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Original Research Article

Screening *Carica Papaya* Compounds as an Antimalarial Agent: *In Silico* Study

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ABSTRACT

Malaria is a highly prevalent infectious disease caused by the Plasmodium parasite transmitted through *Anopheles* mosquitoes, which poses a significant public health challenge worldwide, including in Indonesia. Therefore, a study was conducted to identify potential drug compounds from the *Carica papaya* plant that could inhibit various antimalarial proteins or receptors, such as *Plasmodium falciparum* DXR reductase complex with fosmidomycin, Plasmepsin V *Plasmodium vivax*, *P. falciparum* dihydroorotate dehydrogenase, *P. falciparum* hexose transporter, *P. falciparum* protein kinase 5, and *P. falciparum* dihydrofolate reductase-thymidylate synthase. The researchers used the Pyrx application to dock the *C. papaya* compounds with the targeted antimalarial proteins to determine the binding affinity values. Additionally, they used the Yasara dynamics application to conduct molecular dynamics simulation to ensure the stability of the bonds formed between the ligands and proteins. The results showed that 14 compounds found in *C. papaya*, particularly flavonoids and terpenoids, had the potential to inhibit the six antimalarial proteins with the lowest binding affinity values. Furthermore, the molecular dynamics simulation on 6M20 and 1V0P proteins indicated that the compounds effectively inhibited Plasmodium proteins, as they had an RMSD value below 2.5 Angstrom. The study suggests that *C. papaya* could be a potential source of antimalarial compounds, which could be developed into new drugs to combat this disease.

Keywords: Antimalaria, *Carica papaya*, Molecular Docking, Molecular Dynamic

Introduction

Malaria is a widespread infectious disease that affects many people worldwide, with an estimated 300 million cases reported annually.[1] Indonesia is one of the countries where malaria is endemic, and it is a significant concern for elimination, alongside other diseases like TB and HIV/AIDS. However, the number of malaria cases had decreased from 2018, around 202,176 cases, to only 94,610 cases in 2021.[2] Malaria remains one of the deadliest infectious diseases, caused by Plasmodium protozoan parasites, including *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium knowlesi*.[3]
In Indonesia, *C. papaya*, also known as papaya leaf, is a traditional plant commonly used to treat malaria.[4] It belongs to the Caricaceae family, and some of its species have been used as medicinal plants for various ailments, including antimalarials. *In vitro* studies have demonstrated that *C. papaya* exhibits high and effective antiplasmodial activity against *P. falciparum*, indicating that it is well-suited for traditional malaria treatment.[5]
In silico experiments refer to experiments carried out using a computer. These tests can help determine the interaction between a compound and its molecular target, such as a receptor.[6] Using computational methods, the interaction between compounds and receptors can be visualized, which can aid in identifying the pharmacophore of a compound that can be screened and assist in the synthesis process.[7]

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In the plasmodium synthesis process, the chain cleavage leads to a lack of energy required for metabolism, which ultimately leads to its death. This study aims to identify potential candidate compounds from *C. papaya*, which can be further explored in the search for new antimalarial drugs.

Material and Methods

Materials
The materials used in this study include a Lenovo laptop with 4GB RAM, an Intel Core i3 processor, and software such as Windows 10 operating system, Pyrx, Biovia (Discovery Studio 2021 client), PDB (Protein Data Bank used for searching for plasmodium proteins), and secondary metabolites obtained from papaya leaves, which were downloaded from Pubchem website in the PDB format.

Preparation of Ligand Compounds
To obtain the structure of a compound, the Pubchem program on the website pubchem.ncbi.nlm.nih.gov can be used.[8] This website provides information, including Simplified Molecular Input Life Entry System (SMILE). The downloaded compound structure should be in 3D format with SDF. After obtaining the SMILES from the *C. papaya* compound can be copied and pasted into the PASS Test. The PASS test can be performed on the way2drug.com website by entering the SMILES in the provided box and clicking the "Get prediction" button to view the prediction results. To see the predicted biological activity of a compound, click on the "Pa>0.7" button. A pre-ADMET test can also be performed on the https://PkCSM.com/ website by entering the SMILES in the box and clicking the black pre-ADMEt button.

