)N(C(=O)N2C)C	
Hexadecanoic acid, methyl ester	CCCCCCCCCCCCCCCC(=O)OC	
3-O-Methyl-d-glucose	CCCCCCCCCCCCCCCC(=O)OC	
Desulphosinigrin	C=CCC(=NO)SC1C(C(C(C(O1)CO)O)O)O	

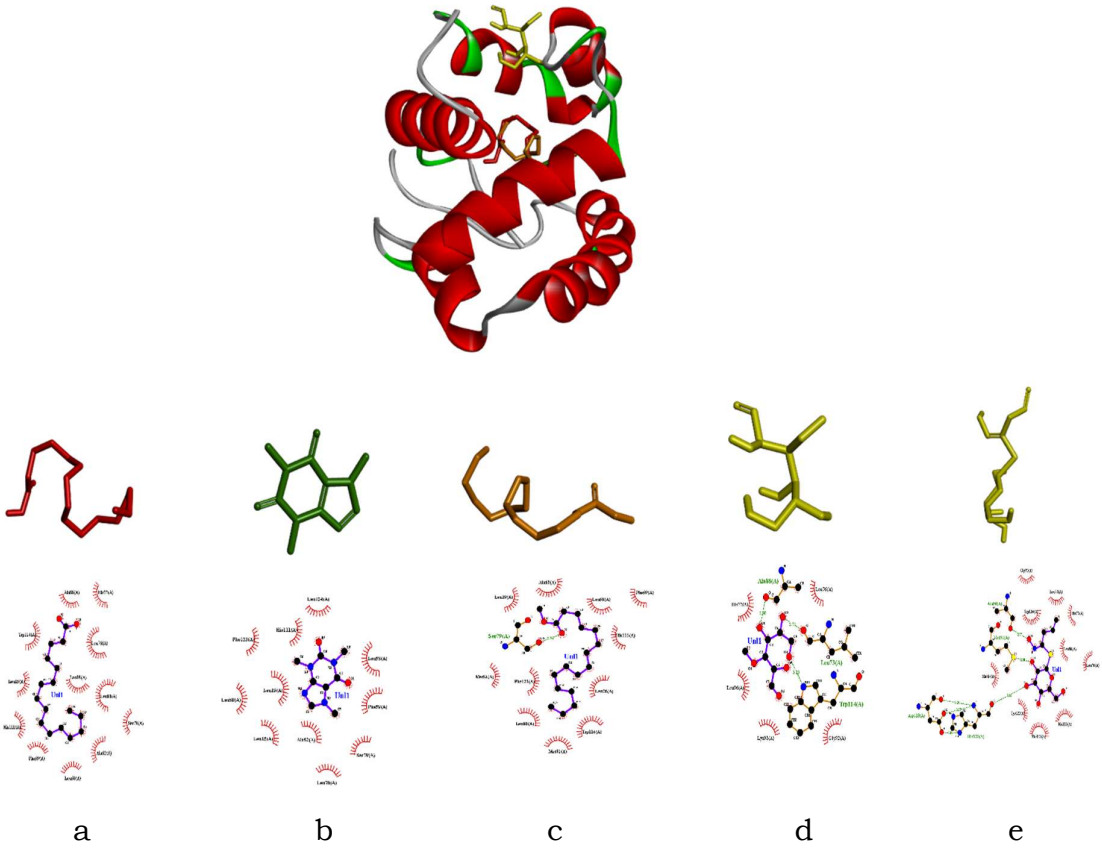


Figure 3. Binding pose of the five active compounds in the binding site on OBP1 protein: N-hexadecenoic acid (a), caffeine (b), hexadecanoic acid-methyl ester (c), 3-O-Methyl-d-glucose (d), and desulphosinigrin (d).

Table 3. Result of molecular docking simulation ligand against OBP1 protein

Ligand	Binding affinity (kcal/mol)	Residues of Amino acid involved in interactions and distance (A)	
		Interactions of Hydrophobic	Hydrogen binding
DEET (Native ligand OBP1 3N7H)	-7,5	Leu73, Leu76, His77, Leu80, Ala88, Met89, Met91, Gly92, Lys93, Leu96, Trp114	
n-Hexadecanoic acid	-5.8	Leu 15, Leu19, Leu58, Phe59, Ala61, Leu76, His77, Ser79, Leu80, Ala88, His111, Trp114	
Caffeine	-5.4	Leu15, Leu19, Leu58, Phe59, Ala62, Leu76, Ser79, Leu80, His111, Phe123, Leu124	
Hexadecanoic acid, methyl ester	-6.1	Leu19, Leu58, Phe59, Ala62, Leu76, Leu80, Met84, Met91, His111, Trp114, Phe123	Ser79
3-O-Methyl-d-glucose	-5	Leu76, His77, Gly92, Lys93, Leu96	Leu73, Ala88, Trp114
Desulphosinigrin	-6.2	Leu19, Leu58, Phe59, Ala62, Val64, Leu76, Ala88, Met91, Trp114, Tyr122	Phe123

The amount of hydrogen bonds shows the binding between amino acid residues and ligands in the interaction. The previous study reported that the oil of Syzygium aromaticum contains the active compound Eugenyl acetate, which can be used as a better repellent than DEET. These compounds form H-bonds with Arg94 and water molecules. Eugenyl acetate remains stable when bound to OBP1 Anopheles gambiae (Affonso et al., 2013). Moreover, the complex formation compound OBP1 with icaridin is an alternative to DEET. Icaridin has a significantly higher affinity for the OBP1 protein because of the higher molecular mass and the specificity of its interaction with OBP1 Anopheles gambiae (Ghavami et al., 2020).

Conclusions

Pulp *Coffea arabica* L. methanol extract contains 30 active compounds, with Desulphosinigrin, Caffeine, n-Hexadecanoic acid, Hexadecanoic acid-methyl ester, and 3-O-Methyl-d-glucose having highest percentage compound. Desulphosinigrin forms hydrophobicity and hydrogen interactions with the OBP1 target protein used in-silico study. Pulp *Coffea arabica* L. methanol extract compounds have the potential as a repellent.

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