Preparation of Proteins Related to Malaria
To prepare the malaria protein for docking, the protein is obtained from the PDB database through the website www.rcsb.org. When selecting the protein, it is essential to consider the resolution limit of 1-3, with a lower limit preferred to avoid missing residues. Additionally, the

Ramachandran plot is checked to ensure that the protein has complete amino acids, and this can be viewed at www.ebi.ac.uk. Once the protein is downloaded, native ligands are searched for using PyMOL. Water molecules are removed to avoid interference during the docking process and ensure the compound and receptor's accuracy.[9]

Molecular Docking

The protein crystal structure, along with the native ligand, was obtained from the Protein Data Bank (PDB). The native ligand was then extracted from the protein with PyMol while retaining polar hydrogen using Kollman charges.[10] AutoDock Vina, which is integrated into PyRx 0.9.9, was used for docking with a grid set to completeness = 8 and sizes of 50, 50, 50 with the center at x = -45.00, y = -35.00, z = -10.00. Docking was considered valid if the RMSD value was less than 2Å.[11]

Molecular Dynamic Simulation

The YASARA Structure version 14.12.2 was utilized to conduct a Molecular Dynamics (MD) simulation on a Microsoft Windows 10 operating system. The simulation employed the YAMBER Force field as its force field of choice, while the Ewald particle algorithm was utilized to calculate the Coulomb distance interaction. The Van der Waals force was restricted to 8 Å, and a cube-shaped simulation box was placed around the simulated molecules at a distance of 5 nm. The simulation box had dimensions of 50 × 50 × 50 Å with a value of n = 6, and its boundary was subjected to periodic conditions. The water density was set at 1 g/cc and a temperature of 298 K. The simulations ran for 10 ns, and snapshots of the system were captured every 100 ps.[12]

Result and Discussion

PASS Test

The present investigation involved a comprehensive review of relevant literature in academic journals to identify specific compounds in papaya leaves. Subsequently, the identified compounds were subjected to the PASS test to evaluate their potential antimalarial properties, including antiprotozoal, antifungal, antiviral, and chemoprotective activities (Table 1). A total of 55 compounds were found to exhibit antimalarial activity based on the screening criteria.

PkCSM Test

The PkCSM test, which includes the Adsorption, Distribution, Metabolism, Elimination, and Toxicity Test (ADMET), is an in silico method used to predict the pharmacokinetic properties and toxicity of compounds in the body.[13] The Lipinski Rule of Five is also employed to evaluate the drug-likeness of the compound, as biological activity and stability alone are not sufficient to determine the potency of a compound as a viable drug candidate.[14] The use of these predictive tests enables researchers to identify potential drug candidates that possess desirable pharmacokinetic and physicochemical properties necessary for drug efficacy.[15]

Table 1. Result of pass test

Coumpound	PASS test	SMILE
Kaempferol 3 – O -pentoside	Chemopreventive, antiprotozoal (leishmania), antifungal	C1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC=C(C=C4)O)O)O)O
Kaempferol-3 – O - rhamnoside	Chemopreventive, antiprotozoal (leishmania), anti fungi	CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC=C(C=C4)O)O)O)O
Kaempferol	Antiseborrheic, chemopreventive	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
Chlorogenic acid	Chemopreventive	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O
Quercetin	Antiseborrheic, chemopreventive	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
Myricetin 3- rhamnoside	Chemopreventive, antiprotozoal (leishmania), antifungal, antiinfective, antiviral	CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C(=C4)O)O)O)O)O)O)O
Quercetin 3 -[rhamnosyl- (1->2) –rhamnosyl - (1>6)-glucoside]	Chemopreventive, antiprotozoal (leishmania), antifungi, antibacterial	CC1C(C(C(C(O1)OC2C(C(C(OC2OCC3C(C(C(C(O3)OC4=C(OC5=CC(=CC(=C5C4=O)O)O)C6=CC(=C(C=C6)O)O)O)O)O)C)O)O)O)O)O
Kaempferol 3-(2Grhamnosylrutinoside)	Chemopreventive, antiprotozoal (leishmania), antifungal, antiviral, antibacterial	CC1C(C(C(C(O1)OC2C(C(C(OC2OCC3C(C(C(C(O3)OC4=C(OC5=CC(=CC(=C5C4=O)O)O)C6=CC=C(C=C6)O)O)O)O)C)O)O)O)O)O
Campesterol	Chemopreventive	CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C

Cholest – 5 -en-3 -ol, 24 – propyliden (3.beta.)	Chemopreventive	CCC=C(CCC(C)C1CCC2C1(CCC3C2C C=C4C3(CCC(C4)O)C)C)C(C)C
Linolenic acid	Antiviral (cmv), antiviral (rhinovirus)	CC/C=C\C/C=C\CCCCCCCC(OCC(O)C O)=O
Kaempferol 3- rutinoside	Chemopreventive, antiprotozoal (leishmania), antifungal, antiviral,	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC 3=C(OC4=CC(=CC(=C4C3=O)O)O)C5= CC=C(C=C5)O)O)O)O)O)O)O
Quercetin 3-rutinoside (Rutin)	Chemopreventive, antiprotozoa(leishmania), antifungal	CC1C(C(C(C(O1a)OCC2C(C(C(C(O2)O C3=C(OC4=CC(=CC(=C4C3=O)O)O)C5 =CC(=C(C=C5)O)O)O)O)O)O)O)O
Gamma.-Sitosterol	Chemopreventive	CCC(CCC(C)C1CCC2C1(CCC3C2CC= C4C3(CCC(C4)O)C)C)C(C)C

Out of the 14 compounds tested, only three, namely kaempferol, quercetin, and linolenic acid, were found to comply with the Lipinski rule of five. The Lipinski rule of five is a set of criteria that must be met for a compound to be considered a viable drug candidate.[16] These criteria include a molecular weight of less than 500 Da to enable the compound to penetrate cell membranes, a lipid solubility with a log P value of less than 5 to ensure easy passage through the lipid bilayer, and less than five hydrogen bond donors and ten hydrogen bond acceptors to reduce the energy required for absorption.[17] In addition to the Lipinski requirements, the toxicity of the compounds was also evaluated, with toxicity values ranging from 1 to 6, where 1 represents high toxicity, and 6 represents no toxicity. Based on the toxicity values, all of the tested compounds have the potential to be non-toxic as their toxicity values are greater than 2. In summary, the results indicate that only a few compounds meet the Lipinski rule of five and that all of the tested compounds have the potential to be non-toxic. These findings suggest further evaluating the potential drug candidates is required to identify the most promising compounds.[18]

The pkCSM test yielded thirty predictive data on fourteen compounds (Table 2.), which included solubility in water, $CaCO_2$ permeability, intestinal absorption in humans, skin permeability, and P-glycoprotein substrate. The solubility in water data predicts a compound's solubility at 25 degrees Celsius and is an essential characteristic for drug absorption in the body.[19] All compounds, except for Campesterol and Cholest-5-en-3-ol, 24-propylidene (3.beta.), had values within the acceptable range of -6 to -2. The $CaCO_2$ permeability data predicts drug absorption when administered orally, and compounds with Papp values greater than 8×10^{-6} cm/s are considered to have good permeability. The model defines good permeability as a value above 0.9. However, several compounds, such as chlorogenic acid, Quercetin, Myricetin 3-rhamnoside, Quercetin 3 - [rhamnosyl - (1->2) – rhamnosyl - (1->6) - glucoside], Kaempferol 3 -(2Grhamnosylrutinoside), and Quercetin 3-rutinoside (Rutin), showed poor $CaCO_2$ permeability.

Intestinal absorption in humans data predicts the percentage of a compound that will be absorbed when administered orally, and it was observed that most of the compounds had good absorption values, with the percentage absorbed being more than 30%, except for Quercetin 3-[rhamnosyl - (1->2) –rhamnosyl - (1->6) - glucoside], Kaempferol 3 -(2Grhamnosylrutinoside), and Quercetin 3-rutinoside (Rutin), which had poor absorption values. Skin permeability data was also assessed to determine a compound's ability to act as a transdermal drug product, with log Kp < -2.5 indicating good permeability.[20] Finally, P-glycoprotein substrate data was analyzed to predict whether the compound could become a Pgp substrate. Out of the 14 compounds, only Campesterol, Cholest-5-en-3-ol, 24-propylidene (3.beta.), Linolenic acid, and Gamma sitosterol were not considered to be Pgp substrates.

The P-glycoprotein inhibitor activity of the compounds was also predicted, along with several other distribution-related data, including Volume of Distribution (Vdss), Fraction Unbound (Fu), Blood-Brain Barrier (BBB) permeability, CNS permeability, CYP450 inhibitor activity, and CYP2D/CYP3A4 substrate activity.[21] Vdss data indicates the theoretical volume of the drug required to distribute evenly in blood plasma, with higher values indicating greater distribution in non-plasma tissues. Compounds with Vdss values below -0.15 are considered a low distribution, while those above 2.81 are considered high. The compound Linolenic acid was found to have a low Vdss value. Fu data predicts the fraction of drugs not bound to plasma, with most compounds having a value of 0 and the highest being 0.658. BBB permeability data predicts the compound's ability to penetrate the blood-brain barrier, which is crucial in determining its toxicity, side effects, and pharmacological activity. Compounds with logBB > 0.3 are predicted to penetrate the blood-brain barrier, while those with logBB values <-1 are predicted to have difficulty distributing in the brain.[22] All compounds except kaempferol are predicted to penetrate the blood-brain barrier.

OCT2 data or Renal Organic cation Transporter 2 shows the prediction of whether the molecule has the potential to become an OCT2 substrate or not. From the data, it is said that all the compounds except for having no potential as OCT2 substrates.[23] The total clearance value, it indicates the clearance of the compound in the body and this is important for the bioavailability data of the compound so that it can determine the dose level to achieve a balanced concentration. The last is the prediction of the toxicity of the compound. There are predictions of LD_{50} in mice, AMES toxicity data, *T. Pyriformis* toxicity data, Minnow toxicity data, MTD data, ORCT data, hepatotoxicity data, skin sensitization dat, and hERG I and hERG II inhibitors. The first is AMES toxicity which determines whether the compound can be potential a compound that causes mutagens and is carcinogenic.[24] The 14 compounds stated that the compound was a non-mutagen compound. For the toxicity of *T. Pyriformis*, it is stated that all compounds are toxic because the value is > -0.5. In Minnow's toxicity, it was also stated that 9 compounds had high toxicity, while the other compounds did not, judging from the log LC_{50} value <-0.3 which was predicted to have high acute toxicity. The Maximum Tolerated Dose (MTD) is a prediction of the dose that causes toxicity. The compound has low toxicity if the maximum dose tolerance is < 0.477, while it will be declared high if the maximum dose tolerance value is > 0.477. It is stated that compounds Kaempferol 3-O-pentoside, Kaempferol-3-O-rhamnoside, Kaempferol, Quercetin, Linolenic acid, and Kaempferol 3- rutinoside, have high toxicity, while other compounds do not have a high toxicity.

The hepatotoxicity data indicates the toxicity of the compounds towards the liver. However, none of the compounds displays hepatotoxicity.[25] Similarly, most compounds are predicted to be non-sensitizers for skin irritation. The hERG I and II inhibitor potential of the compounds is

also assessed, with compounds that inhibit potassium channels via the hERG gene known to cause prolonged side effects such as arrhythmias. Based on the prediction results, compounds including Kaempferol, Quercetin, Linolenic acid, and Chlorogenic acid are not potent hERG II inhibitors. Therefore, some compounds have a good profile as medicinal agents, while others have an unfavourable profile. However, the pkCSM test has its limitations, as it only focuses on the basic sub-structure of the compound rather than the entire compound. Hence, further testing using molecular docking is necessary to determine the potential of these compounds to bind to antimalarial receptors.

Molecular Docking Analysis

Molecular Docking Protein 3I65

Protein 3i65 is a crucial enzyme, namely *Plasmodium falciparum* dihydroorotate dehydrogenase, involved in pyrimidine biosynthesis. This process is responsible for forming DNA and RNA, essential for *P. falciparum's* survival, a malaria causative agent.[26] Specifically, protein 3I65 catalyzes the fourth stage of de novo pyrimidine biosynthesis, which involves the oxidation of dihydroorotate to produce orotate, a pyrimidine precursor.[27] Considering the significance of protein 3i65 in the life cycle of *P. falciparum*, it has been identified as a potential target for developing antimalarial drugs.[28] Targeting this protein could inhibit the malaria cycle during the schizont phase, which acts on the liver stage.[29] The control ligand for protein 3i65 is JZ8, a compound belonging to the triazololpyrimidine derivative. JZ8 has been shown to selectively bind and inhibit protein dihydroorotate dehydrogenase, demonstrating its potential for use in developing antimalarial drugs.

The molecular docking study of the protein 3I65 with different ligands has revealed the highest binding affinity value of -11.9 for the compounds Kaempferol 3-O-pentoside and Kaempferol 3 – O - rhamnoside. However, the control ligand JZ8 has a higher value of -12, indicating the strongest binding affinity with the protein. Interestingly, Kaempferol-3–O-Rhamnoside has been suggested to have greater potential as an antimalarial drug candidate as it produces identical amino acid residues as the control ligand.[30] The amino acid residues produced by the control ligands, on the other hand, include LYS 229, THR249, ASN342, LYS429, ASN458, GLY478, GLY507, TYR528, SER529, ASN458, THR249, ALA225, SER477, and ASN274, forming hydrogen bonds, and ILE263, CYS276, ILE272, and ALA225, forming hydrophobic bonds (Figure 1.).

The amino acid residue TYR528, produced by both the control ligand and Kaempferol-3-O-rhamnose compounds, has been identified as a critical residue involved in the active site of protein 3I65. This residue has been previously defined as part of the active site in the protein-ligand structure of 3I65. Based on this observation, it can be inferred that the Kaempferol-3-O-rhamnose compound may form a stronger bond with the enzyme's active site, resulting in a more efficient reaction in the PfDHODH protein compared to other compounds. Therefore, it can be concluded that Kaempferol-3-O-rhamnose is a potential candidate for developing new antimalarial drugs, as it can interact strongly with the key active site residue TYR528 of the PfDHODH protein, which plays a crucial role in the de novo pyrimidine biosynthesis process.

Molecular Docking Protein 1Q0L

The protein 1Q0L is identified as DXR reductase, which plays a crucial role in the biosynthesis of the isoprenoid in Plasmodium.[31] Specifically, it catalyzes the reduction and rearrangement of DOXP to MEP, thereby suppressing the formation of schizonts and trophozoites, making it a promising target for antimalarial drugs.[32] The FOM control ligand, [formyl (hydroxy) amino] propyl phosphonic acid, belongs to the class of fosmidomycin inhibitors, known as potent and selective inhibitors of PfDXR. Although fosmidomycin is effective against *P. falciparum* in clinical trials, its limited bioavailability and unfavorable drug attributes have restricted its therapeutic potential.[33] Therefore, searching for improved PfDXR inhibitors with better pharmacological/safety profiles is paramount.[34]

The compound Quercetin 3-[Rhamnosyl-(1->2)-Rhamnosyl-(1->6)-Glucoside] showed the highest binding affinity value of -10.1, which is close to that of the control ligand. This compound shares six hydrogen residues (ALA100, ILE101, ASN124, THR10, ALA123, SER12) and four hydrophobic residues (ILE101, ILE13, ALA100, ALA123) with the control, and also has one ionic bond (ASP57) (Figure 2.). Therefore, it is likely that Quercetin 3-[Rhamnosyl-(1->2)-Rhamnosyl-(1->6)-Glucoside] can function similarly to the control ligand. Furthermore, this compound also contains the key residue LYS125, which is found in the NADPH complex.

Molecular Docking Protein 6M20

The *P. falciparum* hexose transporter protein, coded as 6M20, is capable of hindering the growth of the parasite during the trophozoite phase.[35] *P. falciparum* relies heavily on glucose uptake and glycolytic metabolism for its energy needs, and compensates for glucose deficiency by importing glucose from the host bloodstream via the PfHT transporter.[36] BNG, a nonyl beta-D-glucopyranoside compound that is a hexose derivative, serves as the control ligand for this protein. The c336 derivative of this compound exhibits increased PfHT1 inhibition and potential against *P. falciparum* at the cellular level, while maintaining excellent selectivity for human GLUT.[37]

Quercetin exhibits a higher binding affinity value than the control ligand BNG, with a value of -9 compared to the control's value of -7. The *C. papaya* compounds were similar to the control, sharing hydrogen and hydrophobic bonds with specific residues.[38] Quercetin also shares identical residues with the control, such as ASN48, SER317, VAL180, and VAL314, indicating that it can bind to proteins in a similar location as the control ligand. In addition, Quercetin forms hydrogen bonds with amino acid residues ASN48, THR49, and SER317 (Figure 3.). Therefore, Quercetin has substantial potential to inhibit the *P. falciparum* hexose transporter protein.

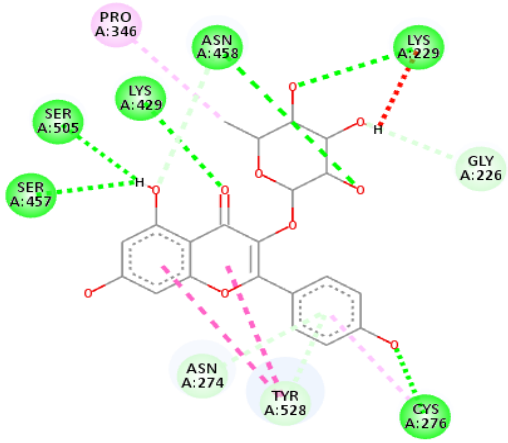

Figure 1: Molecular docking visualization of kaempferol-3-o-rhamnoside with 3I65 protein

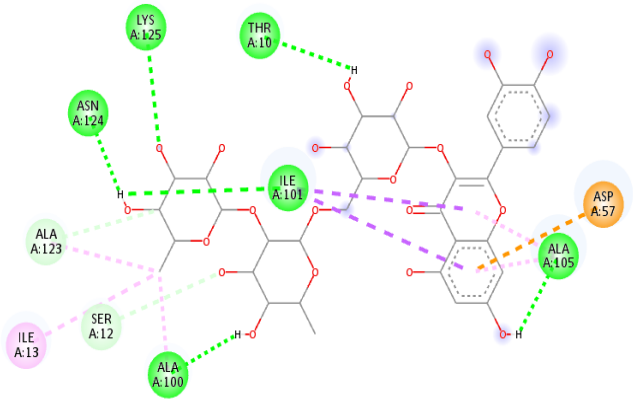

Figure 2: Molecular docking visualization of of Quercetin 3 -[Rhamnosyl - (1->2) – Rhamnosyl - (1->6) -Glucoside] with 1Q0L protein

Table 2: Result of preADMET test

Compound	Molecular Weight	LogP	H-acceptor	H-donor	Toksisitas
Kaempferol 3 – O -pentoside	418.354	0.3946	10	6	_
Kaempferol-3 – O -rhamnoside	432.381	0.7831	10	6	5
Kaempferol	286.239	2.2824	6	4	5
Chlorogenic acid	354.311	-0.6459	8	6	5
Quercetin	302.238	1.988	7	5	3
Myricetin 3 - rhamnoside	463.379	0.1943	12	8	5
Quercetin 3 -[rhamnosyl-(1->2) - rhamnosyl-(1->6)-glucoside]	756.663	-2.8353	20	12	5
Kaempferol 3 - (2Grhamnosylrutinoside)	740.664	-2.5409	19	11	5
Campesterol	400.691	7.6347	1	1	4
Cholest-5 – en – 3 -ol, 24-propylidene (3.beta.)	426.729	8.335	1	1	4
Linolenic acid,	312.45	3.526	4	2	6
Kaempferol 3 - rutinoside	594.522	-1.3927	15	9	5
Quercetin 3 -rutinoside (Rutin)	610	-1.6871	16	10	5
Gamma.-Sitosterol	414.718	8.0248	1	1	4

Molecular Docking Protein 6KOT

The protein *P. falciparum* dihydrofolate reductase thymidylate synthase, with the protein code 6KOT, is essential for the growth of plasmodium and the metabolism of certain amino acids.[39] Folate is crucial for DNA synthesis and DHFR is a critical enzyme in the folate pathway, responsible for producing tetrahydrofolate.[40] DHFR operates as a bifunctional enzyme alongside thymidylate synthase (TS), which uses methylene tetrahydrofolate as a methylating agent to synthesize d-TMP from d-UMP. Inhibiting DHFR will hinder DNA replication and can lead to resistance due to mutations or changes in the gene base sequences of the enzyme, which can cause an amino acid change at a specific position.[41] The enzyme TS is highly conserved, making it a good target for inhibition. The protein has a control ligand called DQ0, which is a dihydropyrimidine compound that effectively inhibits the pfDHFR-TS protein. Dihydropyrimidine acts as an inhibitor of the conversion of dihydrofolic acid, which is necessary for the synthesis of nucleic acids and proteins.[42]

The compound Kaempferol 3-rutinoside exhibits a high binding affinity value of -9.2 and shares similar residues with the control ligand. The control ligand, DQ0, forms hydrogen bonds with SER218, THR130, ASN108, SER111, GLY44, and THR107 and hydrophobic bonds with LEU127, and an ionic bond with ARG129 (Figure 4). Additionally, the compound Kaempferol 3-rutinoside has residues that actively participate in the enzyme's active site, namely ALA16 and LEU46, which have previously been identified in the 6KOT protein's ligand structure. Based on these findings, it can be inferred that Kaempferol 3-rutinoside has the highest potential for inhibiting the DHFR-TS protein among the five compounds studied.

Molecular Docking Protein 1V0P

The *P. falciparum* protein kinase 5 (PfPK5), whose protein code is 1V0P, is involved in the glycolysis process during the schizont phase of the malaria parasite.[43] Its function in ATP production makes it a potential target for developing anti-malarial drugs. PfPK5 is a catalyst in step 5 of the sub-pathway that synthesizes pyruvate from D-glyceraldehyde 3-phosphate.[44] The control ligand of this protein is Purvalanol B, which is derived from oxidolol compounds and has demonstrated effective inhibition of PfPK5.[45] The complex formed by PfPK5 and PVB engages with multiple residues of PfPK5. This knowledge has implications for designing drugs or treatment strategies for malaria by targeting PfPK5 and hindering its activity.[46]

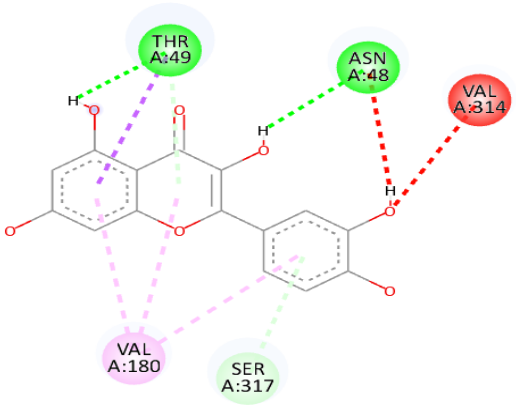

Figure 3: Molecular docking visualization of quercetin with 6M20 protein

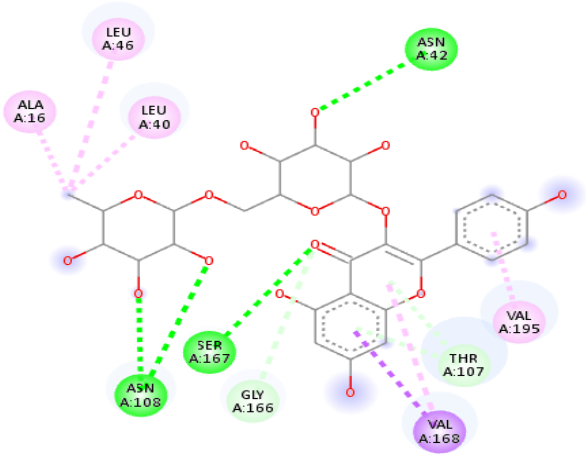

Figure 4: Molecular docking visualization of kaempferol 3-rutinoside with 6KOT protein

Cholest-5-en-3-ol, 24-propylidene-(3.beta.) has the highest binding affinity value of -9.5, while Myricetin 3-rhamnoside has a binding affinity of -9.3. Comparing the residues between the *C. papaya* compounds and the control reveals similarities. The control (PVB) interacts with residues that form hydrogen bonds (ASP85, LEU82, GLU80) and hydrophobic bonds (ILE10, VAL18, ALA30, LEU132, PHE79), and one ionic bond (LYS88). Myricetin 3-rhamnoside shares six residues with the control, including ASP85, LEU82, ILE10, VAL18, ALA30, and LEU132, and has the crucial residue LYS88 that functions as the carbonyl on the protein hinge. Therefore, Myricetin 3-rhamnoside forms a stronger bond with the active site. It can react more efficiently with protein kinase 5, indicating that it binds to the protein more accurately and effectively than the control and other compounds.

Result of Molecular Docking Protein 6C4G

The protein code 6C4G refers to *P. vivax* plasmepsin V, a crucial enzyme responsible for cleaving the pexel (a sequence necessary for exporting effector proteins) to facilitate the remodeling of host erythrocytes and promoting parasite growth and survival.[47] Blocking the activity of plasmepsin V, which is a malarial enzyme, can hinder red blood cell remodeling and ultimately lead to parasite death.[48] The control ligand EQG, also known as benzyl [(6S,7S,10S,13S,18Z)-18-amino-10-cyclohexyl-6-hydroxyl-18-imino-7-(2-methylpropyl)-4,9,12-trioxo-1-phenyl-16-oxa-3,8,11,17-tetraazaoctadecan-13-yl] carbamate, acts as a protease inhibitor for plasmepsin V and has the potential to inhibit its activity.[49]

Quercetin 3 - [rhamnosyl - (1->2) – rhamnosyl - (1->6) - glucoside] shares seven residues with the control, including THR317, GLY315, CYS140, SAP313, CYS140, LEU179, and TYR61. Therefore, it can be inferred that this compound binds accurately to the protein, similar to the control ligand.[50] Additionally, Quercetin 3 - [rhamnosyl - (1->2) – rhamnosyl - (1->6) - glucoside] exhibits a higher binding affinity value and features a crucial residue, GLU141, which indicates its potential as a plasmepsin V protein inhibitor.[51] This compound can be developed as a potential therapeutic agent in the future.

C. papaya is a plant that contains various compounds, such as flavonoids, steroids, alkaloids, and terpenoids. Among these compounds, flavonoids and terpenoids have been found to exhibit high activity in inhibiting malaria proteins.[52] Flavonoids inhibit fatty acid biosynthesis, hinder the entry of L-glutamine and myoinositol into infected erythrocytes, and inhibit membrane formation by plasmodium, thereby preventing the growth of plasmodium and anemia.[53] Conversely, terpenoids interact with Ferriprotoporphyrin IX in the acidic food vacuole of parasites, generating toxic free radical species that kill parasites.[54] The peroxide bridge structure in the artemisinin molecule can be broken by ferrous ions from hemoglobin, leading to the formation of reactive free radicals that are lethal to parasites.[55] These findings suggest that the flavonoids and terpenoids in *C. papaya* can be developed into an effective antimalarial compound.[56]

Molecular Dynamic Simulation

Molecular Dynamic Protein 6M20 and 1V0P

The fluctuations of the system were analyzed concerning simulation time to examine the system's dynamics. The stability of a protein is often evaluated based on the Root Mean Square Deviation (RMSD), as it is an essential factor in determining protein stability.[57] The RMSD values of all the compounds docked on the 6M20 protein were less than 2.5 Angstroms, indicating they are reliable and reasonable.[58] The 6M20 protein had the highest RMSD value of 2.386 Angstroms among the three compounds but was stable at RMSD values between 1000-1100 Angstroms (Figure 7). It was concluded that the compound with the best stability when docked on the 6M20 protein was Cholest-5-en-3-ol, 24-propylidene-(3.beta), as it showed the least number of fluctuations when compared to the other compounds.

Based on the figure 8, it can be inferred that all the compounds docked onto the 1V0P protein are reliable and effective, as their RMSD values were less than 2.5 Angstroms. The 1V0P protein exhibited the most significant RMSD value of 2.386 Angstroms among the Kaempferol compounds, while the Chlorogenic acid and quercetin compounds had the highest RMSD value of 2.253 Angstroms. These three compounds remained stable at RMSD values between 1100-1200 Angstroms. It can be concluded that quercetin is the compound with relatively good stability when docked onto the 1V0P protein, as it displayed the least number of fluctuations compared to the other compounds.

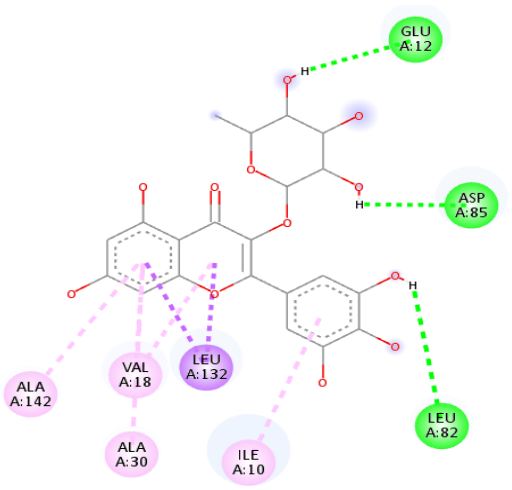

Figure 5: Molecular docking visualization of myricetin 3-rhamnoside with 1V0P protein

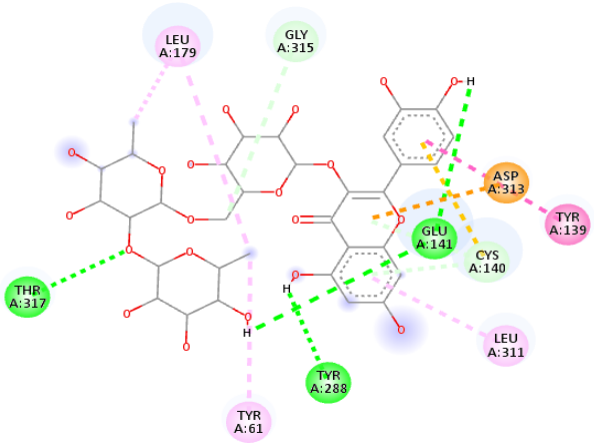

Figure 6: Molecular docking visualization of quercetin 3-[rhamnosyl-(1->2)-rhamnosyl-(1->6)-glucoside] with 6C4G protein

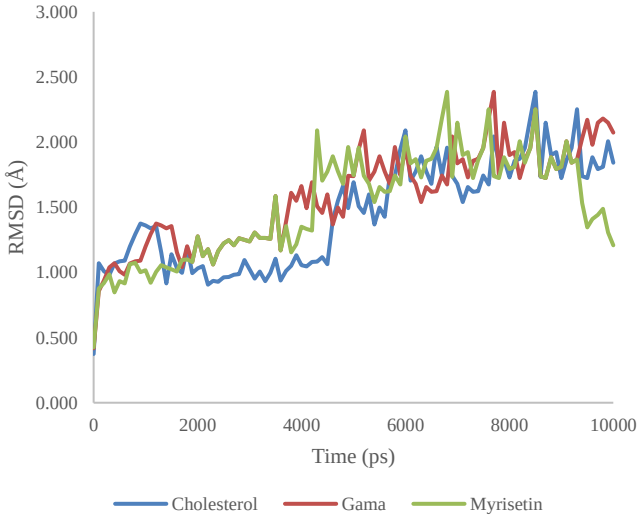

Figure 7: Analysis result of RMSD protein 6M20

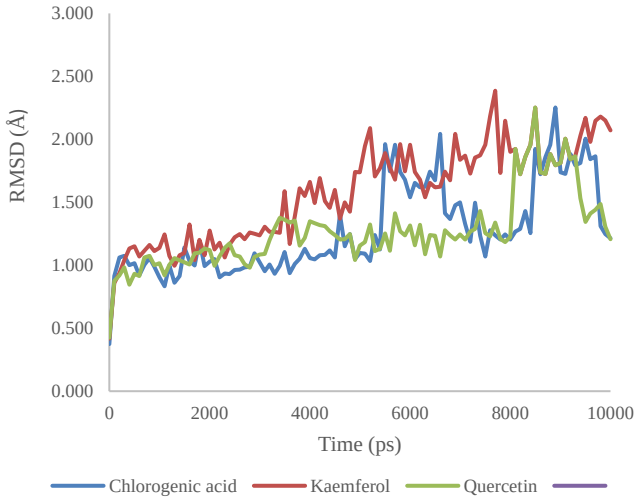

Figure 8: Analysis result of RMSD protein 1V0P

Conclusion

The present study showed that kombucha black tea with gac and mango with the highest sugar content (100g) showed the highest carbohydrate and vitamin C content, was most preferred by the panelists, and possessed significant amounts of phenolic and flavonoids with high antioxidant activity. The result of alcohol content for all samples were presented as less than 0.5% ABV, where all samples were classified as non-alcoholic beverages.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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